Empirical validation of existing human carbon monoxide (CO) exposure models with suggested improvements relevant to groups in the general population

by

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Declaration

I, KE-TING PAN, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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ABSTRACT

Carbon monoxide (CO) poisoning is an important public health issue globally. Several CO exposure models have been built to predict the kinetics of CO uptake and elimination in CO poisoning but based on a limited dataset confined to a handful of young, healthy male volunteers. It is important to improve these models by using a wider range of individuals to seek an optimal performance across a realistic range of characteristics of the general population. Therefore, to expand the validity and practical applicability of CO exposure models to people with different characteristics, such as age, sex, height, weight, smoking status and ethnicity, we carried out three studies to test the relationship between these factors and pulmonary function, represented by the Diffusion capacity of the Lung for CO (DL_{CO}), as well as the rate of CO uptake and CO elimination.

Specifically, we first explored how demographic, physiological and behavioural factors affect CO uptake and CO elimination among young volunteers. The experiment indicated that smoking status did not influence CO uptake and elimination in this group.

Then, we collected pulmonary function test (PFT) data from two hospitals, one in the UK and one in Taiwan. Given the exceptional circumstances of the COVID-19 pandemic in the UK, obtaining ethics approval for hospital data was challenging, however with great effort, PFT data were collected from both hospitals. We found that males had a higher value of DL_{CO} than females, and DL_{CO} was positively associated with height and weight, and negatively associated with age.

Later, the estimated DL_{CO} results from PFT in relatively healthy individuals were used to update established Coburn-Forster-Kane (CFK) models. These showed that, for example, when the CO exposure scenario was the same, CO uptake and elimination rate were higher in a younger male than an older male.

In conclusion, the updated model has the advantage of being able to predict CO uptake and elimination for a wider range of individuals compared to previous CFK models, factoring in their age, sex, height, weight, ethnic group, and smoking status. This could help estimate past CO exposure, and help medical staff recognize CO exposure and design optimal treatments for CO exposure victims.

Keywords: CO poisoning, CO uptake, CO elimination, CO modelling

IMPACT STATEMENT

Carbon monoxide (CO) poisoning is a major public health issue and causes a large health care burden. When people inhale CO, it replaces the oxygen in red blood cells, reducing the amount of oxygen delivered to the vital organs, leading to severe hypoxia consequences and even death. The rate of CO uptake and elimination may influence the severity of the CO poisoning, and may also depend on different demographic, physiological and behavioural factors, such as age, sex, height, weight, smoking status and ethnicity. However, there is a paucity of research thoroughly investigating and discussing these effects.

This research is one of the first studies to explore the potential factors related to CO uptake and elimination and investigate the deeper level of the physiological mechanism involved in the general population. There are many studies working on CO uptake and elimination. However, the studies with not only simulation but also exploration of possible factors affecting CO uptake and elimination and validation with measured data are limited. The other studies are Bruce and Bruce (2003; 2006; 2010), Gosselin et al. (2009) and Kuo et al. (2020). In the study, it clarified the mechanism by conducting three consecutive studies: 1) CO-rebreathing experiment and exhaled CO experiment, 2) pulmonary function data (PFT) collection and 3) modelling in four different settings, the Royal Berkshire Hospital (UK), Southampton Hospital (UK), Tri-Service General Hospital (Taiwan) and UCL (UK).

The main findings show that demographic, physiological and behavioural factors, such as age, sex, height, weight, ethnicity as well as smoking status, affected CO uptake and elimination through pulmonary function. These findings have several critical implementations. In academia, this research provides a better understanding of CO poisoning and fills a research gap regarding the possible factors and mechanisms that affect CO uptake and elimination.

In practice, the information obtained from the present study could provide valuable guidance for public health, modellers, health care and the built environment sectors. From the public health point of view, if the exhaled CO concentration for a non-smoker is equal to, or is above 5 ppm, and for a smoker with the last cigarette more than 4 hours ago is above 10 ppm, the subject might have exogenous CO exposure and this should trigger a home investigation. For the modellers, the CO exposure models in our study could be simulated with various CO sources and take

account of important factors affecting CO uptake and elimination that have been found, such as; age, gender, height, weight, ethnicity and pulmonary function. Therefore, future models, such as empirical models or microenvironmental exposure models, could be simulated for different population demographics, especially for different susceptible groups (individuals who have a longer CO elimination time, such as people with older age and those with lower DL_{CO} values). Also, for public health policymakers, when designing regulations related to CO exposure, they should require settings for susceptible groups to have stricter limits for ambient CO than others.

In health care, the findings from the present study could help medical staff identify susceptible CO patients and provide more accurate treatment strategies tailored to the different characteristics of individuals in different CO exposure scenarios. In the built environment, architects and engineers should be particularly careful in designing settings for susceptible groups, such as nursing homes or hospices. Generally, anywhere that contains a CO source should be planned with sufficient ventilation to reduce potential CO exposure. CO poisoning is about human health and air quality at the same time. If governments pay more attention to these issues, they could help improve the health of the environment and populations on a global scale.

Part of the work has been published in a scholarly journal and presented at major academic and research events (see Appendix 10.3) in the PhD stage to gain feedback from external experts, which significantly improved the work. Further publications and presentations are currently in preparation as follows,

1) Pan, KT., Leonardi, G. S., Ucci, M. and Croxford, B. (2021) 'Can Exhaled Carbon Monoxide Be Used as a Marker of Exposure? A Cross-Sectional Study in Young Adults', 18(22), 11893 (Journal paper, which is based on Section 4, published after viva).
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3) Comparison of factors affecting pulmonary function parameters between Taiwan and UK (Journal paper in preparation).
4) Modification of CO models with factors related to CO uptake and elimination and comparison of the simulations (Journal paper in preparation).

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List of Abbreviations

Abbreviation	Meaning
APPCOG	All-party parliamentary carbon monoxide group
AQS	Air quality standard
BGA	Blood gas analyser
BSA	Body surface area
BTPS	Body temperature (37°C or 99°F), pressure (ambient pressure), water vapour saturated
CPAP	Continuous positive airway pressure
CFK	Coburn-Forster-Kane model
CO	Carbon monoxide
COHb (%)	Carboxyhaemoglobin
MbCO	Carboxymyoglobin
COPD	Chronic obstructive pulmonary disease
DL_{CO} (ml/mmHg/min)	Diffusing capacity of the lung for CO
DM	Diabetes mellitus
DNS	Delayed neurological sequelae
EPA	Environmental Protection Agency
ERV (L [a])	Expiratory reserve volume
FEV_1 (L [a])	Forced expiratory volume in first one second
FEV_1/FVC (%)	The ratio of forced expiratory volume in first one second to forced expiratory volume
FRC (L [a])	Functional residual capacity
FRC/TLC (%)	The ratio of functional residual capacity to total lung capacity
FVC (L [a])	Force vital capacity
Hb	Haemoglobin
HBO	Hyperbaric oxygen
HFNC	High flow nasal cannula
HIM	Human inhalation models
IC (L [a])	Inspiratory capacity
IRV (L [a])	Inspiratory reserve volume
K_{CO} (ml/mmHg/min/L)	Diffusion capacity of the lung for CO divided by alveolar volume (V_A), the K_{CO}, also known as the transfer coefficient (k_{CO}), an index of the efficiency of alveolar transfer of carbon monoxide
N_2	Nitrogen
NAAQS	National ambient air quality standards
O_2	Oxygen
O_2Hb	Oxyhaemoglobin
PaO_2	Partial pressure of oxygen
PBPK	Physiologically based pharmacokinetic model
PFT	Pulmonary function test
RBH	Royal Berkshire Hospital
RV (L [a])	Residual volume
RV/TLC (%)	The ratio of residual volume to total lung capacity
SEE	Standard error of the estimate
SIDS	Sudden infant death syndrome

STPD	Standard temperature (0°C or 273K) and pressure (760 mm Hg) and dry
TAQMD	Taiwan Air Quality Monitoring Database
TLC (L [a])	Total lung capacity
TSGH	Tri-Service General Hospital
US EPA	United States environmental protection agency
V_A (L)	Alveolar volume
V_{AR} (ml/min)	Alveolar ventilation rate
V_{BL}	Blood volume
VC (L)	Vital capacity
VT (L)	Tidal volume
WHO	World Health Organization
[COHb]	Concentration of COHb
[O_2Hb]	Concentration of O_2Hb

Note: [a] L, Litre

1. INTRODUCTION

This introduction provides an overview of the research background, research questions and research design. Section 1.1 introduces the background of the thesis. Section 1.2 gives the research questions, aim and objectives. Section 1.3 describes the research design and structure of the thesis.

1.1 Background

Carbon monoxide (CO) poisoning is an important global public health issue and a leading cause of fatal poisonings reported in many countries (Raub et al., 2000). In Taiwan's National Mortality Registry, it showed 439 deaths from unintentional CO poisoning registered during 1997-2003 (Shie and Li, 2007). In the UK, the Cross Government Group on Gas Safety and CO Awareness reported about 25 deaths annually from accidental CO poisoning in England and Wales (Cross Government Group on Gas Safety and CO Awareness, 2019). The health effects of CO exposure mainly result from the high affinity of CO and haemoglobin (Hb), which leads to the formation of carboxyhaemoglobin (COHb) thus causing Hb to lose the ability to carry oxygen throughout the body (Dolan, 1985; Blumenthal, 2001). With increased CO exposure, people may suffer from headaches, fainting, confusion and even loss of consciousness or death (WHO, 1999; Townsend and Maynard, 2002; Kao and Nanagas, 2004). Moreover, patients who have recovered from CO poisoning may experience long-lasting effects: around 25% of CO poisoned patients may experience delayed neurological sequelae (DNS) (Weaver et al., 2002; Pepe et al., 2011). These include neuropsychological sequelae and cognitive and psychological sequelae, such as Parkinson-like syndromes, hearing loss, memory loss, dementia, depression and anxiety (Pepe et al., 2011). Therefore, patients who suffer from CO poisoning may experience not only the initial acute health effects but also continuous sequelae. Both have the potential to inflict large health and economic burdens on society.

When treating CO poisoning, the main objective is to reduce the amount of CO accumulating in the body as soon as possible. Therefore, it is important to understand the rate of CO uptake and elimination in the human body. Some scholars have speculated that sex, age, height, weight and smoking status might affect the rate of CO uptake and elimination (Burney et al., 1982; Weaver et al., 2000; Cronenberger et al., 2008; Zavorsky et al., 2014; Pan et al., 2020). However, the effects of these factors on the rate of CO uptake and elimination are controversial in the literature, since previous

research mainly focuses on the correlations without considering the underlying mechanism.

Therefore, to uncover the demographic, physiological and behavioural factors and possible underlying mechanisms, we investigated the mechanisms and parameters used in CO exposure models. There are several CO exposure models to predict the COHb in CO uptake and elimination in the human body (Forbes et al., 1945; Coburn et al., 1965; Bruce and Bruce, 2003; Gosselin et al., 2009). The most widely used model is the Coburn-Forster-Kane (CFK) model (Coburn et al., 1965). It contains parameters such as CO concentration, duration of CO exposure, alveolar ventilation rate, DL_{CO} (the diffusing capacity of the lungs for CO), endogenous CO rate, blood volume, concentration of Hb and initial COHb value (Coburn et al., 1965). In 2009, Gosselin et al. built a modified version of the CFK model. In these two models and the literature, the pulmonary function has been proven to affect CO uptake and elimination (Filley et al., 1954; Coburn et al., 1965; Gosselin et al., 2009; Pan et al., 2020).

DL_{CO}, an important pulmonary function in the CFK models, is affected by sex, age and height (Paoletti et al., 1985; Park et al., 1986). In addition, other factors may also influence DL_{CO}, including weight, Hb and smoking status (Talaminos Barroso et al., 2018; Frans et al., 1975; Saydain et al., 2004) and thus could also influence the rate of CO uptake and elimination. For example, when the value of DL_{CO} of a patient increases, so does the rate of CO uptake and elimination (Filley et al., 1954; Coburn et al., 1965; Bruce and Bruce, 2003; Gosselin et al., 2009).

However, these models above have been only developed and tested with people that are generally young, healthy, white and male. The impacts of different characteristics among the general population may be neglected. It is necessary to improve these CO exposure models to predict the COHb value for a wider range of these characteristics across the population to seek an optimal investigation and more personalised treatment for CO poisoned patients.

Therefore, in this research, we aimed to empirically validate the CO exposure models to enable them to cover a wider population by revealing the effects of demographic, physiological and behavioural factors on the rate of CO uptake and elimination. We studied whether these factors affected pulmonary function and thus impacted the rate of CO uptake and elimination. The results could provide guidance for health care workers, allowing them to give more accurate assessment and treatments to CO poisoned patients depending on their unique characteristics.

1.2 Research question, aim and objectives

This section presents the aims and objectives of the research. The overarching research question is 'Does improving our understanding of the effect of variations in age, sex, height, weight, ethnicity and smoking status on the prediction of CO exposure models provide useful information that could help in assessing CO poisoned patients?' The research aims to empirically validate the CO exposure models to enable them to cover a wider population than possible so far. Additionally, it seeks to investigate the relationship between demographic, physiological and behavioural factors and pulmonary function, and then how the lung function affects the rate of CO uptake and elimination. To achieve this aim, the following objectives were developed, and the related hypothesis were tested as follows:

- To understand the factors that affect CO uptake and elimination as per the literature.
- To investigate the relationship between demographic, physiological and behavioural factors (particularly smoking status and smoking habits) and exhaled CO value (and thus to inferred CO uptake and elimination). In particular, I hypothesize that:
    - Demographic factors and smoking status will affect CO uptake and CO elimination in the human body.
    - Heavy smokers have a longer CO half-life than light smokers.
- To analyse the relationship between selected demographic, physiological and behavioural factors (including smoking status), PFT parameters and disease with regard to DL_{CO}, V_A and K_{CO}. In particular, I hypothesize that:
    - Age, sex, height and weight influence DL_{CO}, V_A and K_{CO} in a quantifiable way.
    - Smoking decreases K_{CO} and DL_{CO} in a quantifiable way.
    - Groups of individuals attending TSGH (Asian) and RBH (Caucasian) have similar factors affecting DL_{CO}, V_A and K_{CO}.
- To update the original CFK (1965) and modified CFK (2009) models with estimated DL_{CO} from the prior investigations in the PFT study and

use the models to simulate several different scenarios. In particular, I hypothesize that:

- There are quantifiable differences in CO uptake rate and CO elimination rate between males and females, old and young, tall and short, smokers and non-smokers.

1.3 Research design and structure of the thesis

1.3.1 Research design

To answer the central research question, a variety of methods were used across the different parts of the research. This section presents the flow of research in logic details and provides background details for each dataset used in the present study.

Research steps and the logic flow of the thesis

There are three main parts to the thesis (see Figure 1-1). First, experiments were conducted to compare CO uptake and elimination before and after CO exposure before analysing the data to ascertain if any factors affect CO uptake and elimination rate. Second, demographic, physiological and behavioural factors and pulmonary function test (PFT) data were collected from two hospitals, the Tri-Service General Hospital in Taiwan and the Royal Berkshire Hospital in the UK to see which factors affect PFT data, including DL_{CO}, V_A and K_{CO}. Finally, we used individuals' different pulmonary function results to predict their CO uptake and elimination; these predictions were then compared with measured data from literature, our CO-rebreathing experiment and our exhaled CO experiment. Next, the demographic, physiological and behavioural factors and PFT data were used in CFK models to predict the CO uptake and elimination for individuals in different CO exposure scenarios.

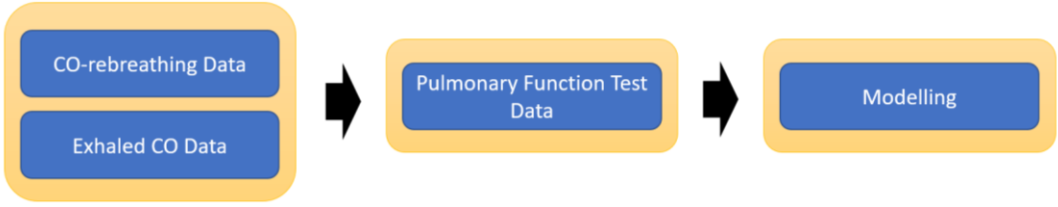

Figure 1-1. Research steps

Collaborations and datasets

The research took advantage of connections made by the researcher and the supervisory team; three partners, in particular, were essential to the research:

- Tri-Service General Hospital, National Defense Medical Centre, Hyperbaric Oxygen Centre, Taiwan
- Royal Berkshire Hospital, Respiratory Department, UK
- Southampton General Hospital, Anaesthesia and Critical Care Research Unit, UK

The CO-rebreathing experiment was conducted at Southampton General Hospital. The exhaled CO experiment was conducted at UCL, and the PFT data were collected from the Tri-Service General Hospital and the Royal Berkshire Hospital. Figure 1-2 shows the basic characteristics of the different datasets used.

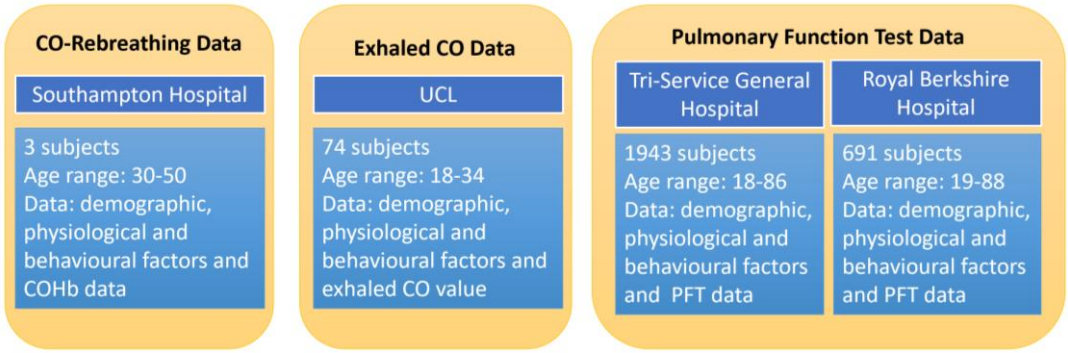

Figure 1-2. Basic characteristics of the different dataset used in the present study

1.3.2 Structure of the thesis

The thesis has nine chapters. Following the introduction (which now includes the research questions, aim, and objectives, Chapter 2 presents the literature review, including an overview of CO poisoning, CO exposure assessment, factors affecting CO uptake and elimination, modelling of CO exposure and CO gas used in clinical tests. The main studies were divided into 4 chapters;

- 3- Collecting and analysing the COHb elimination data from CO-rebreathing experiment,

- 4- Investigating the effect of smoking on CO uptake and elimination time in smokers,

- 5- Gathering and analysing the primary data on pulmonary function and

- 6- Updated existing CO uptake and elimination model and simulation in different CO exposure scenarios.

And finally, Chapter 7 draws the general discussion and Conclusions, along with the corresponding recommendations and gives some suggestions for future work. References are given in Chapter 8 and Appendix are provided in Chapter 9.

2. LITERATURE REVIEW

This literature review provides an overview of CO poisoning, CO exposures models and factors related to CO uptake and elimination. Section 2.1 introduces the characteristics of CO, its health effects on the human body and the prevalence, treatment and prevention of CO poisoning. Section 2.2 describes CO exposure assessment and measurements, CO exposure standards and typical scenarios of CO exposure in humans. Section 2.3 continues with models of CO uptake and elimination in the human body. Section 2.4 gives an overview of the factors related to CO uptake and elimination. This section has been published as an academic journal paper by the author (see Appendix 9.3). Section 2.5 introduces some methods that use CO as a clinical testing gas to determine various uptake and elimination rate constants in clinics, research laboratories and hospitals, and is followed by a summary Section 2.6.

2.1 Overview of CO poisoning

CO is a colourless, odourless, tasteless, combustible and potentially toxic gas. Because of these characteristics, it is known as a 'silent killer' (Penney, 2007). CO behaves similarly to oxygen in the body but has a much higher affinity to the same oxygen transporter molecule in the blood (Cobb and Etzel, 1991). The affinity of CO to haemoglobin (Hb) is around 200-260 times higher than oxygen (Dolan, 1985; Blumenthal, 2001). After an individual breathes in CO, it replaces the oxygen in red blood cells and forms carboxyhaemoglobin (COHb). At high exposure, CO can cause insufficient oxygen transport in the body and can lead to hypoxia (lack of oxygen) and a series of adverse health effects, such as headaches, respiratory dysfunction, tissue damage and even death (Kao and Nanagas, 2004; Weaver, 2009; Rose et al., 2017).

2.1.1 Epidemiology of CO poisoning

To get a better understanding of the importance of CO poisoning, this section provides an overview of CO poisoning worldwide and introduces different sources of CO.

CO poisoning in different countries

CO poisoning can broadly be split into two categories, unintentional (accidental) and intentional (generally means suicide) CO poisoning. A worldwide epidemiological study from the Global Health Data Exchange registry reported that the global incidence

of CO poisoning has remained stable, while mortality has declined over the last 25 years (Mattiuzzi and Lippi, 2019). However, CO poisoning is still one of the leading causes of fatal poisonings, and it causes a large number of deaths annually both in Europe and in the United States (Valent et al., 2002). In the United States, there were a total of 24,890 CO poisoning deaths (including unintentional and intentional) from 1999 to 2014, and the age-adjusted death rate was approximately 0.49/100,000 per year (Hampson, 2016). Another study has shown that there are around 8.6-40.4 emergency department visits per 100,000 for CO poisoning annually (U.S. CDC, 2005; Hampson and Weaver, 2007).

From 1980 to 2008, a survey was conducted to better understand CO-related mortality and morbidity in Europe. The national data on CO poisoning was provided by the member states of the WHO European Region. In the report, annual CO-related deaths were recorded at 140,490, with an annual death rate of 2.2 per 100,000, as provided by 28 member states (Braubach et al., 2013). In the UK, there were about 25 deaths from accidental CO poisoning annually in England and Wales (Cross Government Group on Gas Safety and CO Awareness, 2019). Between 2000 and 2010, Ghosh et al. (2016) reported 5,312 total admissions to hospital for CO poisoning in England. Then, from 2002 to 2016, there were a total of 3,399 unintentional non-fire related (UNFR) CO poisoning hospital admissions and the UNFR CO poisoning hospital admissions decreased for both males and females. There is a seasonal pattern to UNFR CO-related monthly hospital admissions with fewer admissions in summer (5% of total admissions in June and July) than in winter (approximately 15% of the total in December) (Roca-Barceló et al., 2020). Similar patterns of results were also found in the United States, China and Taiwan (Henn et al., 2013; Pan et al., 2013; Li et al., 2015). Moreover, besides seasonal pattern, Roca-Barceló et al. (2020) also mentioned socio-demographic characteristics, housing stock characteristics and legislation may also have contributed to the UNFR CO poisoning hospital admissions. Generally, vulnerable groups (lower social-demographic characteristics, such as deprived) may have a higher risk of UNFR CO poisoning hospital admission.

In the Far East, CO poisoning is also an important public health issue. In China, CO poisoning is one of the main toxic agents causing deaths (Liu et al., 2009). In Wuhan City, 156 deaths due to CO poisoning were reported from 2010 to 2014, around 1.6 per 100,000 per year (Li et al., 2015). In Taiwan, there were 439 deaths from unintentional CO poisoning between 1997 and 2003 (Shie and Li, 2007). Also, deaths

from CO poisoning doubled between 2001 and 2003 in Japan, although this may be related to an increase in CO related suicides (Yoshioka et al., 2014): the number of suicides linked to exposure to CO from charcoal burning has been increasing in East Asian countries such as Hong Kong, Taiwan and Japan since the late 1990s (Liu et al., 2007; Yoshioka et al., 2014; Chang et al., 2019; Yoshioka et al., 2019; Kinoshita et al., 2020).

Exogenous sources of CO poisoning

CO in the environment can arise from both natural and human-made sources, with natural processes accounting for about 40% and human activities for 60% (U.S. EPA, 1991). Natural sources include forest fires, volcanoes, the by-product of the metabolism of plants and the incomplete or partial oxidation of methane (CH4) and other non-methane hydrocarbons (Penney, 2007). Human-made CO is created by a variety of human activities, including cooking, smoking tobacco, using water heaters, driving, barbecues, creating industrial emissions and using generators (Manel et al., 1999; Henn et al., 2013; Fisher et al., 2013; Pan et al., 2019; Ashcroft et al., 2019; Kinoshita et al., 2020). If people are close to the combustion sources, they may be at risk of breathing in a high concentration of CO, depending on the CO source and the duration of exposure.

Table 2-1. Sources of CO (including natural sources and human-made sources, compiled from various references mentioned in the text by the author)

Natural sources	**Human-made sources**	
	Outdoors	**Indoors**
Forest fires **Volcanoes** **Natural gases** **Emission from plants** **Oxidation of methane and other non-methane hydrocarbons**	Motor vehicle exhausts BBQs Industrial emissions Cigarette smoking Generators	Heating systems Cooking appliances Gas and oil boilers Fireplaces Generators Cigarette smoking

Table 2-1 shows that most CO sources are indoors. In the UK, Fisher et al. (2013) and Close et al. (2015) have reported that a high proportion of CO poisoning incidents occur in private dwellings, and that central heating systems, water boilers and gas are the primary sources. In Taiwan, Pan et al. (2019) have found charcoal burning and gas boilers or water heaters are the main CO sources.

In Taiwan, Lin et al. (2021) have reported that the outdoor CO was around 0.5 ppm (daily average) between 2000-2012 from Taiwan Air Quality Monitoring Database (TAQMD). In the UK, the annual mean of the outdoor CO concentration was about 0.1-0.5 ppm from Defra's UKAir between 2015 and 2020 (Defra's UKAir, 2021). Generally, the average indoor CO concentration is relatively low (less than 3 ppm) both in Taiwan and the UK (Harrison et al., 2002; Volans et al., 2007; Chen et al., 2016). However, in Volans et al.'s (2007) report, the indoor CO concentration could exceed 5 ppm when cooking, which is a common source of CO in the house.

Endogenous sources of CO poisoning

There is also endogenous CO in the human body. In healthy people, small amounts of CO are formed endogenously from the catabolism of Hb and other haem proteins, such as myoglobin (Ilano and Raffin, 1990; Penney, 1996; Wu and Wang, 2005). Endogenous CO works as a neural messenger and has both biological and physiological functions in the human body. According to Wu and Wang (2005), endogenous CO is related to cardiac function, vascular contractility, the nervous system, platelet aggregation and monocyte activation. This is why people have many adverse health effects when there is an increase in CO concentration in the body. Usually, in healthy people, blood COHb concentration is between 0.4 and 0.7% due to endogenous CO production (Coburn et al., 1965). However, the endogenous CO production rates may range from less than 0.01 to 0.06 mL/min in different conditions and diseases (Owens, 2010). In some situations, the endogenous CO production increases and the COHb level rises to 4 to 6%, such as in bodies where hypermetabolism and haemolytic anaemia are present (Owens, 2010; Kinoshita et al., 2020).

2.1.2 Pathophysiology of CO poisoning

To understand CO and its health effects, this section presents the pathogenesis of CO poisoning, signs and symptoms of CO poisoning and descriptions of acute and chronic CO poisoning and its associated delayed neurological sequelae (DNS).

Pathogenesis of CO poisoning

Healthy individuals need small amounts of endogenous CO production to maintain general physiological function in the body (Wu and Wang, 2005). However, if the CO concentration exceeds the body's tolerance level, its effect becomes toxic.

CO affects the human body in a two-step process. First, after people inhale CO, CO concentration increases in the alveolar gas in the lungs. This gas is then transferred to the lung capillaries and, as the gas diffuses through the capillaries into the bloodstream, it binds with Hb to form COHb. In the second step, the CO in the blood is transferred and binds to cellular haemoproteins in the tissues, such as cytochrome P-450, cytochrome c oxidase and myoglobin (Penney, 1996; Kao and Nanagas, 2004; Kinoshita et al., 2020).

The first step is mainly related to Hb. Hb is an iron-containing protein in the blood cell, primarily used as an oxygen-transport system for the whole body. When people inhale CO, CO forces Hb to form COHb rather than oxyhaemoglobin (OHb) due to its high affinity with Hb (Dolan, 1985; Varon et al., 1999). A COHb molecule cannot transport oxygen, thus reducing the total capacity of oxygen transport, which can lead to a lack of oxygen (hypoxia) in organs and tissues (Meredith and Vale, 1988; Weaver, 2009).

In addition to Hb, CO also binds to other haem-containing proteins, such as myoglobin, cytochrome and guanylate cyclase. These haem-containing proteins (other than COHb) account for approximately 10-15% of the total CO in the human body (Ilano and Raffin, 1990). When CO binds to myoglobin, this reduces the amount of oxygen stored in muscle tissue which can cause adverse effects, possibly resulting in arrhythmias and cardiac dysfunction in the myocardium. Furthermore, CO also impairs oxidative metabolism in cells if it binds with cytochrome, leading to tissue damage. A further effect is that elevating CO concentration increases nitric oxide (NO) activity, which boosts the formation of free radicals that can also cause oxidative damage (Dolan, 1985; Ilano and Raffin, 1990; Kao and Nanagas, 2004). Overall, the pathogenesis of CO poisoning can be separated into three phases: Hb binding, direct cellular toxicity (protein-binding) and nitric oxide effects (Kao and Nanagas, 2004; Roderique et al., 2015). Figure 2-1 shows an overview of the pathogenesis of CO poisoning.

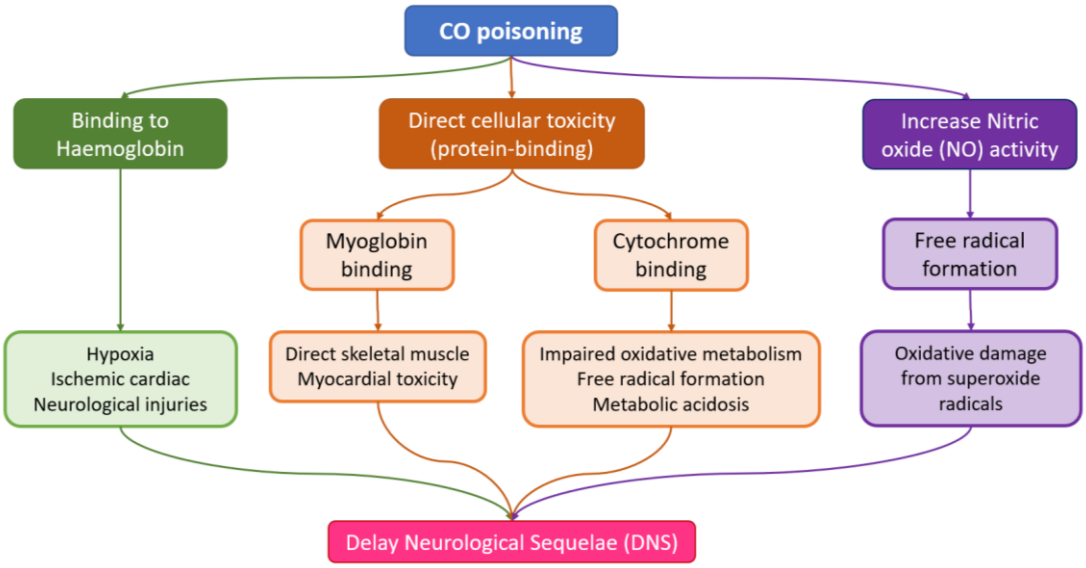

Figure 2-1. Overview of the pathogenesis of CO poisoning (adapted from Kao & Nanagas, 2004)

Smoking and COHb

Tobacco smoking produces tar, nicotine and CO (Rickert et al., 1983). Smokers usually have a higher value of COHb in the blood – around 6-9% – compared to 1-3% in non-smokers (Castleden and Cole, 1975; Friedman et al., 2015). Therefore, in general, CO poisoning is defined as COHb levels over 5% in non-smokers and over 10% in smokers (Ilano and Raffin, 1990).

Signs and symptoms of acute and chronic CO poisoning

CO poisoning itself can be broadly divided into two grades: acute and chronic. Acute CO poisoning means people are exposed to a high concentration of CO, usually more than 200 ppm (over 30% COHb), that causes immediate effects, such as headache, fainting, confusion and loss of consciousness (WHO, 1999; Townsend and Maynard, 2002; Kao and Nanagas, 2004), see Figure 2-2. As organs with very high oxygen demand, the brain and heart are more susceptible to tissue hypoxia resulting from acute CO poisoning (Kinoshita et al., 2020).

Chronic (persistent and long-term) exposure to lower levels of CO may sometimes go unrecognised. In 2012, Clarke et al. found that around 4% of Accident and Emergency (ER) attendees had a raised COHb concentration, but with non-specific CO exposure symptoms (Clarke et al., 2012). The symptoms were milder than those seen in acute CO poisoning, such as headache, nausea, dizziness, fatigue and sleepiness, difficulty concentrating and memory problems, as well as changes in mood (Townsend and Maynard, 2002; Kao and Nanagas, 2004).

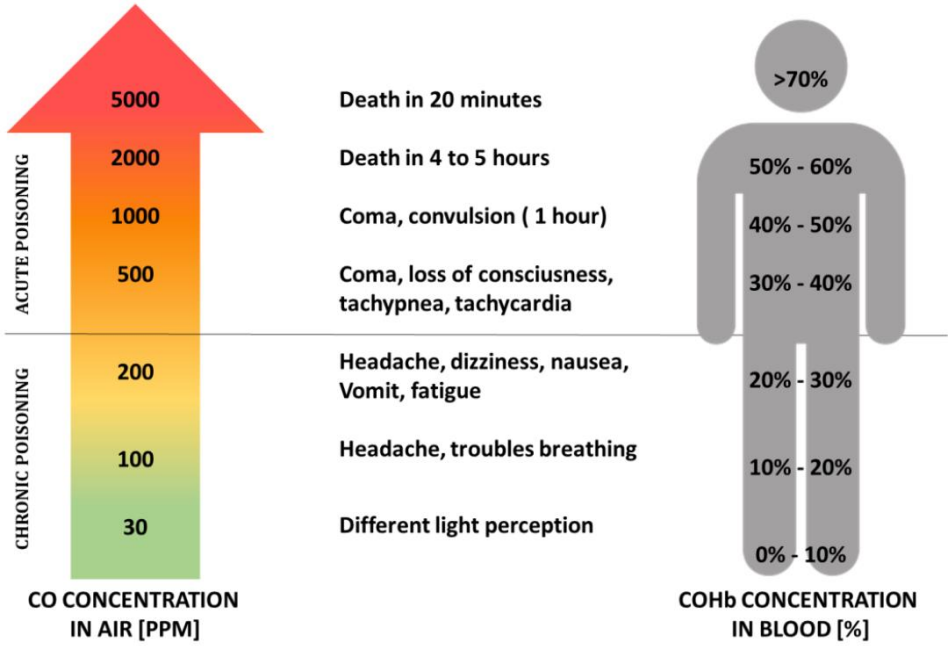

Figure 2-2. Symptomatology associated with reference levels for carbon monoxide (CO) in parts per million (ppm) and carboxyhaemoglobin in % (Adapted from WHO guidelines, 1999)

Many studies have also reported that CO poisoning is associated with neuropsychological functions (Townsend and Maynard, 2002; Croxford et al., 2008; Weaver, 2009). In particular, CO poisoning can result in delayed neurological sequelae (DNS) such as Parkinson-like syndromes, cognitive impairment, hearing loss, memory loss, etc. DNS generally occur within 20 days after an initial complete clinical recovery from CO poisoning and it is estimated that about 25% of CO poisoned patients might have permanent DNS (Weaver et al., 2002; Pepe et al., 2011). The consequences represent an important issue in public health.

2.1.3 Treatment for and Prevention of CO poisoning

This section describes the conventional treatments for CO poisoning. It also provides an overview of methods for preventing CO poisoning.

Treatment for CO poisoning

The primary way to treat CO poisoning is to remove the CO from the body as quickly as possible. When people breathe clean, ambient air, the COHb half-life (the time taken for the concentration to drop by 50%) is around four hours (Ernst and Zibrak, 1998; Kao and Nanagas, 2004). If poisoned individuals are administered 100% oxygen at one atmosphere, this reduces the half-life of COHb to about 90 minutes. With high-pressure oxygen, known as hyperbaric oxygen (HBO) therapy, the half-life

of COHb drops even further, to less than 30 minutes (Pace et al., 1950; Ernst and Zibrak, 1998).

Several methods can be utilised to apply 100% oxygen, including using a rebreathing reserve mask, a high-flow nasal cannula (HFNC), and oxygen therapy with continuous positive airway pressure (CPAP). All of these result in a shorter half-life of COHb in the body than breathing ambient air only (Weaver et al., 2002; Olson and Smollin, 2008; Kim et al., 2019; Bal et al., 2019).

HBO therapy is the use of oxygen under elevated atmospheric pressure. In Weaver et al.'s (2002) study, the results showed that CO poisoned patients who received three hyperbaric oxygen sessions within 24 hours had a lower cognitive sequelae rate after six weeks and 12 months than those who received normobaric oxygen therapy. However, treating CO-poisoned patients with HBO therapy continues to be controversial (Raphael et al., 1989; Weaver et al., 2002; Buckley et al., 2011; Pan et al., 2019). In the Cochrane Database of Systematic Reviews, it was concluded that there is insufficient evidence to support the use of hyperbaric oxygen for the treatment of patients with CO poisoning (Buckley et al., 2011). These differences of opinion have led to different policies and protocols in various centres and countries. Some countries (e.g. Taiwan) still treat CO poisoned patients with HBO, while others (e.g. UK, Germany) do not encourage the treatment (Mutluoglu et al., 2016; Pan et al., 2019).

Prevention of CO poisoning

Methods to prevent CO poisoning can be separated into three categories based on the CO source: ambient (air pollutants), source-based (e.g. heating and cooking appliances) and intentional CO poisoning (e.g. suicide as a result of charcoal burning or venting a car exhaust pipe back into the interior of the vehicle).

For the first category (ambient CO/air pollutants), in the US, Mott et al. (2002) found that CO-related mortality rates declined from 20.2 to 8.8 deaths per million person-years between 1968 and 1998, possibly as a result of the Clean Air Act of 1970 (U.S. EPA, 1970). Then, in 2010, WHO released the indoor air quality (IAQ) guidelines for selected pollutants (WHO, 2010). Now many countries have set their own CO standards, guidelines, and regulations tried to reduce the CO exposure (Health Canada, 2010; Taiwan EPA, 2012). Another more recent factor that probably contributes to lower CO exposure is that many countries have now banned smoking in

enclosed public places and workplaces (Health promotion administration (Taiwan), 2009; HM Government, 2006).

In the second category (source-based), many policies or regulations have been developed. Since many CO poisonings happen indoors, a CO alarm installation is important in enabling people to act on the presence of the gas as soon as possible. In England, the Smoke and CO Alarm (England) Regulations (2015) required private sector landlords to set CO alarms in all properties containing a solid fuel-burning appliance and the regulations has been extended to both social and private rented sector landlords in 2021 (HM Government, 2015; 2021). A CO alarm not only detects high concentrations of CO but in some cases can also indicate if occupants have been exposed to a low level for a longer duration (McCann et al., 2013). In the US, Christensen et al. (2020) have analysed data from the National Poison Data System on unintentional CO exposures in residences in Wisconsin from 2014 to 2016 and found that individuals without CO detectors were prone to more severe medical outcomes than those who had one. Regular checking and maintenance of appliances and CO alarm(s) are required to reduce the chance of CO exposure (Fisher et al., 2014; Fisher et al., 2013; McCann et al., 2013). Moreover, ventilation also plays an important role in CO poisoning: people should pay special attention to ensuring sufficient ventilation when cooking and using heaters (Johnson et al., 2014).

The third category (intentional CO poisoning) is very different from the others since suicide is a very different topic. Liu et al. (2007) suggested that the media should report intentional CO poisoning judiciously and responsibly due to copycat suicides. However, this may not be enough. Another way to prevent such deaths is to consider limiting charcoal sale to people with suicide attempt. Moreover, like other suicide prevention methods, taking care of the people around you and reporting unusual situations are always important in avoiding tragedy.

In summary, Section 2.1 has covered all aspects of CO poisoning from a general description of CO, epidemiology, pathophysiology, and treatment and prevention. It should be noted that some specific aspects (e.g. cellular toxicity of specific enzymes' reaction to CO) are outside the scope of this thesis.

2.2 CO exposure assessment, guidelines and scenarios

A variety of assessment and measurement methods are used to detect CO in the environment and in the human body. Section 2.2.1 introduces CO exposure assessment and measurement methods that are widely used. Section 2.2.2 then presents common CO exposure scenarios for various human activities, and Section 2.2.3 provides information on standards, guidelines and regulations for environmental CO.

2.2.1 CO exposure assessment and measurement

There are several types of CO exposure assessments and measurements. This section gives an overview of CO exposure assessment methods and associated exposure measurement devices, including biological monitoring, personal monitoring, questionnaires/diaries, environmental monitoring and environmental modelling (Oliverio, 2020).

Biological monitoring

In biological monitoring of CO, CO exposure is determined by the percentage of COHb in the human body.

1. **Blood gas analyser (BGA):** an analyser using electrodes to determine pH, partial pressure of carbon dioxide, CO and partial pressure of oxygen in blood samples (Dukić et al., 2016). It is commonly used for diagnosis and treatment in clinics, research laboratories and hospitals. It was also used in our CO-rebreathing study (see Section 3).
2. **Breath CO monitor:** a CO detector with an electrochemical sensor to measure the CO level of the expired air. Exhaled CO concentration can be monitored easily and noninvasively, offering a relatively low-cost and quick way to measure CO levels (Jarvis et al., 1980; Jarvis et al., 1986). This method is now widely used for research and clinical usage. It has acceptable validity as a proxy for total CO concentration in the body (Jo and Oh, 2003; Deveci et al., 2004; Maga et al., 2017) and was also used in our exhaled CO study (see Section 4).

   COHb levels can be calculated for smokers and non-smokers from the empirical relationship formula between CO ppm and COHb (see Section 4.2.4 for the formula) (Carlsten et al., 1954; Cohen et al., 1971; Jarvis et al., 1980; Jarvis et al., 1986; Scherer, 2006). It is valid for normal healthy

smokers (correlation test compared the exhaled CO with COHb: correlation coefficient = 0.98, sample size= 182).

3. **Pulse CO-oximeter:** a spectrophotometer that determines Hb derivatives in the blood, such as COHb and O_2Hb, by measuring absorbance at selected wavelengths (Zaouter and Zavorsky, 2012; Kinoshita et al., 2017).

Personal monitoring

Personal monitoring is carried out using personal CO monitors, portable CO monitors (CO data loggers), CO passive diffusion tubes and real-time gas monitors.

1. **Personal CO monitors:** a monitor that is attached to a person during the measurement period and from which all measured data can be selected for usage (Alm et al., 1994).
2. **Portable CO monitors:** a monitor that a person can carry with them throughout the measurement period, which often has a larger battery than the personal monitors (Dunn et al., 2013).
3. **CO passive diffusion tubes:** a device that can be attached to an individual or, more commonly, to key locations reflecting local exposure. It consists of a tube, open at one end, which contains a sorbent that absorbs the pollutant at a rate controlled by molecular diffusion over the observation period (Nash and Leith, 2010; Commodore et al., 2013).
4. **Real-time gas monitors:** a monitor that measures the CO concentration continuously. The readings are saved or sent to the computer directly (Ashok et al., 2014). Normally uses infra-red spectroscopy as the sensing method.

The primary difference between biological monitoring and personal monitoring is the time period. Biological monitoring usually shows how the body reacts, the biomarkers, to a given concentration in the air, while personal monitoring can reflect concentrations over time. Both methods are widely used.

Questionnaires/diaries

For questionnaires and diaries, participants record the possible CO sources they may be exposed to, such as cooking appliances, heaters, boilers and other CO-generating appliances. Also, the duration of CO exposure and sometimes the symptoms they experience may be recorded (Georgoulis et al., 2002; Croxford et al., 2008).

Environmental monitoring

Environmental monitoring methods include CO alarms, ambient CO monitors, CO data loggers and fixed site (FS)/central site (CS) monitors.

1. **CO alarm:** this will sound an alarm if it detects the presence of CO but must be checked/replaced periodically. It is commonly installed in houses, offices or places that may have an excessive concentration of CO (McCann et al., 2013). Generally, this uses an electrochemical fuel cell as the sensing element.
2. **Ambient CO monitors:** a device for continuously monitoring CO concentrations. Several types of sensors and analyses are used, although., electrochemical fuel cell-based sensors are the most common (Gluschko et al., 2019; Chen et al., 2016).
3. **Fixed site (FS)/central site (CS) monitors:** a monitor that is usually placed based on population distribution (higher density in urban areas than rural areas) and aims to give a representative measurement for an area (Son et al., 2010). For example, the information from environmental monitoring could be found in Defra's UKAir monitoring site in the UK and TAQMD in Taiwan (Defra's UKAir, 2021; TAQMD, 2021).

Environmental modelling

Environmental modelling is used to estimate CO exposure over larger areas; methods include regional modelling and micro-environment modelling. The difference between personal monitor measurements and environmental monitoring/modelling is that personal monitors focus on the individual CO exposure and monitoring/modelling assumes that a specific population group in a particular micro-environment is exposed to the same average CO concentration for a selected time period, such as daily, monthly or annually (Zou et al., 2009).

1. **Regional modelling:** a method that makes a detailed prediction for a target area by focusing its resolution on the target area and the immediate area (Staniforth, 1997).
2. **Microenvironment modelling:** a method that attempts to model a micro-environment, defined as an area where individuals would have the same exposure. However, this approach fails to take into account the potential variability in exposure concentration as a result of different human behaviours

in the same micro-environments (Dimitroulopoulou et al., 2006; Lioy and Weisel, 2014; Shrubsole et al., 2017).

Overall, this shows that every method has its pros and cons. Before deciding which method to use, it is advisable to consider the required output briefly: a combination of different methods may be necessary and useful. As shown by Oliverio's (2020) review, most CO exposure assessment research uses a combination of methods.

2.2.2 CO exposure scenarios

This section introduces several common CO exposure scenarios involving different human activities. When people are outdoors, they are usually exposed to low CO concentrations; exceptions are areas near traffic and parking areas. If people stay indoors, CO sources can be heating or cooking appliances (WHO, 2010). In 2004, Bruinen de Bruin et al. published a report about proportional contributions of micro-environments to 48-hour personal exposures: they found that approximately 82% of the total CO exposure time was indoors, while time spent in traffic accounted for 7.5% (Bruinen de Bruin et al., 2004).

Figure 2-3 separates the common CO exposure scenarios into different locations and exposure characteristics. The locations are the three main places where people are exposed to CO – residences, workplaces, and transport. There are four types of CO exposure characteristics: low CO ppm/short duration, high CO ppm/short duration, low CO ppm/long duration and high CO ppm/long duration. The high CO ppm/long-duration type was not included in the section, as this implies a case of severe CO poisoning and it is not a common CO scenario for general population (except intentional CO poisoning).

For example, some CO exposure events could occur due to appliances with problems, with peak CO concentrations of up to 53-100 ppm being measured (WHO, 2000). Faruk Tekbaş et al. (2001) found that broken hot boiler systems may cause a range of 54-300 ppm CO indoors while Volans et al. (2007) found that a mean of 20 ppm and a peak of 80 ppm can occur if gas appliances have problems, such as damage or incorrect installation.

In the workplace, office building CO concentration (e.g. commercial office buildings, schools) can generally be expected to be low, and not exceed ten ppm (Reynolds et al., 2001; Chaloulakou et al., 2003) although some places may have

higher concentrations, such as buildings near heavily used roads, underground car parks, road tunnels and similar locations (Wallace, 1983; Cattaneo et al., 2010; Hagler et al., 2010). However, in Sydney, researchers found that COHb levels in traffic police officers may be more related to smoking habits than their exposure to motor vehicle emissions (Bisby et al., 1977).

The highest CO exposures may occur when workers operate appliances that release CO. Those working in warehouses with propane-fuelled forklifts were found to have suffered clinical symptoms of CO exposure, such as headaches, dizziness, nausea, chest pain or tightness, etc. CO exposure concentration ranged from 220-88,770 pm when propane-fuelled forklifts are idle and 861-72,840 ppm when at working speed. (Fawcett et al., 1992; Ely et al., 1995). Firefighters are also assumed to be exposed to high concentrations of CO but, fortunately, they are generally well-equipped to deal with such exposures. Their CO exposure was found to average around 1.0 ppm, with a peak of 42.9 ppm for a full shift (Kirkham et al., 2011).

There was a big variation between different types of commuting, places and times (Flachsbart et al., 1987). For example, in Mexico City, the CO concentration was as high as 57.5 ppm for car passengers and 58.6 ppm for minivans (Fernandez-Bremauntz and Ashmore, 1995). However, through the air quality monitoring, it showed the average outdoor CO concentration was about 0.5 ppm in Taiwan (Lin et al., 2021; TAQMD, 2021) and around 0.1-0.5 ppm from Defra's UKAir between 2015 and 2020 (Defra's UKAir, 2021). The lower CO value is presumably because the street is an open area rather than an enclosed one such as the inside of a vehicle.

Furthermore, the WHO has reported that environmental tobacco smoke in homes, workplaces and transport vehicles can raise the eight-hour average CO concentration to 20-40 ppm (WHO, 2000). Vellopoulou and Ashmore's (1998) study also showed a difference in CO concentration in the office and at home, depending on whether cigarette smoking is permitted or not. If cigarette smoking is permitted, the mean CO concentration is around 7.1 mg/m^3 (6.2 ppm) in the office and 6.4 mg/m^3 (5.6 ppm) at home; if cigarette smoking is not permitted, the mean CO concentration is approximately 2.9 mg/m^3 (2.5 ppm) in the office and 2.1 mg/m^3 (1.8 ppm) at home. Fortunately, today an increasing number of regulations ban smoking indoors: for example, the UK banned smoking in all enclosed public places and workplaces in 2007 (HM Government, 2006).

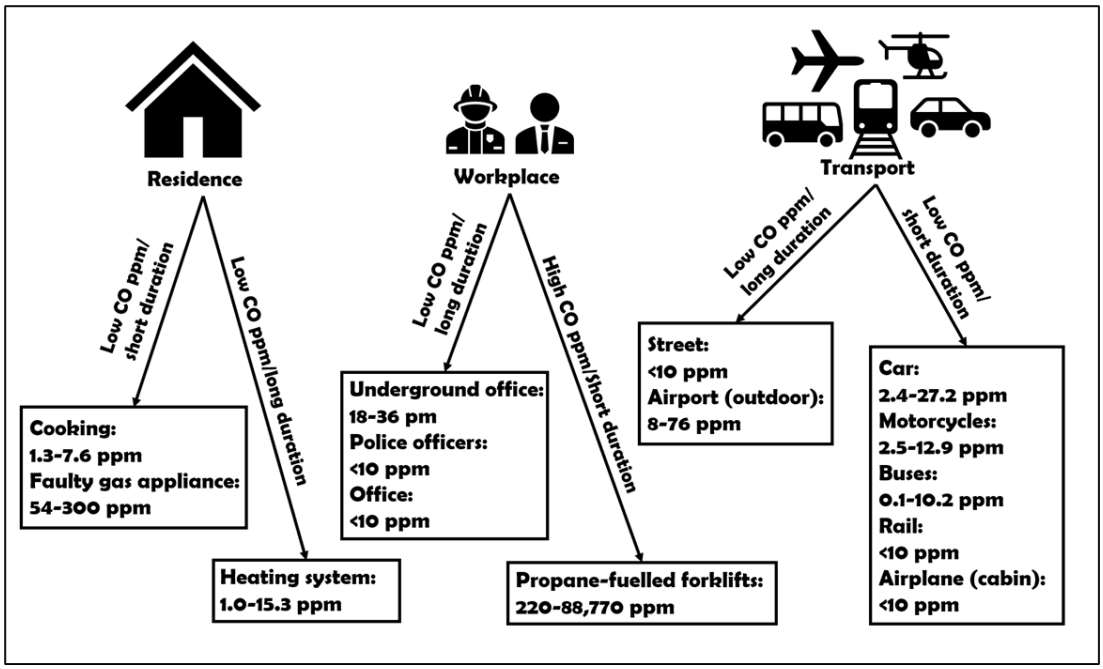

Figure 2-3. Different common scenarios of CO exposure, generated by the author from various sources, including Bellin and Spengler (1980), Wallace (1983), Flachsbart et al. (1987), Poulton (1987), Fawcett et al. (1992), Ott et al. (1994), Fernandez-Bremauntz and Ashmore (1995), Faruk Tekbaş et al. (2001), Reynolds et al. (2001), Chaloulakou et al. (2003), Arnold et al. (2004), Dimitroulopoulou et al. (2006), Volans et al. (2007), Cattaneo et al. (2010), MacDonald et al. (2010), Kirkham et al. (2011), Potchter et al. (2014), Shrubsole et al. (2017).

2.2.3 Standards and guidelines for exposure to ambient CO

Because of CO's adverse health effects on the human body and different potential CO exposure scenarios around individuals, governments and health organisations have set standards and guidelines for ambient CO concentrations.

Setting up standards and guidelines

In 1971, the United States Environmental Protection Agency (U.S. EPA) established the National Ambient Air Quality Standards (NAAQS). The NAAQS for CO aimed to prevent COHb from exceeding 2% in the blood of healthy, non-smoking adults and high-risk groups, such as the elderly, infants, pregnant women and people with coronary disease, lung disease and anaemia (Penney, 2007). The relationship between the concentrations of CO exposure and COHb in the human body could be calculated by Coburn-Forster-Kane (CFK) model (Coburn et al., 1965). WHO also worked on air quality guidelines and the reports published as early as 1957 to the series of editions (WHO, 1958; 1964; 1972; 1987; 1999; 2000). Then, the CFK model was used to predict the limited concentration of CO that could be exposed for different time intervals without exceeding 2% COHb for resting, normal individuals (WHO, 2010).

The WHO guidelines for CO exposure are focused on indoor air (WHO, 2010). The indoor CO guidelines they produced are presented in Table 2-2.

Table 2-2. WHO carbon monoxide guidelines for indoor air

Average time	Concentration (mg/m^3)	ppm
15 minutes	100	87
1 hour	35	32
8 hours	10	9
24 hours	7	6

Adapted from the report by the World Health Organisation, 2000. The covert factor is 1 mg/m^3 equal to 0.873 ppm (WHO, 2010)

Based on the WHO guidelines, every country in the world also announced its regulations and recommendations for CO exposure limits although different countries set different standards. For example, Health Canada published a Residential Indoor Air Quality Guideline in 2010 (Table 2-3) which recommended that people avoid exposure to CO concentrations of more than 25 ppm in any one hour and more than ten ppm in any 24 hours. This guideline is based on model developed by Gosselin et al. (2009), which is a modified model from CFK model built by Coburn et al. (1965).

They have used the model to predict the relationship of CO exposure and COHb in the body for different gender and age groups and found both situations would result in COHb blood levels above 2.0% and incur potential health effects (Health Canada, 2010; Gosselin et al., 2009).

Table 2-3. Residential maximum exposure limits for CO in Canada

Average time	Concentration	
	mg/m³	ppm
1h	28.6	25
24h	11.5	10

In the UK, besides the regulations for indoor CO limits (Table 2-4), the government also has set regulations regarding CO alarms that both social and private rented sector landlords should set CO alarms in rooms with a fixed combustion appliance (excluding gas cookers) mandatorily (HM Government, 2019; 2021).

Table 2-4. Residential maximum exposure limits for CO in the UK

Average time	mg/m³
15 minutes	100
1 hour	30
8 hour	10

In 2012, Taiwan's Environmental Protection Administration (EPA) announced air quality standards (AQS) for CO (see Table 2-5 (Taiwan EPA, 2012)).

Table 2-5. Air quality standard for CO in Taiwan

Average time	ppm
1 hour	35
8 hours	9

Table 2-6. Number of countries with air quality standards (AQS) for CO

Average time	Countries with AQS for CO	Countries without AQS for CO	AQS for CO unknown
15 minutes	8 (4%)	157 (82%)	26 (14%)
1 hour	52 (27%)	116 (60%)	26 (13%)
8 hours	87 (45%)	81 (42%)	26 (13%)
24 hours	12 (6%)	155 (80%)	27 (14%)

In 2017, Kutlar Joss et al. (2017) conducted a study to provide an overview of air quality regulations worldwide. They reviewed ambient AQS for 194 countries worldwide for six air pollutants: $PM_{2.5}$, PM_{10}, ozone, nitrogen dioxide, sulphur dioxide and CO. Table 2-6 shows that fewer than 10% of countries have AQS for CO

exposures of 15 minutes and 24 hours while 13-4% have no known standards for any CO exposures.

Even though the WHO has recommended air quality standards for CO and some countries have implemented even more stringent standards, the majority do not follow or, perhaps, value these standards. Possible reasons are that some countries prefer to rely on their own experts' opinions while in others, it simply may not be practicable/affordable. Air quality plays an important role not only in the environment but also for human health. Governments worldwide should emphasise the effects of air quality and continue to work to improve air quality locally, nationally and globally.

Moreover, the air quality standards and guidelines for CO are based on several studies and human CO exposure models (Coburn et al., 1965; Health Canada, 2010; Gosselin et al., 2009). Some of the factors have been considered when developing the standards and guidelines, such as age and gender. However, there may be other factors that affect the rate of CO uptake and elimination (see Section 2.4) in the human body that should be considered.

2.3 Human CO exposure models

CO can have lethal consequences, even in relatively small, unnoticed doses. Therefore, the relationship between the concentration of environmental CO and that in the human body is important and many equations and models have been developed to estimate this relationship. These modelling approaches can be roughly split into three types: the empirical model, the Coburn-Foster-Kane (CFK) equation and the multi-compartment model.

The three types of CO models are given below.

2.3.1 The empirical model

Over the last 80 years, many different empirical models have related ambient CO exposure to blood CO concentration. However, in this section, only two empirical models are presented to give an overview of the concept.

The first empirical model

Forbes et al.'s (1945) model predicts COHb level based on subjects (seven healthy Caucasian males of age between 20 and 40) being exposed to various CO concentrations and taking exercise at sea level. Their study shows that the CO uptake is, by nature, exponential and the initial rate of formation of COHb increases proportionally to the increased level of ambient CO. The final (equilibrium) value of CO is still related, but less so, to the ambient level of CO (Penney, 1996). Forbes' formula from 1945 is given below.

$$\text{Rate of CO uptake} = \text{K} \times \text{PIco}\left\{\frac{COHb\%\ at\ equilibrium - COHb\%\ at\ time\ t}{COHb\%\ at\ equilibrium - COHb\%\ at\ time\ zero}\right\}$$

a. K is a constant which changes with the activity level of subjects
b. PI_{CO} (mmHg) is the partial pressure of inspired CO

Moreover, the equilibrium value was defined based on enzyme kinetics, the Haldane relationship, which was first recognized by Haldane (1930 cited in Mellors, 1976).

Haldane relationship equation

$$PC_{CO} = \frac{PC_{O2}[COHb]}{M[O_2Hb]}$$

In the equation, M (Haldane's coefficient which quantifies the relative affinity of CO and O_2 for Hb) and PC_{O2} (partial pressure of O_2) were assumed to be 210 and 98 mmHg (Forbes et al., 1945). Other factors are PC_{CO} (partial pressure of CO),

[COHb] (COHb concentration) and [O_2Hb] (O_2Hb concentration). The Haldane relationship equation above is widely used in the empirical model as well as the CFK equation and the multi-compartment model.

The second empirical model

The second empirical model was designed by Peterson and Stewart (1970). They used the data from resting subjects exposed to (a narrow range of) CO from 25 to 512 ppm with a duration of 0.5 to 24 hours.

$$\text{COHb}(\%) = \left\{\frac{\text{CO (ppm)}^{0.858} \times t^{0.63}}{197}\right\} \times 10^{-0.0094t'}$$

a. t' is the time after exposure (COHb ranged from around 2-25%).

Although the empirical models described above can predict a certain COHb concentration level, they are only accurate for the conditions under which their data was gathered. Also, most of the subjects were healthy white males of age between 24 and 42: therefore, these models may miss some important physical and physiological variables (Penney, 1996). Further research should consider a wider variety of CO exposure scenarios and wider physical and physiological variables to provide a more useful model for predicting the concentration of COHb in varied situations.

2.3.2 Coburn-Forster-Kane (CFK) model

Coburn, Forster and Kane created the Coburn-Forster-Kane (CFK) equation in 1965. This model tries to predict COHb, including terms for endogenous and exogenous CO sources in the human body and was tested with data from three healthy males and three anaemic patients (patients with low concentrations of haem). The model has been used to predict the concentration of CO for a wide variety of situations with an acceptable prediction (r=0.917, n=150), including CO uptake and elimination (Peterson and Stewart, 1970; Peterson and Stewart, 1975; Bruce and Bruce, 2003; Gosselin et al., 2009).

This CFK model shows that the blood [COHb] changes with time with a baseline at time t=0 $[COHb]_0$.

$$\frac{\frac{[COHb]\overline{Pco2}}{[O2Hb]M} - Vco\left[\frac{1}{DLco} + \frac{PB - PH2O}{V_{AR}}\right] - PIco}{\frac{[COHb]0\overline{Pco2}}{[O2Hb]M} - Vco\left[\frac{1}{DLco} + \frac{PB - PH2O}{V_{AR}}\right] - PIco} = e^{-\frac{t\overline{Pco2}}{MV_{BL[O2Hb]}(\frac{1}{DLco} + \frac{713}{V_{AR}})}}$$

a. V_{CO} (ml/min) is the rate of production of CO in the human body in millilitres STPD per minute.
b. DL_{CO} (ml/min/mmHg) is the pulmonary diffusing capacity in millilitres per minute per millilitres Hg.
c. $\overline{PC}co$ (mmHg) is the mean CO tension equal to the concentration of COHb in the pulmonary capillaries.
d. PAco (mmHg) is the alveolar CO tension.
e. V_{AR} (ml/min) is the alveolar ventilation rate in millilitres STPD per minute.
f. PIco (mmHg) is the inspired tension of CO in millilitre Hg.
g. P_B (mmHg) is barometric pressure.
h. P_{H2O} (mmHg) is the vapour pressure of water.
i. [COHb] is the concentration of COHb in the blood in millilitres gas STPD per millilitres blood.
j. [O_2Hb] is the concentration of O_2Hb in the blood in millilitres gas STPD per millilitres blood.
k. M is the ratio of the affinity of blood for CO over O_2.
l. 713 (mmHg) = P_B-P_{H2O}=760-47, which is the barometric pressure minus the vapour pressure of water.

In the CFK model, all parameter values were derived from the literature and presented at standard BTPS conditions (Coburn et al., 1965; Peterson and Stewart, 1975; Gosselin et al., 2009) (see List of Abbreviations and Appendix 9.2.1 for details).

Peterson and Stewart (1970) tested the CFK model with observed data of participants exposed to a range of CO concentrations and exposure periods. CO concentrations varied from less than one CO ppm to 1000 CO ppm, and the period from 1.5 to 24 hours. They found that the CFK model was more accurate than other CO-predicting equations in their study. However, there are some limitations to the CFK model. First, the model assumes that CO exchange only happens in the lungs. However, CO exchange may also take place in tissues and cells. In reality, when people breathe in CO, it spreads throughout the entire body and is not confined only to the lungs. CO may be stored in tissue, and different tissue types have different CO diffusion rates. For example, myoglobin in the muscle can store around 10-15% of the CO in the human body (Ronald and Coburn, 1970). Peterson and Stewart's (1970) study found that the half-life of CO elimination might be 30% longer than that calculated with the CFK model (320 versus 252 minutes). They believe that this might be due to the lack of the myoglobin effect in the CFK model.

Moreover, because Coburn et al. (1965) assumed that CO could mix rapidly in the extravascular compartment, the CFK model calculated all possible extravascular compartments as a single compartment (Luomanmäki and Coburn, 1969) and the

washout of CO follows a simple mono-exponential curve. However, CO washout may not be mono-exponential in reality.

Second, this model does not predict either arterial or venous COHb separately. It predicts the average level of COHb. However, in a real-life situation, the difference in COHb level between arterial and venous blood should be considered. The literature reports differences ranging from around 2.3% to 12.1% (Smith et al., 1994, Touger et al., 1995)

Third, Coburn et al. (1965) did not extend the model to smokers. Smokers may have a different reaction to non-smokers as their mean COHb concentration is higher (Meredith and Vale, 1988; Ilano and Raffin, 1990; Weaver, 2009).

Fourth, the model is poor at predicting the COHb level in a sharply increasing CO exposure situation. Bruce and Bruce (2003) found that the CFK model resulted in a more accurate prediction when people were in a stable CO exposure environment.

In summary, the CFK model can make acceptable predictions for many situations and has been widely used for more than 50 years. However, the limitations of the CFK model mean that further CO uptake and elimination models have been developed since its creation.

2.3.3 Multi-compartment models

The multi-compartment model is a type of mathematical model used to describe how materials, energies or natural chemical substances are transferred between the compartments of a system. In these models, it is assumed that the human body can be conceptually divided into multiple compartments. For example, the lungs, blood system and tissues may be considered to be separate compartments in the human body. This section reviews the modified CFK model by Gosselin et al. (2009), and Bruce and Bruce's multi-compartment model (2003, 2006, 2008, 2010).

Gosselin's (2009) modified CFK model

Since the CFK model was developed in 1965, many researchers have tried to improve upon or modify it. In 2009, a modified CFK model was created by Gosselin et al. The original CFK model only considered the human body as a single compartment (Coburn et al., 1965). However, the modified CFK model divided the CO circulation into three: alveoli, blood and the extravascular compartment and considers the interaction between these different compartments using the three

differential equations they developed as described below (Gosselin et al., 2009). Their model is used to simulate CO uptake, distribution and elimination. Figure 2-4 shows the kinetics of CO in the human body.

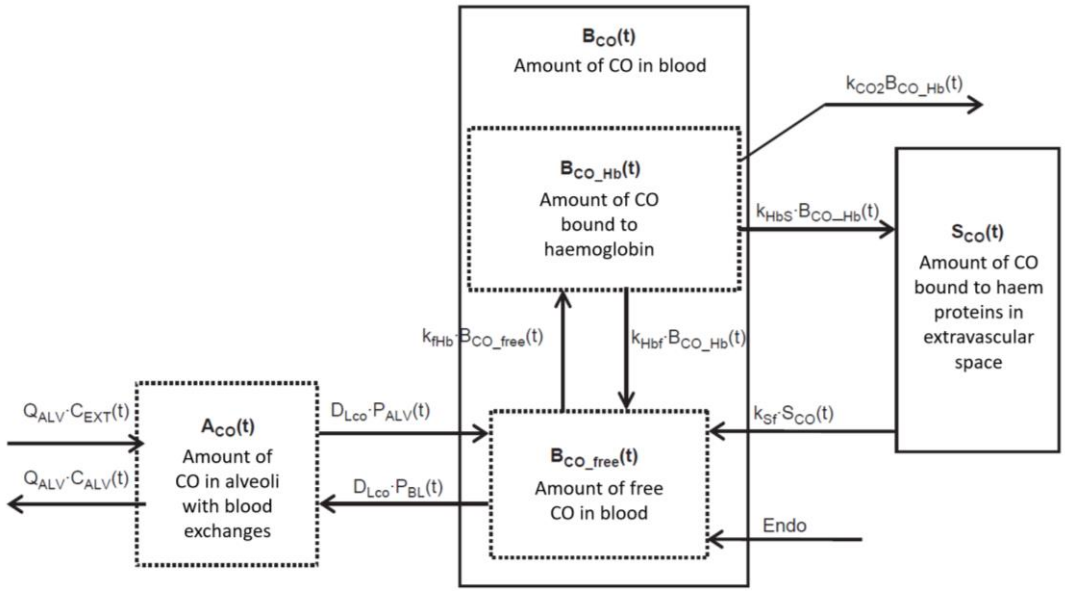

Figure 2-4. Model of the kinetics of CO from Gosselin et al. 2009

The following three differential equations are derived from Figure 2-4, using three state variables: the CO amount in alveoli $A_{CO}(t)$, the CO amount in the blood $B_{CO}(t)$, and the CO amount in extravascular space $S_{CO}(t)$ (see Appendix 9.2.1 for details of equation parameters).

CO uptake and the elimination model of Gosselin et al. (2009)

$$\frac{dA_{CO}(t)}{dt} = Q_{ALV} \times \left[C_{EXT}(t) - \frac{A_{CO}(t)}{V_{ALV}}\right] - DL_{CO} \times \left[\frac{A_{CO}(t)}{V_{ALV}} \times R \times T - \frac{B_{CO}(t) \times P_{O2}}{M \times \left(B_{co_{Hb}}^{Max} - B_{CO}(t)\right)}\right]$$

$$\frac{dB_{CO}(t)}{dt} = DL_{CO} \times \left[\frac{A_{CO}(t)}{V_{ALV}} \times R \times T - \frac{B_{CO}(t) \times P_{O2}}{M \times \left(B_{co_{Hb}}^{Max} - B_{CO}(t)\right)}\right] - \mathrm{k_{HbS}} \times \mathrm{B_{CO_{Hb}}(t)} + \mathrm{k_{Sf}} \times \mathrm{S_{CO}(t)} - \mathrm{k_{CO2}} \times \mathrm{B_{CO\_Hb}(t)} + \mathrm{Endo}$$

$$\frac{dS_{CO}(t)}{dt} = \mathrm{k_{HbS}} \times \mathrm{B_{CO_{Hb}}(t)} - \mathrm{k_{Sf}} \times \mathrm{S_{CO}(t)} + \mathrm{k_{CO2}} \times \mathrm{S_{CO}(t)}$$

a. A_{CO} (ml_{CO}) means the amount of CO in alveoli.
b. B_{CO} (ml_{CO}) means the amount of CO in the blood.
c. S_{CO} (ml_{CO}) means the amount of CO bound to haem proteins in the extravascular spaces.

d. Q_{ALV} (ml_{air} /min) means the alveolar ventilation rate of inhalation.
e. C_{EXT} (ml_{CO}/ml_{air}) is the concentration of CO in ambient air.
f. C_{ALV} (ml_{CO}/ml_{air}) is the concentration of CO in alveoli.
g. DL_{CO} (ml_{CO} /min/mmHg) means the diffusing capacity of lungs for CO.
h. P_{ALV} (mmHg) means the partial pressure of CO in alveoli.
i. P_{BL} (mmHg) means the partial pressure of CO in lung capillaries.
j. Endo (ml_{CO}/min) represents the rate of endogenous production of CO.
k. k_{HbS} (min^{-1}) represents the capture rate of CO from the blood to haem proteins in extravascular spaces.
l. k_{CO2} (min^{-1}) represents the capture rate of CO from blood to haem proteins in extravascular spaces.
m. k_{Sf} (min^{-1}) represents the release rate of CO from haem proteins in extravascular spaces into the blood. A_{CO} (ml_{CO}) means the CO amount in alveoli.

After developing the modified CFK model, Gosselin et al. tested its validity and sensitivity with published data of male adults and found the results to be acceptable. It showed that the goodness-of-fit slope between predicted and experimental data was close to 1 and presented a fairly narrow confidence interval (0.95-1.04). They also compared the predicted data from the original (1965) CFK model and their modified CFK model (2009) with existing experimental data from the literature and the results showed that the data from their model fit the data better than the CFK model (CFK model [r= 0.917, n=150] and modified CFK model [r= 0.996, n=150]) (Gosselin et al., 2009).

However, there are some limitations to the modified CFK model. First, it assumes that Hb is completely saturated with either CO or O_2 and thus it can only be valid under normal atmospheric pressure (Gosselin et al., 2009). Second, it does not consider the difference between COHb in arterial and venous blood. Third, it predicts the CO level in tissue without having been tested with real data. Fourth, the model does not consider the time delay for transport. Fifth, its predictions were only tested with young, male participants.

The Bruce and Bruce's multi-compartment model (2003, 2006, 2008 and 2010)

In 2003, Bruce and Bruce developed a multi-compartment model to estimate COHb and COHb responses to the inhalation of CO in the human body. The model contains five compartments: lungs, mixed venous blood, arterial blood, non-muscle tissue and muscle tissue (Figure 2-5). In the model, Bruce and Bruce emphasised the potential effects of carboxymyoglobin (MbCO) on CO uptake and elimination.

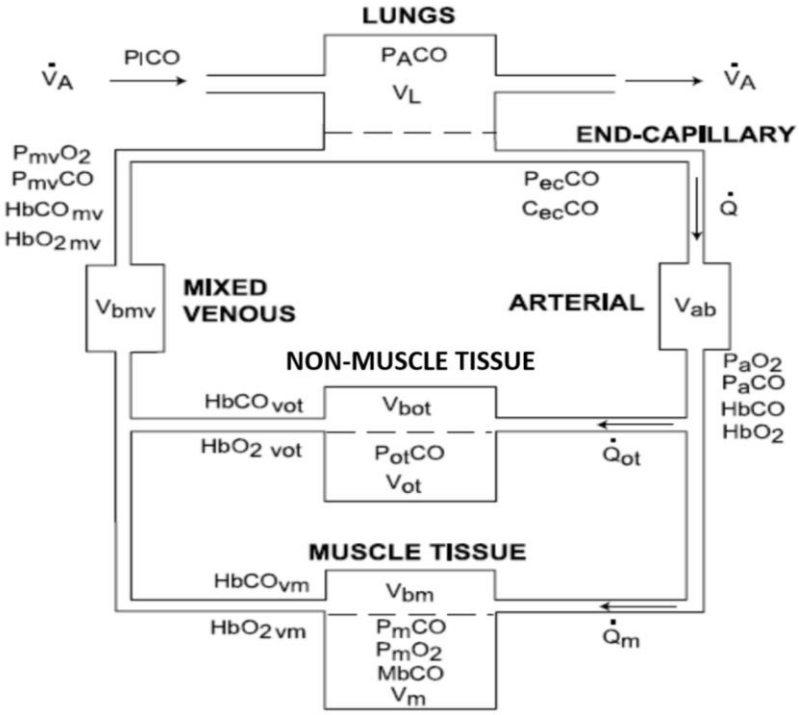

Figure 2-5. Bruce and Bruce's multi-compartment model (Bruce & Bruce, 2003)

There are various assumptions present in the multi-compartment model, including that ventilation, blood flow, pH and partial pressure of alveolar O_2 are constant, and the partial pressure of O_2 in the arterial blood is the same as the partial pressure of O_2 in the end-capillary. In the model, Bruce and Bruce demonstrated that the mass of CO could be balanced in all compartments. There are several equations in each step, and Bruce and Bruce have continued to modify and improve the model, with updates in 2006, 2008 and 2010 (Bruce and Bruce, 2006; Bruce et al., 2008; Erupaka et al., 2010).

The strength of Bruce and Bruce's model is its ability to predict the difference between arterial and venous COHb separately: levels in arterial blood were considerably higher than in venous blood during CO exposure in Smith et al.'s (1994) study.

There are still some limitations to this model. First, it does not consider the metabolism within the tissue or the CO distribution in the tissue. Second, the model assumes that cardiac output, muscle metabolism and blood flow in the muscle remain the same when exposed to CO. Third, the parameter values have to be re-tested for each dataset to provide the best fit on each prediction, and some values (e.g. blood flow for each compartment) cannot be measured directly.

In conclusion, the Bruce and Bruce model tries to give an overview of each compartment related to CO uptake and elimination. The results are validated by exposing subjects to CO for up to 5000 minutes (Bruce and Bruce, 2003), but the demographic characteristics of subjects were limited as they were normal young volunteers. Also, the model is complicated by the addition of more and more assumptions: the most recent model uses 24 main equations and more than 20 additional equations for simulations (Erupaka et al., 2010). Therefore, it is not easy to apply.

2.3.4 Comparison of the three different models

The CFK model has been used for more than 50 years, tested by many researchers and is still considered good at predicting different situations (Coburn et al., 1965) although Gosselin et al. (2009) found their modified CFK model to be slightly better at predictions. Both CFK models have been used to set the standards and guidelines of exposure limits of CO (Barn et al., 2018). The Bruce and Bruce multi-compartment model is good at predicting when the concentration of ambient CO changes rapidly or when the blood has not mixed completely. It is also better at predicting the CO washout time than the CFK (1965) model (Bruce and Bruce, 2003).

Table 2-7 compares the three different models. All three included the parameters of blood volume and subject weight but none considered smoking habits or lung diseases as a parameter. Gosselin et al. predicted the CO uptake and elimination of smokers using the modified CFK (2009) model but they did not consider the potential difference between smokers and non-smokers, such as differing red blood cells production, COHb concentration and lung functions (Frans et al., 1975; Yang, 1993; Graham et al., 2002; Najeeb, 2010).

Bruce and Bruce's (2003, 2006, 2008) model tried to calculate the CO amounts in each compartment. However, they could only predict an average level of CO in the compartment across different tissues beds due to lack of available experimental data on CO levels in tissues. Also, the Bruce and Bruce (2010) model became increasingly complicated with more than 40 equations were included, making it difficult to apply. Thus, the CFK (1965) model and the modified CFK (2009) model are the most widely used for predicting CO uptake and elimination in different situations.

Table 2-7. Comparison of parameters in three different models

	CFK (1965) model	Modified CFK (2009) model	Bruce and Bruce model
Compartment	Single-compartment	Multi-compartment	Multi-compartment
Age	None	Included	Included
Height	Included	Included	Included
Weight	Included	Included	Included
Blood volume	Included	Included	Included
Hb	Included	Included	Included
Tissue	None	All haem proteins	Only myoglobin
Free CO	None	Included	Included
Smoking status	None	None	None
Steady CO exposure	Good prediction	Good prediction	Good prediction
Rapidly varying CO exposure	Worse prediction	Better prediction	Better prediction
Elimination time	Good prediction	Good prediction	Good prediction
Application	Widely used	Widely used	Not widely used

2.4 Factors related to CO uptake and elimination

A version of this literature review section was published in the *International Journal of Environmental Research and Public Health* in January 2020 (Pan et al., 2020) (see Appendix 9.3). It reviews a total of 39 publications in which the related factors covered different dimensions, from environmental exposure and physiological metabolisms to treatments.

2.4.1 Summary of the factors found from the literature

From the review, the factors can be divided into four categories: environmental, demographic and behavioural, physiological and treatment. Table 2-8 and Table 2-9 give an overview of each of these four categories. However, some factors that may be related to both CO uptake and elimination are not included, such as genetics, the presence of disease, vulnerability and special features of age (e.g. young children and the elderly).

After the paper was published, the researcher received valuable comments from correspondents. Zavorsky et al. (2012) found that CO half-life decreased with exercise by increasing isocapnic (a state of constant carbon dioxide in the blood or tissues) ventilation. They also suggested that a combination treatment for CO exposure which includes mild exercise, hyperventilation and normobaric hyperoxia (100% oxygen inhalation) may increase CO elimination for some patients.

2.4.2 Updated information on factors affecting CO uptake and elimination

As well as those factors mentioned in the review, other factors may be related to CO uptake and elimination, such as infancy, smoking and chemistry of haemoglobin. Also, people in different phases of pregnancy and infancy have distinct physiological reactions compared to other healthy adults.

For babies less than three days of age, Stevenson et al. (2019) found that CO elimination rates were faster than those of adults. Although the starting point of CO exposure was slightly different for each baby in the study, the findings indirectly suggest that CO elimination rates for babies are much faster than for adults and that the time to achieve a steady-state is shorter.

For smoking status, even though a past study had not shown a strong relationship between smoking and CO half-life (Burney et al., 1982), Cronenberger et al. (2008) found that smokers' CO half-life had a median of 30.9 hours and ranged from 7.13-

367 in adult smokers, much longer than the 4 hours of CO half-life for non-smokers found by Kao and Nanagas (2004).

The chemistry of haemoglobin may also have an impact on CO uptake and elimination. The dynamic of competing for the binding of Hb for oxygen and CO is complex. When CO binds to the Hb, it occupies the site for binding to oxygen and it increases the affinity of the free haem molecules for oxygen. This means less oxygen could be carried and released to the tissue (WHO, 1999). Also, Longo (1970) found that the affinity of Hb for CO varied between foetus and adults. However, the effects of the chemistry of haemoglobin on CO uptake and elimination is not fully understood.

Other factors, such as height and weight, may also affect the rate of CO uptake and elimination through pulmonary function and blood characteristics (Coburn et al., 1965; Peterson and Stewart, 1975; Roca et al., 1990; Brown et al., 1997; Gosselin et al., 2009). Generally, taller and heavier people may breathe in and out more air which would increase the rate of CO uptake and elimination (Roca et al., 1990; Gosselin et al., 2009; Talaminos Barroso et al., 2018). However, taller and heavier people may have more blood volume which lead to decrease the rate of CO uptake and elimination (Nadler et al., 1962; Brown et al., 1997; Gosselin et al., 2009). Therefore, the effects on the rate of CO uptake and elimination from height and weight is hard to predicted.

'Susceptible groups' are people with chronic obstructive pulmonary disease (COPD), anaemia, heart failure or multiple co-morbidities, and persons of advanced age (Barn et al., 2018). The review from Barn et al. (2018) showed that when exposed to 100 CO ppm for 1 hour, subjects with COPD might have a higher mean of COHb concentration compared to healthy subjects and subjects with cardiovascular disease from different studies. However, the details of the characteristics of each subject were missing. It is still hard to conclude the effects of diseases on CO uptake and elimination.

Moreover, the effects of some factors are still not clear, such as ethnicity, genetics, the chemistry of haemoglobin, the presence of disease, and membership of susceptible groups. If we can understand CO uptake and elimination among these people, it may be possible to adjust the CO standard and provide information to improve CO poisoning treatment. An updated table for factors related to CO uptake and elimination is presented in Table 2-8 and Table 2-9.

Table 2-8. Factors related to CO uptake (adapted from Pan et al., 2020)

Field	Factor	Results	Experiment	Control	Reference
Environment	CO concentration increase	CO uptake rate increases	Range: 0.01%-0.2 % CO		Forbes et al. (1945)
			Range: 0-523 CO ppm		Peterson and Stewart (1970)
			Range: 8.7-1000 CO ppm		Peterson and Stewart (1975)
	Longer duration of exposure	CO uptake amount increases	Range: 0-270 min		Forbes et al. (1945)
			Range: 15-480 min		Peterson and Stewart (1970)
			Range: 0-1440 min (50 CO ppm)		Benignus et al. (1994)
	O_2 concentration increase	CO uptake rate decreases	Oxygen	Air	Forbes et al. (1945)
	Altitude increase	CO uptake rate increases	16,000 ft; 40,000 ft	0 ft	Forbes et al. (1945)
		No difference	2134 m	Sea level	Horvath and Bedi (1989)
Demography and behaviour	Exercise increase	CO uptake rate increases	Hard work	Rest	Forbes et al. (1945)
			Light exercise; moderate exercise	Resting	Filley et al. (1954)
		No difference	moderate exercise	Low exercise	Tikuisis et al. (1992)
Physiology	Ventilation rate increase	CO uptake rate increases	Range: 6-30 L/min		Forbes et al. (1945)
			Range: 5.8-105 L/min		Filley et al. (1954)
	Diffusion capacity of CO (DL_{CO}) increase	CO uptake rate increases	36.3 c.c./min/mmHg	16.9 c.c./min/mmHg	Filley et al. (1954)
			Range: 5-30 ml/min/torr		Bruce and Bruce (2003)
			-	-	Coburn et al. (1965)
			-	-	Gosselin et al. (2009)
	Blood volume increase	CO uptake rate decreases	-	-	Pace et al. (1946)
	The diffusion rate of CO flux from blood to muscle compartment increase	CO uptake rate increases	Range: 0-100 ml/min/torr		Bruce et al. (2008)
	More muscle mass	Less important	-	-	Bruce and Bruce (2006)
	Anaemia	CO uptake rate increases	haematocrits of 18% and 30%	haematocrits of 42% and 60%	Woehlck et al. (2001)
	Pregnancy (Mother and foetus)	CO uptake rate decreases (lag)	Infant	Mother	Hill et al. (1977)

Table 2-9. Factors related to CO elimination (adapted from Pan et al., 2020)

Field	Factor	Results	Experiment	Control	Reference
Environment	CO concentration increase	COHb half-life increases	200.8 CO ppm for 60 min	51.6 CO ppm for 60 min	Peterson and Stewart (1970)
	Longer exposure duration	COHb half-life increases	1,250 CO ppm for 40 min	10,000 CO ppm for 5 min	Bruce and Bruce (2006)
			(same CO dose in two groups)		
	O_2 concentration increase	COHb half-life decreases	100% oxygen	-	Weaver et al. (2000)
			2.5 atm, 100% oxygen (HBO)	-	Pace et al. (1950)
Demography and behaviour	Age	No difference	Range: 9-86 years old		Burney et al. (1982)
			>40 years old	<40 years old	Weaver et al. (2000)
		COHb half-life decreases	4-12 years old	-	Klasner et al. (1998)
		COHb half-life decreases	Infant	Adults	Stevenson et al. (2019)
	Sex	No difference	Female	Male	Burney et al. (1982)
			Female	Male	Weaver et al. (2000)
		COHb half-life decreases	Female	Male	Pace et al. (1950)
			Female	Male	Zavorsky et al. (2014)
	Smoking	No difference	Smokers	Non-smokers	Burney et al. (1982)
		COHb half-life increases	Smokers	Non-smokers	Cronenberger et al. (2008)
	Exercise increase	COHb half-life decreases	Various exercise intensities	Rest	Zavorsky et al. (2012)
Physiology	Ventilation rate increase	COHb half-life decreases	Range: 4-10 L/min		Coburn et al. (1965)
			15 and 30 L/min	3 and 6 L/min	Selvakumar et al. (1993)
			Range: 5-20 L/min		Kreck et al. (2001)
			Range: 4-40 L/min		Zavorsky et al. (2014)
	Chronic obstructive pulmonary disease (COPD)	No difference/ COHb half-life slightly longer	COPD patients	Normal subjects	Crowley et al. (1989)
	Blood volume increase	COHb half-life increase	-	-	Coburn et al. (1965)
			Range: 0.3-0.7 (V_{BL}/VAwo)		Bruce and Bruce. (2006)
	Total Hb mass increase	COHb half-life increase	Male	Female	Zavorsky et al. (2014)
	The diffusion rate of CO flux from blood to muscle compartment increase	COHb half-life decreases	Range: 0-2 ml/min/torr		Bruce et al. (2003)
	Muscle mass	Less important	-	-	Bruce and Bruce (2006)

Field	Factor	Results	Experiment	Control	Reference
	Anaemia	COHb half-life decreases	Anaemia	Polycythaemia	Zavorsky et al. (2014)
	Pregnancy (Mother and foetus)	COHb half-life increases	Infant	Mother	Hill et al. (1977)
Treatment	100% oxygen	COHb half-life decreases	100% oxygen	-	Weaver et al. (2000)
	High flow nasal cannula (HFNC)	No difference	100% oxygen with high flow	100% oxygen	Kim et al. (2019)
	Continuous positive airway pressure (CPAP)	COHb half-life decreases	100% oxygen with positive pressure	100% oxygen	Bal et al. (2019) Caglar et al. (2019)
	Hyperbaric oxygen (HBO) therapy	COHb half-life decreases	2.5 atm, 100% oxygen	-	Pace et al. (1950)
			3 ATA, 100% oxygen	1 ATA, 100% oxygen	Peterson and Stewart (1970)
	Isocapnic hyperpnea (IH)	COHb half-life decreases	Hyperventilation (6% CO_2 in O_2)	without isocapnia	Takeuchi et al. (2000) Fisher et al. (2011) Zavorsky et al. (2012) Sein Anand et al. (2017)

2.5 Clinical tests using CO gas

This section provides an overview of two different clinical tests using CO gas; the pulmonary function test (PFT) with a diffusing capacity of CO (DL_{CO} or TL_{CO}) test and the CO-rebreathing method. Both tests were used in our research (see Section 3 and 5) to understand CO uptake and elimination in the human body.

2.5.1 Overview of Pulmonary Function Test (PFT)

The pulmonary function test (PFT) is widely used in clinics and hospitals. The purpose of a PFT is to understand how well the lungs work, as well as diagnosis of symptomatic diseases and screening for early, asymptomatic diseases (Hughes, 2008; Ranu et al., 2011; Strong, 2014a). A standard PFT has three parts, measuring lung function (spirometer test), lung volume (lung volume test) and the diffusion capacity for CO (DL_{CO} test or TL_{CO} test) (Ranu et al., 2011; Strong, 2014a), please see details as follows.

Spirometry test

A spirometer is a medical device that measures the volume of air inspired and expired by the lungs and then provides information that can be used to diagnose a patient's lung function. The most critical parameters in the test are FEV_1 (forced expiratory volume in one second, which is the volume of breath exhaled with effort in that timeframe after full inspiration), FVC (forced vital capacity is the full amount of air that can be exhaled with effort in a complete breath) and FEV_1/FVC (the ratio of FEV1 to FVC).

Lung volume test

For a lung volume test, different methods can be utilised. One involves the use of body plethysmography where Boyle's law is used to calculate the lung volumes. (Wanger et al., 2005; Ranu et al., 2011; Strong, 2014b). Other measurements, such as nitrogen washout or helium dilution, may also be used. In the nitrogen (N_2) washout technique, the person is asked to breathe in pure oxygen (O_2) and then exhale into a monitor to record the amount of nitrogen 'washed out' which allows the lung volume to be calculated (Wanger et al., 2005; Strong, 2014b). In the helium dilution technique, the amount of helium is determined before the test and remains constant

during the test so lung volumes can be calculated after the equilibrium is reached (Wanger et al., 2005; Strong, 2014b).

DL_{CO} Test

In the DL_{CO} test, the diffusing capacity of the lungs for CO or the lung diffusion coefficient for CO (DL_{CO}, mL/min/mmHg) is measured. DL_{CO} is also known as the transfer factor of the lungs for CO (TL_{CO}, mmol/min/kPa). The DL_{CO} measures the ability of gas to cross the alveolar membrane to the blood in the pulmonary capillaries. In the DL_{CO} test, a person is asked to do a single breath technique by inhaling test gas (typically including 0.3% CO, 21% O_2, 0.3% methane, helium or other tracer gas and N_2 to make up the balance) rapidly, holding it for 10 seconds and then exhaling (Cheung and Cheung, 2015). Then, the alveolar volume (V_A) and K_{CO} (DL_{CO}/V_A) were measured. The alveolar volume (V_A) is the total number of contributing alveolar units and K_{CO} (the diffusion capacity of the lung for CO normalised by alveolar volume), is an index of gas exchange efficiency) (Hughes and Pride, 2012). DL_{CO} is calculated from V_A and K_{CO}, and indicates the ability of gas transfer from inspired gas to the red blood cells (Ranu et al., 2011; Strong, 2014c; Graham et al., 2017).

PFT parameters and their indications

There are several parameters in PFTs, including lung volumes and lung capacities. Figure 2-6 shows the phases of the breathing curve, and from this, lung volume and capacity can be calculated. When people inhale air in comfortable and normal conditions, the volume is called tidal volume (VT). Inspiratory reserve volume (IRV) is the maximal amount of additional air that a person can breathe into their lungs after a normal inspiration. The expiratory reserve volume (ERV) is the maximal amount of extra air that a person can breathe out from their lungs after a normal expiration. Residual volume (RV) is the amount of air left in a person's lungs after maximal exhalation – this is the amount of air required to prevent alveolar collapse (Cotes et al., 2006; Hughes, 2008; Ranu et al., 2011; Strong, 2014a; Jimenez, 2019).

Various lung capacities can be calculated, including total lung capacity, vital capacity, functional residual capacity and inspiratory capacity. Total lung capacity (TLC) is the sum of all lung volumes. Vital capacity (VC) is the maximal volume of air that person can breathe in from RV or breathe out from TLC. Functional residual capacity (FRC) is the volume of air that remains in the lungs after a normal expiration.

Inspiratory capacity (IC) is the maximal volume of air that a person can breathe in after normal expiration (Cotes et al., 2006; Hughes, 2008; Ranu et al., 2011; Jimenez, 2019).

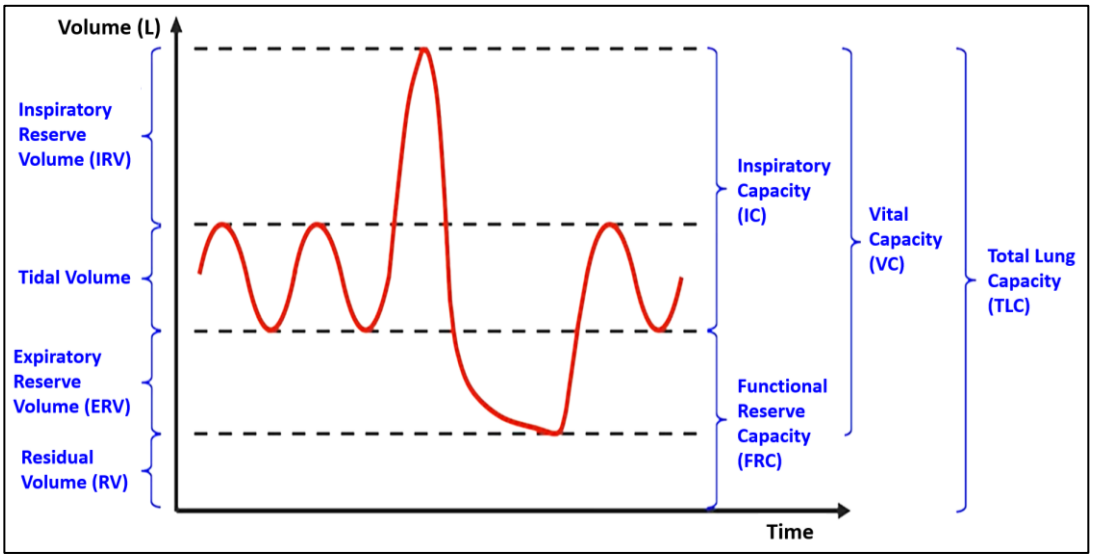

Figure 2-6. Lung volumes and capacities as determined by a PFT (adapted from Eric Strong (Strong, 2014a), https://www.youtube.com/watch?v=6TApeMJ-rkc)

As well as the measurements that come directly from the PFT, some derived values could also indicate lung condition. For example, a higher value of RV/TLC or FRC/TLC indicates a worse airflow obstruction, more gas trapping or a higher possibility of pulmonary hyperinflation (Gagnon et al., 2014; Kendrick, 2015; Shin et al., 2015); while a lower ratio of V_A to TLC may reflect poor gas mixing (ventilation inhomogeneity), airway closure or airway narrowing (van der Lee et al., 2006; Kaminsky et al., 2014).

Figure 2-7 provides the PFT parameters and their indications for lung function (Strong, 2014d; Tseng et al., 2017). In a typical clinical situation, clinic staff start by calculating the FEV_1/FVC from the PFT. After checking this, they then go on to check the FVC and TLC values. At this point, they can approximately diagnose differentiating between obstructive lung diseases (difficulty exhaling all of the air from the lungs), restrictive lung diseases (difficulty expanding one's lungs when inhaling), a mix of the two, or normal lung function. The DL_{CO} value may then be checked to enable a more detailed diagnosis of different lung diseases, such as emphysema (a lung condition that causes shortness of breath), chronic bronchitis (a type of COPD that makes it hard to breathe), asthma, interstitial lung disease (ILD, which is a disorder that causes scarring or fibrosis of the lungs), chest wall and pleural

disorders, neuromuscular disease, pulmonary vascular disease, pulmonary hypertension and normal lung function (Hughes, 2008; Ranu et al., 2011; Strong, 2014d; Tseng et al., 2017; Miller and Enright, 2012).

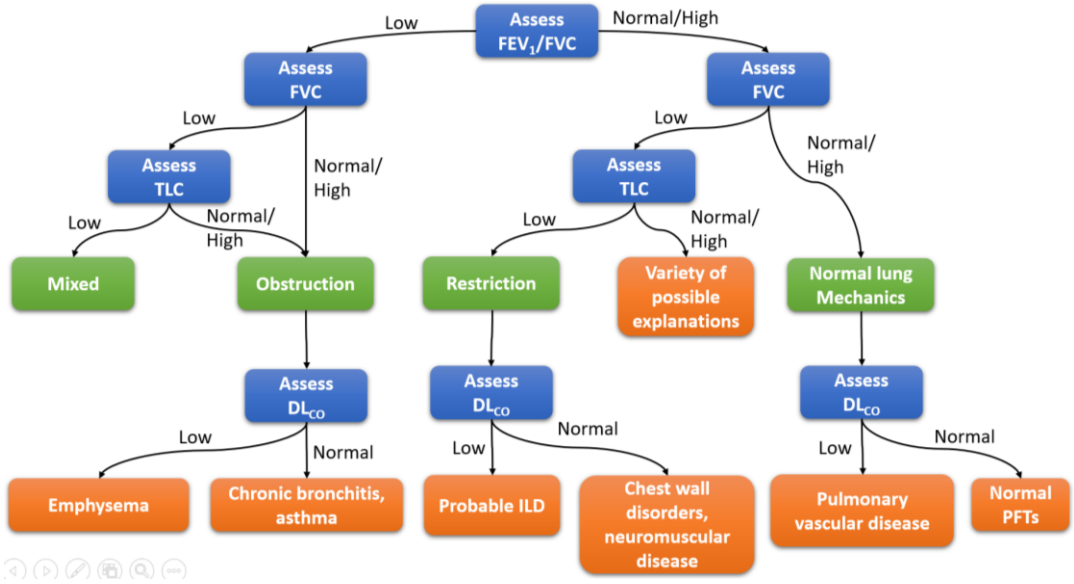

Figure 2-7. PFT parameters and their indications for lung function (adapted from Strong, 2014e & Tseng et al., 2017)

Figure 2-7 shows that FEV_1/FVC, FVC, TLC and DL_{CO} are the most important parameters when using PFTs for diagnosis. PFT interpretation follows a simple rule: if the measured value is 80% to 120% of the reference value, then it is considered a normal value (Wyka et al., 2011). Johnson and Theurer (2014) suggested the normal range of FEV_1/FVC should be more than 70%. The FEV_1/FVC indicates airflow limitation, and a result of lower than 70% is considered a sign of COPD (Mannino and Buist, 2007). Overall, there are slightly different values of normal pulmonary function from study to study (Wang et al., 1997; Barreiro and Perillo, 2004; Stanojevic et al., 2010; Wyka et al., 2011; Johnson and Theurer, 2014).

2.5.2 Factors affecting pulmonary function

Talaminos Barroso et al (2018) tried to understand which factors may affect lung function: their study divided the factors affecting pulmonary function into six fields – age, sex, height, weight, ethnic group and body position.

Age has long been proven to affect lung function: the lungs mature when people are around 20 to 25 years old and the number of alveoli, alveolar ducts and capillary segments become stable (Weibel and Gomez, 1962; Bowdish, 2019). FEV and FEV_1 decline with age because the lungs' compliance (their ability to stretch and

expand) decreases, muscle strength declines, and there is a growing tendency of the smaller airways to close during forced expiration (Lalley, 2013). DL_{CO} and K_{CO} also decline due to declining gas exchange between the alveolar surface and reduced blood volume (Burrows et al., 1961; Talaminos Barroso et al., 2018).

Generally, males have a higher value for FRC, VC, TLC, RV and DL_{CO} than females because males have larger lungs and a larger number of bronchi, a greater surface area of alveolar and a wider airway tube (Townsend et al., 2012; Talaminos Barroso et al., 2018). Height affects many lung function parameters since many parameters (e.g. TLC, VC, RV, FVC, FEV_1 and DL_{CO}) are related to body size and body surface area (Blakemore et al., 1957; Quanjer et al., 2014; Talaminos Barroso et al., 2018). Weight has more complicated effects on lung function; usually, excess fat causes displacement of the diaphragm toward the chest cavity and results in an increase in IC and a decrease in RV (Babb et al., 2008).

Many studies have analysed the effects of ethnicity in PFT data. The American Thoracic Society (ATS) and the European Respiratory Society (ERS) suggest using the PFT reference differently according to ethnicity (Pellegrino et al., 2005; Whitrow and Harding, 2008; Stanojevic et al., 2010; Kiefer et al., 2011). However, Kiefer et al. (2011) reported that ethinicity only explain less than 1% of the lung function variability in the US population. The ethnic differences in lung function may be partially explained by trunk length/standing height, but the differences in chest sizes and respiratory muscle strength should also be considered (Pellegrino et al., 2005). Whitrow and Harding (2008) found that the length of the upper body segment could explain more of the ethnic differences in adolescent lung function than standing height (Whitrow and Harding, 2008).

2.5.3 Factors affecting DL_{CO} (TL_{CO}) and K_{CO}

Of the factors that affect lung function, age, sex, and height may also influence DL_{CO} (Peces-Barba et al., 2004; Quanjer et al., 2014; Talaminos Barroso et al., 2018). However, it is less clear how weight affects DL_{CO}: Blakemore et al. (Blakemore et al., 1957) found the value of DL_{CO} increased with increased weight while, in contrast, Enache et al. (Enache et al., 2011) found that obese people had lower DL_{CO} due to alveolar volume declining or structural changes caused by increased lipid deposition and Sharp et al., (1964) showed that DL_{CO} remains normal.

Moreover, body surface area may affect the value of DL_{CO} (Burrows et al., 1961; Peterson and Stewart, 1975; Park et al., 1986; Yang, 1992). In Peterson and Stewart's (1975) study, as the body surface area increased, so did the value of DL_{CO}. However, Park et al. (1986) showed that age and height might have a more significant effect on the value of DL_{CO} directly.

DL_{CO} also varies between different groups due to ethnic differences in lung function (Paoletti et al., 1985; Pellegrino et al., 2005; Stanojevic et al., 2010; Chhabra et al., 2016). Therefore, each country or ethnic group might have their own reference data to calculate the value of lung function parameters in PFTs (Graham et al., 2017). Korotzer et al. (2000) found that the values of FVC, FEV_1 and V_A were lower in Asians than Europeans, but the values of DL_{CO} and K_{CO} were similar. Besides ethnicity, Sakornsakolpat et al. (2018) also found that genetic differences may be associated with DL_{CO}.

Hb affects the value of DL_{CO} due to the amount of CO binding to Hb. More Hb may result in more CO diffusing to the blood (2006). People with anaemia have a lower DL_{CO} than people without anaemia (Rankin et al., 1961), while people with polycythaemia vera (too many red blood cells) have a higher DL_{CO} than people without polycythaemia vera (Herbert et al., 1965). Therefore, the adjustment of Hb for DL_{CO} should be considered (Marrades et al., 1997). In females, the menstrual cycle may also influence DL_{CO} value. The peak of DL_{CO} usually occurs at the beginning of menstruation and is followed by a rapid decline by day three of the cycle, with a mean difference between them of 9%. The change is due to differing blood volume in the blood capillaries over a menstrual cycle (Sansores et al., 1995; Farha et al., 2007; Talaminos Barroso et al., 2018).

Smoking not only worsens all spirometric parameters but also decreases DL_{CO} and K_{CO} (Cotes et al., 2006; Najeeb, 2010; Sill, 2016). However, the DL_{CO} value improves very soon after people stop smoking (Sansores et al., 1995; Najeeb, 2010); it also increases with an increase in exercise level (Blakemore et al., 1957).

Additionally, diseases such as asthma and COPD may affect the value of DL_{CO}. Those with asthma may have a normal or increased DL_{CO}, and those with COPD may have a lower DL_{CO} (Saydain et al., 2004; Magnussen et al., 2017).

Besides all the factors that mentioned above, pulmonary function test based on CO diffusion could be affected by CO exposure, leading to misinterpretation as lung disease what in reality was CO poisoning (Chiang and Wang, 1970; Graham et al.,

2017). Therefore, this is why all the standard protocol of PFT would advise the patients to stop smoking or avoid CO exposure on the day of PFT to avoid misleading (Graham et al., 2017).

Normal DL_{CO} values

This section introduces some equations used to predict the value of DL_{CO} and the normal range for healthy general people. Paoletti et al. (1985) conducted a study to develop reference equations for DL_{CO} of white people in north Italy; the general value of DL_{CO} is shown in Table 2-10. They separated the predictive equations into different age ranges to allow for the physiological effects of lung development and maturation (Weibel and Gomez, 1962; Bowdish, 2019).

Table 2-10. DL_{CO} (ml/min/mmHg) for different ages and sexes (Paoletti et al. 1985)

Age	Female	Male
10	18.9	17.9
20	28.8	41.7
40	27.4	37.8
60	26.1	33.9

Several other studies have also been conducted to develop reference equations for PFT in different populations. However, Stanojevic (2018) stated that even though there is a general idea of PFT reference data, differences between individuals should still be considered when interpreting PFT data and corroborating with laboratories, clinics and other sites.

2.5.4 CO-rebreathing model

CO is used in CO-rebreathing experiments to measure the total Hb mass in the human body. The CO-rebreathing method is frequently used to determine the effects of training and altitude exposure on the body's oxygen uptake ability (Heinicke et al., 2001; Siebenmann et al., 2017) and provides precision in terms of total Hb mass (Durussel et al., 2013). This method is also used to measure blood volumes and total Hb mass in persons with certain diseases, such as heart failure patients and those with chronic liver disease (Ahlgrim et al., 2018; Plumb et al., 2020).

Heinicke et al. (2001) asked subjects to rest while seated for 15 minutes and then connected them to a Krogh spirometer (Student Spirometer, ZAK, Germany) and asked them to breathe normally for 15 minutes, still in a seated position. The Krogh spirometer bag was filled with oxygen and a known mass of CO. Blood

samples were also taken to measure the blood COHb levels (ABL 520, Radiometer, Denmark). The experiment stopped when the COHb concentration reached a plateau. The researcher then calculated the CO remaining in the Krogh spirometer and used it and the blood samples to determine the total Hb mass in the human body.

In 2005, an optimised CO-rebreathing method (oCOR-method) was developed by Schmidt and Prommer to determine the total Hb mass. The oCOR-method has subjects inhale a bolus (or known mass) of CO for two minutes, to reach the same concentration of COHb at around five minutes compared to the previous method that took 15 minutes. After testing using the oCOR-method, they found that it could be used to calculate the total Hb mass without reducing validity and reliability (Schmidt and Prommer, 2005) (see Appendix 9.2.1 for details of the equations for total Hb mass).

Generally, total Hb mass is affected by sex, height and weight. Males have a higher value of total Hb mass than females. As height and weight increases, so does the value for total Hb mass (Zavorsky et al., 2014).

In summary, Section 2.5 has introduced the PFT and CO-rebreathing methods, both of which use breathing in CO to measure their targets' values. In PFT, CO is used to calculate DL_{CO}, V_A and K_{CO}; and in the CO-rebreathing method, it is used to calculate total Hb mass in the blood. Therefore, both approaches are utilised in this dissertation to understand the CO uptake and elimination in the human body.

2.6 Summary of the literature review and research gap

The literature review revealed that CO poisoning is still a big issue in public health. It causes not only acute adverse health effects but also long-lasting consequences, such as DNS. The health effects of CO are related to the CO uptake and elimination. Several CO exposure models have been created to measure the rate of CO uptake and elimination and set suitable guidelines for indoor CO to avoid CO poisoning. However, these models are often based on a limited number of people that are generally healthy, white and male.

Through the literature review, we have learnt that demographic, physiological and behavioural factors, such as age, sex, height, weight, smoking status and ethnicity, have been shown to have effects on CO uptake and elimination (Burney et al., 1982; Weaver et al., 2000; Cronenberger et al., 2008; Zavorsky et al., 2014; Pan et al., 2020). However, the effects of these factors are not clearly understood – there is still a knowledge gap about the underlying physiological mechanism (pulmonary function) connecting demographic, physiological and behavioural factors and the rate of CO uptake and elimination. If the study could provide a better understanding of the relationship between demographic, physiological and behavioural factors, pulmonary function and the rate of CO uptake and elimination, and then to empirically validate the existing CO exposure models, it would be possible to expand these CO exposure models to people with wider range of different characteristics and improve the assessment and treatment of CO poisoned patients.

3. COLLECTING AND ANALYSING THE COHB ELIMINATION DATA FROM CO-REBREATHING EXPERIMENT

3.1 Introduction

This part of the research arose as a result of discussions and collaboration with Jamie Plumb, a medical doctor and researcher at Southampton General Hospital. As mentioned in the literature review in Section 2.5.4, the CO-rebreathing method is used to measure the total Hb mass in the human body with an amount of CO inhalation (Schmidt and Prommer, 2005; Durussel et al., 2013). Therefore, after the participants attended the CO-rebreathing experiment, the researcher could obtain the total Hb mass data and also the decay COHb data of CO elimination. This experiment had several objectives:

- To explore if the total Hb mass could affect the COHb elimination rate
- To collect the COHb decay data to be compared with the predicted data using the CO exposure models produced during the research.

3.2 Methods

3.2.1 Ethical approval

The study was conducted as part of an ongoing project by Southampton General Hospital. Ethical approval for the project was granted by the hospital's Research Ethics Committee (London Surrey NHS Research Ethics Committee). The Southampton Respiratory Biomedical Research Unit is funded by the National Institute for Health Research (NIHR V1.2 07/01/2018). Participants signed a consent form (see Appendix 9.1.1) in the hospital, and all of the procedures were performed by the clinic staff at Southampton General Hospital.

3.2.2 Protocol and data collection

Plumb was conducting tests to estimate total Hb mass, and this process also allowed the researcher to gather detailed data on COHb decay in the human body. By participating in the project, the researcher was able to gain a valuable understanding of the methods used. The data gathered through this method required

frequent blood samples being taken and, as this is an invasive method, it had to be undertaken by appropriately trained medical professionals.

The power calculation was based on Malczewska-Lenczowska et al.' (2013) study. The researcher defined the sample size required in the CO-rebreathing experiment as obtained from the procedure to evaluate a comparison of two means, with the power required in the study as 80%, while the significance value was 0.05. The sample size was calculated using STATA software, whereby the researcher estimated that 4 participants were needed for each group (male and female). On 6th November 2017, the three subjects were informed about the study and then signed informed consent forms. The method followed is shown in Table 3-1.

Table 3-1. CO-rebreathing method protocol (Schmidt and Prommer, 2005; Otto et al., 2017)

	Protocol
1	Take baseline data: – Measure height, weight; – Ask about fitness level (athlete/medium/no particular exercise); – Insert cannula to take blood samples; – Determine background breath CO levels using Drager Pac 6500 Reusable Single Gas Detector (Draeger Safety UK Ltd, UK); see Figure 3-1 and Figure 3-2. Take three readings and determine average results. For each reading, the subject breathes in fully then exhales; – Take initial baseline blood sample and label. Agitate sample to prevent clotting.
2	Administer known volume (for standard test use 1ml of CO for each kg of body weight in pure oxygen) of pure CO to raise the COHb value around 4-6%.
3	Apply nose clip to avoid breathing through the nose. In ambient air, subjects are required to inhale fully, then exhale fully. After full exhale, the participant is connected to a well-sealed Krogh spirometer (Spico-CO Respirations-Applikator, Blood Tec, Germany) and a 3 liter anesthetic bag pre-filled with 100% oxygen; while inhaling, the CO dose is injected into the spirometer, and the subject completes a full inhale to take in the CO and oxygen and breathes in for as long as possible, see steps 4 to 6 (Figure 3-1 and Figure 3-2).
4	t=0, the starting point, is the moment of first breathing in CO.
5	The participant then holds his or her breath for about 10 seconds.
6	The participant starts normal respiration (tidal volume), still using the spirometer, which means rebreathing the oxygen and CO mixture for about 1 min 50s (Steps 4 to 6 are around 2 minutes in total).
7	At the end of this period, the subject fully inhales and then fully exhales to fill the spirometer bag, and then the valve is closed. The participant is disconnected from the spirometer.
8	At this point, breath CO readings are re-taken (the test is conducted three times and recorded).

	Protocol
	The CO concentration in the filled sample bag is measured, using the CO breath detector, which allows an estimation of the amount of CO taken up by each subject.
9	Blood samples are taken via the cannula, at 6, 8, 10, 12 and 30 mins and around 1, 2, 3 and 4 hours.
10	Using a blood gas analyser (Radiometer ABL 800 Flex PH Blood Gas Analyzer) (see Figure 3-2), the COHb levels in each sample are measured three times. The results are read and recorded.

Figure 3-1. Experiment photos of the CO-rebreathing method (all photos are copyright Ke-Ting Pan and subjects agreed to their use)

Figure 3-2. Equipment for CO-rebreathing method: (a) Krogh spirometer and bag (b) 7-liter calibration syringe, Series 4900 (c) Blood gas machine, radiometer ABL 800 flex PH blood gas analyser (d) Drager handheld monitor.

3.3 Results

The total Hb mass was calculated from the CO-rebreathing experiment, and the COHb elimination rate post-experiment was tracked over time and the data for three subjects recorded. The researcher, one of the supervisors and a research colleague were the participants; their profiles and the results are provided in the section.

3.3.1 CO-rebreathing experiment data

Table 3-2 shows that the three subjects had different demographic, physiological and behavioural factors and total Hb masses. Subject A (female) had the lowest blood volume and total Hb mass, while Subject C (male) had the highest blood volume and total Hb mass. In general, the reference range for Hb is 12.0-16.0 g/dL for women and 13.5-17.5 g/dL for men (Fatemi and Clayton, 2008; Shinkawa et al., 2009; Kawai et al., 2017); the reference values for venous haematocrit are 41-53% for males and 36-46% for females (Fatemi and Clayton, 2008). Of the three subjects, only Subject A's Hb concentration was lower than the reference value.

The fitness levels were athlete, medium and no particular exercise. If the participant's fitness level was athlete, the CO dose weight was multiplied by 1.1 ml/kg; for medium, by 1.0 ml/kg; and for no particular exercise, by 0.9 ml/kg. The exhaled CO was 0.8 ml for Subject A, 0.6 ml for Subject B and 2.6 ml for subject C.

Table 3-2. *Subject profiles, experiment data and calculated total Hb mass in CO re-breathing experiment*

	Subject A	**Subject B**	**Subject C**	**Reference value**
Age	30	42	50	
Ethnicity	Asian	Asian	Caucasian	
Sex	Female	Female	Male	
Height (cm)	169.9	162.2	177.2	
Weight (kg)	55.3	51.0	78.0	
Smoking status	Non-smoker	Non-smoker	Non-smoker	
BMI	19.2	19.4	24.8	
Fitness Level (Athlete/Medium/No particular exercise) (self-reported by participants)	No particular exercise	Medium	Medium	
Blood volume (ml)	3624	3745	5667	
Hb concentration, [Hb] (g/dL)	11.0	14.6	14.8	M: 13.5-17.6 F: 11.3-15.2
Total Hb mass (g)	362.7	497.6	763.2	

	Subject A	Subject B	Subject C	Reference value
Total Hb mass (g/kg)	6.6	9.8	9.8	
Venous haematocrit (%)	36.2	42.6	42.7	M: 40-54 F: 36-46
CO dose (ml)	50	50	78	
Dose/kg (ml/kg)	0.9	1.0	1.0	
CO remaining in Krogh spirometer system (ml)	3.3	3.3	1.3	
CO exhaled (ml)	0.8	0.6	2.6	
CO-Myoglobin (ml)	0.7	0.7	2.65	
CO in bloodstream (ml)	44.22	44.4	70.41	

Note: M: male; F: female

3.3.2 COHb elimination rate data from the CO-rebreathing experiment

As well as calculating the total Hb mass, the experiment also followed and recorded the COHb elimination in the blood several hours after exposure. Each participant had nine blood samples drawn at points from baseline to 240 minutes after breathing in CO.

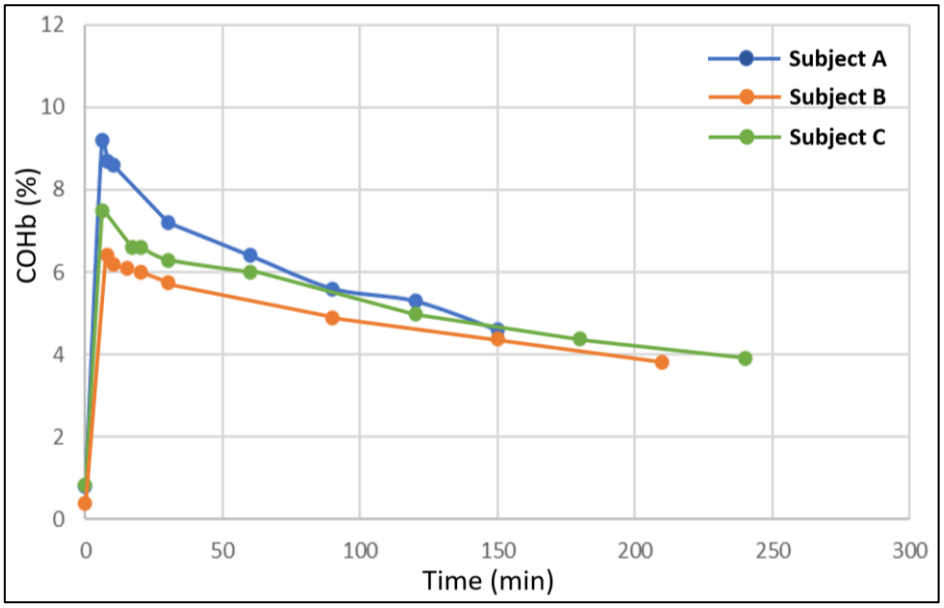

Figure 3-3. COHb (%) readings from the CO-rebreathing results of the three subjects

Figure 3-3 shows that Subjects A (0.8 %) and C (0.8 %) have a similar baseline COHb and Subject B (0.4 %) has a lower baseline COHb. The COHb half-life was between 120 to 150 minutes for Subject A, more than 210 minutes for Subject B and between 180 to 240 minutes for Subject C. Subject A, with the lowest Hb

concentration (11.0 g/dL), blood volume (3624 ml) and total Hb mass (362.7 g), had a shorter COHb half-life than Subjects B and C. All had data measured at the 150 minutes; however, only Subject C's COHb was measured at 240 minutes (the other two participants had to leave because of personal reasons). All of the data were used to test the simulation of the CFK and modified CFK models in Section 6.

3.4 Discussion and Conclusion

3.4.1 Discussion

In the CO-rebreathing experiment, Subject A, with the lowest Hb concentration, blood volume and total Hb mass, had a shorter CO half-life (between 2 to 2.5 hours) compared to Subjects B (more than 3.5 hours) and C (between 3-4 hours) and also shorter than average CO half-life from the literature (Kao and Nanagas, 2004). Zavorsky et al. (2014) also found that more total Hb mass could prolong the CO half-life due to more CO stores in the body. Moreover, anaemia (decreased Hb concentration compared to normal) may increase the rate of CO uptake and elimination (Woehlck et al., 2001; Zavorsky et al., 2014). Blood volume also plays a role: as it increases, CO uptake and elimination rates decrease (Coburn et al., 1965; Bruce and Bruce, 2006). All of the above factors influence the amount of CO stored in the body.

3.4.2 Limitations

The researcher planned to continue the project with further participants as the aim was for the recorded data to be used not only to test the simulation models but also to investigate the role of total Hb mass in CO uptake and elimination. However, despite several attempts to continue, the project was shut down in May 2018 due to a lack of available facilities and clinical staff. Thus, there were only three sets of results to use in comparing the various models produced during the research.

Therefore, it was difficult to determine the relationship between Hb concentration, blood volume, and total Hb mass, and CO elimination. However, the results did show that Subject A, with the lowest Hb concentration, blood volume and total Hb mass, had a shorter CO half-life after breathing CO, which is similar to previous studies (Coburn et al., 1965; Woehlck et al., 2001; Bruce and Bruce, 2006; Zavorsky et al., 2014).

3.4.3 Conclusion

In the study, three sets of results were gathered for use in testing various aspects of the models developed during this research. The CO-rebreathing experiment reported the COHb concentration in the bloodstream at each time step, the calculated blood volume and the calculated total Hb mass. Even though we could see the effects of having the lowest Hb concentration, blood volume and total Hb mass on Subject A, it is difficult to draw any hard and fast conclusions regarding the effects due to the small sample size. Future studies should recruit more subjects and examine each factor separately, thus allowing the effects of each to become clear.

4 INVESTIGATING THE EFFECT OF SMOKING ON CO UPTAKE AND ELIMINATION TIME IN SMOKERS

4.1 Introduction

The variation in smoking-related factors between non-smokers and smokers may affect CO uptake and CO elimination in the human body (Kao and Nanagas, 2004; Cronenberger et al., 2008). Therefore, the researcher recruited smokers and non-smokers among the university student population to understand CO uptake and elimination in both by measuring exhaled CO at various time intervals. A version of this exhaled CO experiment (Section 4) was published in the *International Journal of Environmental Research and Public Health* in November 2021 (Pan et al., 2021) (see Appendix 9.3).

Between January and June of 2019, the researcher collected data from smokers and non-smokers using questionnaires and CO monitoring of exhaled breath. This data included age, sex, height, weight, ethnicity, smoking status and exhaled CO values. This experiment had several objectives:

- To measure the difference in the baseline of exhaled CO values between smokers and non-smokers
- To record the change in exhaled CO values over time in smokers before and after smoking
- To explore the difference in CO uptake and elimination between light smokers and heavy smokers.

4.2 Methods

4.2.1 Ethical and data protection approval for exhaled CO data collection

The study followed the ethical application procedure at UCL and was approved by the UCL Research Ethics Committee and registered with the UCL Data Protection Registration Service (Appendix 9.1.4). The researcher began to write the study proposal in August 2018 and applied for data protection registration in September 2018. The application was approved by the UCL Research Ethics Committee on 25th January 2019 (Project ID: 14201/001) (see Table 4-1 for timeline).

Table 4-1. UCL ethical and data registration procedure

Time	Task
May-July 2018	Project proposal developed
August 2018	All documents prepared
September 2018	Application submitted
October 2018	Received comments on the application
October-November 2018	Revised documents
December 2018	Re-submitted the application
25 January 2019	Approval received

4.2.2 Protocols for the exhaled CO study

Before the observation, the researcher checked the CO value present in the study room. Also, the researcher checked the monitor and the screen and ensured correct operation. The researcher then gave each participant a new cardboard mouthpiece to use with the monitor. Figure 4-1 shows the study protocol.

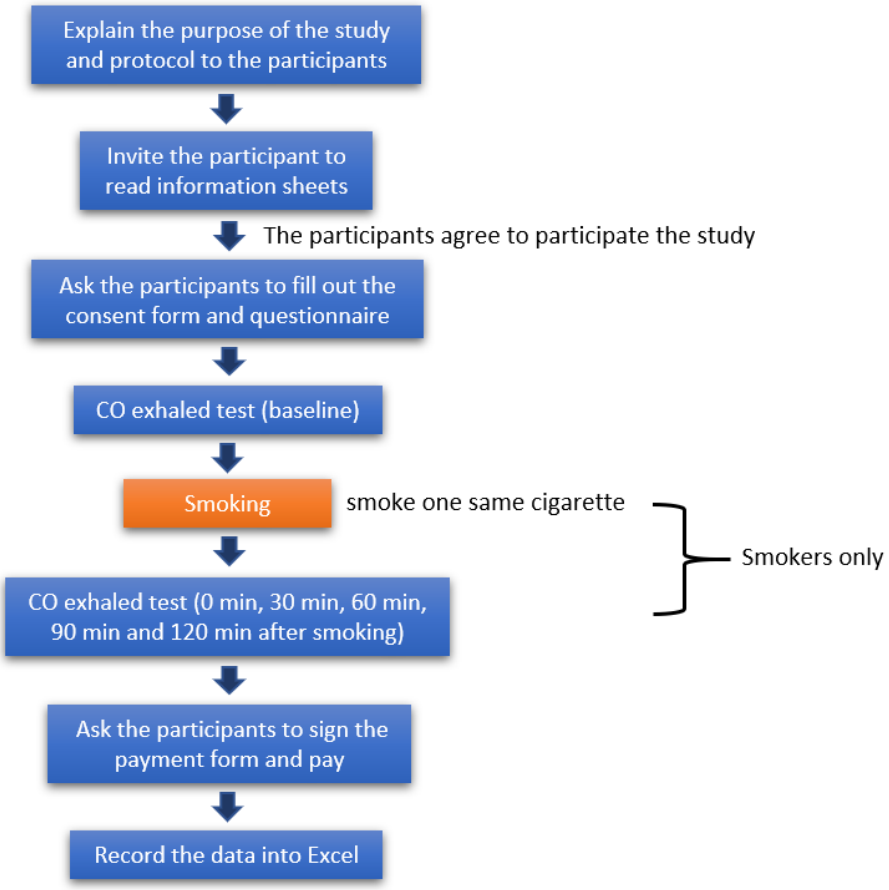

Figure 4-1. Protocols for the exhaled CO study

The researcher explained the purpose of the study and the protocol to the participants who then read the information sheet and consent form, having been informed that if they had any questions, they could ask the researcher directly. After

agreeing to participate in the study, the researcher gave them the questionnaires to complete (see Appendix 9.1.4, Supplementary Figure 9-8).

The above exhaled CO test was based on the literature (West, 1984; Biglan et al., 1986). The results suggested that holding the breath for 20 seconds is optimal; at this stage, the exhaled CO concentration equilibrates with the CO concentration in the blood.

For each exhaled CO test, the researcher guided the participant, seated, to inhale deeply through his or her nose. Then, the participant was asked to hold his or her breath for 20 seconds and then exhale all of the gas through their mouths into the CO monitor. The results of exhaled CO levels were then recorded.

All participants did the exhaled CO tests for the baseline, while the smokers were asked to smoke a cigarette after their baseline test; see Figure 4-1. The researcher then recorded the time they started and finished smoking. After smoking outdoors (due to smoking being banned in enclosed public places in the UK), the participants immediately returned to the study room to repeat the exhaled CO test and then every 30 minutes until 120 mins after smoking. The choosing of 120 mins exhaled CO concentration follow-up after smoking is based on the pilot study, in which the CO decay could be seen and from which the exhaled CO half-life was calculated.

At the end of the experiment, the participants were asked to sign a payment form, and their mouthpieces (single-use products) disposed of. The researcher cleaned the whole monitor with cleaning swabs between uses to further prevent any risk of infection between participants. All of the data from the questionnaires and the tests were recorded in Microsoft Excel for analysis.

In the exhaled CO study, the financial incentive was £30 for smokers and £5 for non-smokers. The big difference in financial incentives between smokers and non-smokers is because of the time spent and measurements were taken. Only smokers would be asked to smoke one control cigarette and record the decay of exhaled CO concentration for up to 2 hours. It took around three hours in total if including the baseline CO measurement. For non-smokers, they were only asked to attend the baseline exhaled CO concentration measurement. Therefore, the total experiment time would be around 30 min.

Figure 4-2. Experiment photos of the exhaled CO study

4.2.3 Recruitment of participants

This section includes the definitions of smokers and non-smokers in the study, the recruitment of participants, and the comparison of the sample size between the literature and the present study.

Definitions of smokers and non-smokers

Non-smokers were defined as subjects without a history of active smoking before participating in the study and without a history of passive smoking in the previous four months (Moscato et al., 2014). Smokers were defined as those who have smoked more than 100 cigarettes throughout their entire life until the present, and have ever smoked within the past 30 days (Sargent and Dalton, 2001; Starr et al., 2005). There is a wide range of definitions of light smokers, from those that smoke less than four cigarettes per day to those that smoke 10–20 cigarettes per day. For this study, the definition of a light smoker is someone who smokes 1-9 cigarettes per day. The definition was based on Biener and Albers (2004) and Husten (2009), whose research was more focused on young adults, so the target group was more similar to our participants. Heavy smokers were defined as smoking ten or more cigarettes per day (Biener and Albers, 2004).

Sample size and recruitment of participants

From the sample size calculated using data from a previous study (Maga et al., 2017), the researcher defined the sample size required in this study as that obtained from the procedure to evaluate a comparison of two means, with the power required in the study as 80%, while the significance value was 0.05. The sample size was calculated using STATA software, whereby the researcher estimated that 13 participants were needed for each group. For this study, we recruited participants

through physical posters (Appendix 9.1.4) placed at Central House, UCL and Goodenough College, London, and through a general e-mail to all BSEER students. These approaches, however, did not result in many respondents. Therefore, the researcher went around campus every week, approached smokers, shared study information and invited participants to participate in the study providing small flyers. If the smokers were interested in the study, the researcher would send them the details of the study after they agreed.

Table 4-2. Recruitment criteria for participants

Inclusion criteria	**Exclusion criteria**
Aged 18 to 34 Not pregnant Healthy with no history of lung function illness	Pregnancy Major medical diagnosis A history of lung function illness Inability to fill in the questionnaire

The recruitment criteria of the study are given in Table 4-2: they are based on Maga et al. (2017) and Windsor-Shellard et al. (2019).

A person's lung function matures around mid-twenties, remains until around middle-age and then decreases later in their lives (Bowdish, 2019). Also, even light smokers aged 35-49 may have a higher risk of fatal and non-fatal myocardial infarction (Bjartveit and Tverdal, 2005). Therefore, the researcher decided to focus on young adults and to recruit participants aged 18-34 – to prevent including ageing lung function and diseases as factors that may affect the results (Windsor-Shellard et al., 2019).

Moreover, to reduce the last cigarette's effects, smokers who participated in the experiment were asked to attend at least four hours after they last smoked (Warner, 2005; Najeeb, 2010), the time period being determined based on the half-life of COHb in people breathing natural air (Kao and Nanagas, 2004). The researcher recorded the time since the last cigarette before the exhaled CO test of each participant and took the variable into account in the analysis.

The study period was 26 January to 30 June 2019, and in total, 84 participants took part, 57 smokers and 27 non-smokers. After exclusions, data on 74 participants remained available for analysis (Figure 4-3).

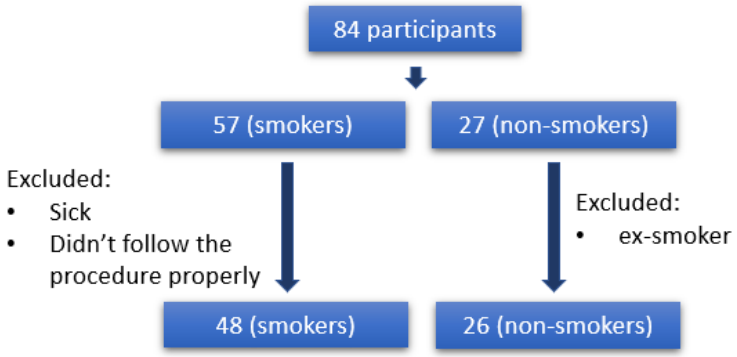

Figure 4-3. Flow chart for recruitment of the exhaled CO study

Sample size comparison

The data from 48 smokers and 26 non-smokers were analysed. The average baseline exhaled CO was compared with the results from Maga et al. (2017) (see Table 4-3). Their exhaled CO values were measured with the use of PiCO+ Smokerlyzer tools (Bedfont Scientific Ltd, England), which is the same series of Smokerlyzer used in the present study (Micro+ Smokerlyzer, Bedfont Scientific Ltd, England). Although fewer participants were recruited for the present study compared to Maga et al. (2017), when comparing the results, the baseline exhaled CO of smokers and non-smokers showed similar values: the 95% CI overlap and the mean are similar.

Table 4-3. Comparison of baseline exhaled CO (ppm) between the present study and Maga et al. (2017)

		Mean	SD	95% Confidence Interval	
				Lower	Upper
Present study	Smokers (n=48)	6.9	4.9	5.51	8.29
	Non-smokers (n=26)	1.9	0.5	1.71	2.09
Maga et al. (2017)	Smokers (n=90)	6.5	4.0	5.67	7.33
	Non-smokers (n=318)	1.1	0.8	1.01	1.19

Generally, the exhaled CO concentration is lower than 5 ppm in non-smokers. If their exhaled CO concentration is equal to, or above 5 ppm, they might have exogenous CO exposure. For smokers, their exhaled CO concentration is usually above 5 ppm and could be more than 10 ppm. Therefore, it is harder to determine if smokers have additional CO exposure besides smoking, compared to non-smokers (PHE, 2014; British Lung Foundation, 2020).

4.2.4 Questionnaire and equipment for the study

The researcher designed the questionnaire by following Boynton and Greenhalgh (2004) who created a hands-on guide to questionnaire research. The questionnaire also included a section to record the exhaled CO value (see Appendix 9.1.4, Supplementary Figure 9-8 for details).

Designing and developing the questionnaire

The researcher included age, sex, ethnicity, height and weight in the questionnaire. These variables comprise the basic demographic, physiological and behavioural information of each participant, and have been reported to possibly relate to the rate of CO uptake and elimination (Gosselin et al., 2009; Zavorsky et al., 2014; Verbanck et al., 2016). For the diet question, the options included vegetarian, vegan, gluten-free, pescatarian and none of above (Clarys et al., 2014).

The smoking status section had three options: non-smokers, smokers and ex-smokers (U.S. CDC, 2011). The "Ex-smoker" option was included so this category could be identified and then excluded from analysis, to enhance the contrast between smokers and non-smokers. Smokers were asked to provide the year they started smoking and the average number of cigarettes smoked per day and per week, so the researcher could understand their smoking habits.

The questionnaire also asked about recent activities to ascertain if participants had recently exercised and/or whether had been exposed to CO before starting the study so that these factors could be considered as variables in the study (see Appendix 9.1.4, Supplementary Figure 9-8).

Female participants were invited to answer questions related to their menstrual cycles as this is associated with CO concentration in the body. Exhaled CO concentration is lower during menstruation than outside of menstruation (Delivoria-Papadopoulos et al., 1974; Antczak et al., 2012).

To record the exhaled CO value, the researcher recorded the CO value in the study room. Then, when the participants went to smoke, they were asked to record when they started smoking, the time they finished smoking and the number of puffs they took in the questionnaire. All of the data gathered from the questionnaire were recorded in Microsoft Excel. Data was stored on the researcher's laptop and password protected; a backup copy was stored on an encrypted USB stick which was

placed in a locked drawer in the office. Moreover, all data will be deleted two years after the publication of this thesis.

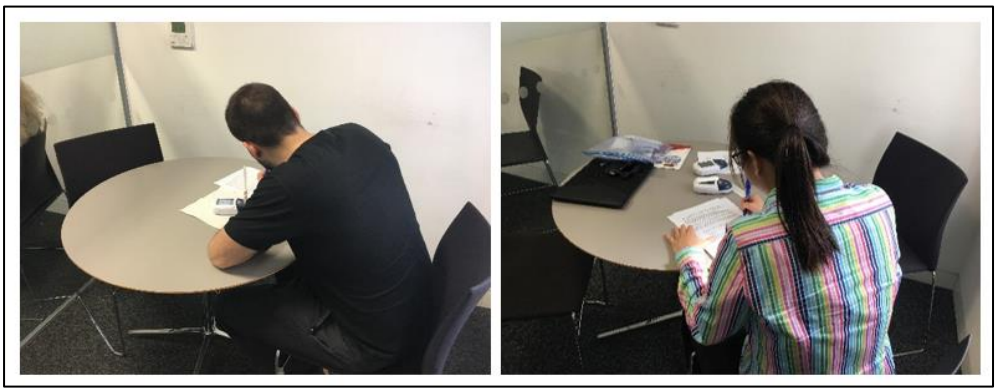

Figure 4-4. Exhaled CO study; participants complete the questionnaire

Equipment for exhaled CO monitoring

The researcher used a breath CO monitor; specifically, the 'Micro+™ Smokerlyzer®' (Bedfont Scientific Ltd, England), as shown in Figure 4-5, which has an accuracy of less or equal to ±2ppm. The monitor measures the amount of CO in a person's breath and has been already used in many studies and clinics (Moscato et al., 2014; Maga et al., 2017). It can detect CO concentrations from 0 to 500 ppm and calculates the COHb as per Jarvis et al.'s study (1986). The equation is described below.

Regression between exhaled CO and COHb:

$$COHb\ (\%)\ =\ 0.63 +\ 0.16 \times [value(ppm)\ of\ Micro\ Smokerlyzer]$$

Several Micro+™ consumables were used in the study, including SteriBreath™ mouthpieces, D-pieces™ and monitor cleaning wipes. Every participant had a self-use mouthpiece to control the risk of infection. The D-piece™ is a filter that includes a one-way valve, so the air is not drawn back into the monitor. Moreover, the unique design of the D-piece™ allows it to remove more than 99% of airborne bacteria and more than 96% of viruses and moisture from the participant's breath. Alcohol-free monitor cleaning wipes were used to maintain the monitor's performance.

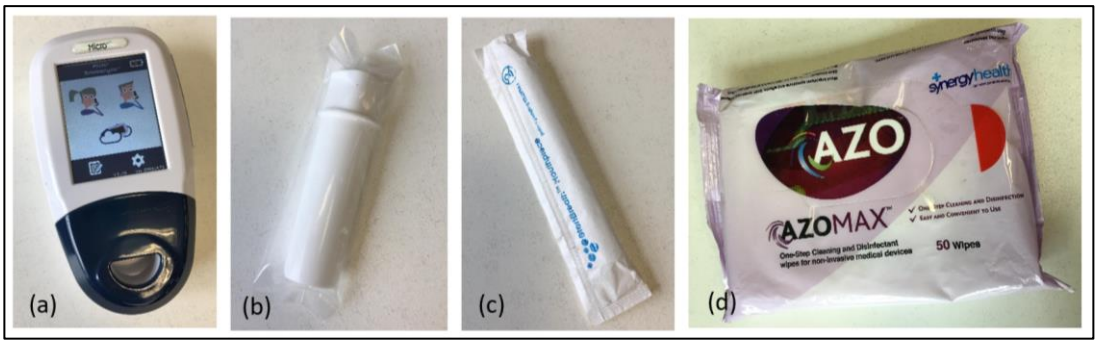

Figure 4-5. (a) Micro+™ Smokerlyzer® (b) D-piece™ (c) SteriBreath™ mouthpiece (d) Monitor cleaning wipes

For the cigarettes used in the study, the researcher reviewed cigarette descriptions on internet shops to find the CO amount in each cigarette. It is not possible for roll-up cigarettes to produce a constant amount of CO, so at the design stage the researcher excluded such cigarettes from consideration. In the study, the researcher focused on the level of CO in the cigarette. The CO level found in cigarettes is reported as a range of around 5.0-20.2 mg/cigarette (Russell et al., 1975; Hsu et al., 2011). Therefore, the control cigarette (Seven Stars, Japan Tobacco) has been chosen because it contains a typical CO level among all the cigarettes, which is around 10 mg/cigarette.

4.2.5 Analysis of the exhaled CO data

All of the data were recorded using Microsoft Excel (Microsoft Excel Office 365, USA) and analysed and modelled using the statistical software IBM SPSS Statistics 26 (IBM, Armonk, New Y, USA) and StataIC 15 (TX: StataCorp LLC, USA). The exhaled CO data were analysed as shown in Figure 4-6. First, descriptive statistics were used to calculate mean and proportion of characteristics of the exhaled CO dataset.

A univariable analysis was then carried out to understand the relationship between each variable, including baseline exhaled CO, exhaled CO half-life, exhaled CO increased after smoking and exhaled CO decreased 2 hours after smoking. When analysing the continuous results, if the independent variable had two categories (e.g. sex, smoking status, etc.), the t-test was applied; if the variable had more than two categories (e.g. ethnicity, type of cigarette, etc.), an ANOVA test was applied; and for continuous independent variables, linear regression was applied (Kirkwood and Sterne, 2003). When analysing the relationship between categorical variable data, the

chi-square test was applied (Kirkwood and Sterne, 2003). However, when the number of subjects observed in a category was too small (e.g. sample size < 20 or < 10 per group), a nonparametric analysis was used for comparisons of median values (Corder, 2014; Warner, 2008). Non-parametric analyses included the Mann-Whitney U test and the Kruskal-Wallis H test (Corder, 2014). A p-value of <0.05 was assumed to be statistically significant, and all p-values were presented for two-sided tests.

After the univariable analysis, a Pearson correlation test and a multicollinearity test was run to investigate the relationships between the variables. If there is a statistically significant linear relationship between two variables or the variance inflation factor (VIF) exceeds 10 within two variables, one of the variables should be excluded to avoid the effects of collinearity (Asuero et al., 2006; Hair Jr et al., 2014). The exclusion of variables from the multivariable regression model was based on the results from the VIF and correlation test and the literature. A multivariable regression was then applied to investigate the possible factors related to baseline CO, the CO half-life and CO uptake and elimination. In the multivariable regression model presented, a standardised beta coefficient was used to rank the most important variables. A p-value of <0.05 was assumed to be statistically significant (Hackshaw and Kirkwood, 2011).

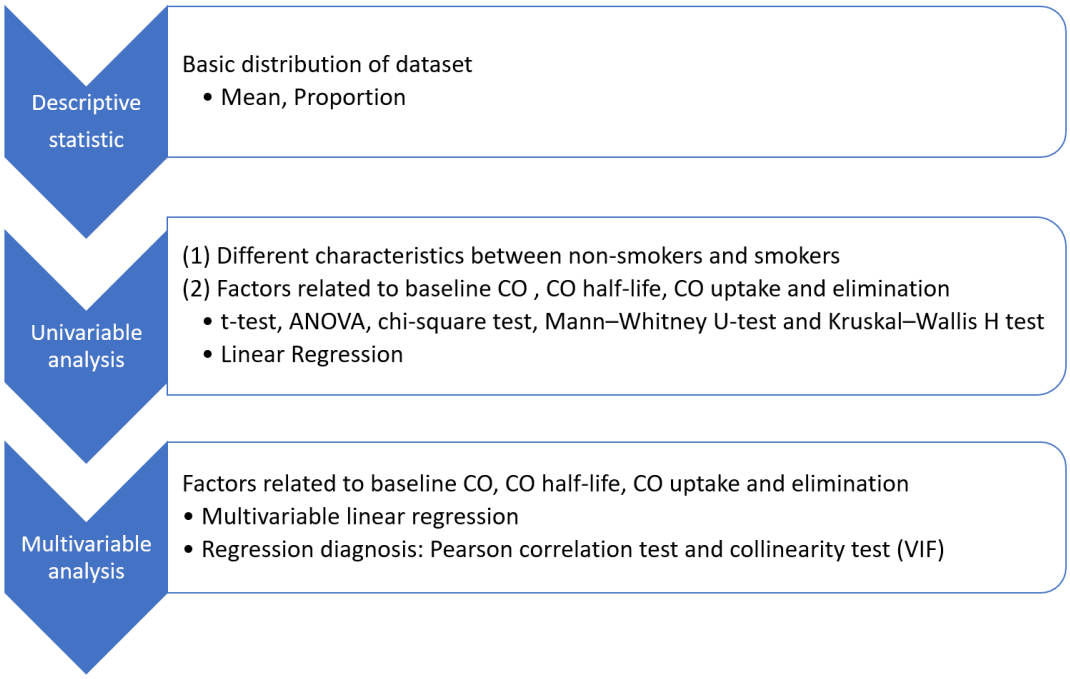

Figure 4-6. Analysing the exhaled CO data

Calculation of CO half-life

For the smokers, the half-life of COHb in the blood was calculated using a method from the literature independently described by Weaver and Ozturan (Weaver et al., 2000; Ozturan et al., 2019). The two-point method for measuring the COHb half-life (i.e. the time required for COHb levels to drop by 50%) uses concentrations of COHb taken at two different points. This method assumes that CO is eliminated from the body following a mono-exponential curve. In the equation below, if concentration 1 (c1) and concentration 2 (c2) are the levels of COHb taken at time 1 (t1) and time 2 (t2) during CO 'wash-out' time, then the half-life of COHb (HL(COHb)) in the blood is shown below. The exhaled CO half-life is also calculated as follows:

The half-life of COHb (exhaled CO),

$$HL(COHb\ or\ exhaled\ CO) = ln\ (2) \times (t2 - t1)/[ln(c1) - ln(c2)]$$

In the present study, the researcher used two time points; one time point was the time that immediately after smoking (t1) and the other time point was the time at two hours after smoking (t2). Two exhaled CO concentration used in the study were the exhaled CO concentration at the time immediately after smoking (c1) and the exhaled CO concentration at two hours after smoking (c2).

4.3 Results

In total, 84 participants were involved in this study, 57 smokers and 27 non-smokers. After exclusions were taken into account, 74 participants (48 smokers and 26 non-smokers), were analysed. The aim was to investigate differences, including baseline CO value, between smokers and non-smokers, and factors affecting CO uptake and CO elimination in smokers.

The results are separated into three parts. The first shows the demographic, physiological and behavioural factors of smokers and non-smokers and factors related to baseline exhaled CO. The second and third parts show factors related to CO uptake, CO elimination and CO half-life of smokers.

4.3.1 Overview of demographic, physiological and behavioural factors and factors related to baseline exhaled CO

An overview of the full exhaled CO experiment data is presented in Table 4-4 and Table 4-5. The average age was 27.1, with 28% females and 72% males. The

dataset included 26 non-smokers and 48 smokers (28 light smokers and 20 heavy smokers).

Table 4-4. Basic characteristics between smokers and non-smokers by t-test in the exhaled CO experiment dataset

Variable	Total (n=74) mean ±SD	Smokers (n=48) mean ±SD	Non-smokers (n=26) mean ±SD	p-value
Age (years)	27.1 ±4.0	26.6 ±4.5	27.9 ±2.7	0.202
Height (cm)	173.0 ±9.3	174.3 ±8.1	170.6 ±10.9	0.100
Weight (kg)	69.1 ±13.5	**72.1 ±13.8**	**63.2 ±11.1**	**0.007****
BMI (kg/m2)	23.1 ±3.3	**23.6 ±3.6**	**21.8 ±2.3**	**0.026***
Baseline exhaled CO [a] (ppm)	5.2 ±4.6	**6.9 ±4.9**	**1.9 ±0.5**	**<0.001*****

*Note: Where a significant factor was found, these values are shown in bold; *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1; [a] result measured from exhaled CO experiment.*

Table 4-5. Basic characteristics and comparison of characteristics between smokers and non-smokers by chi-square test in the exhaled CO experiment dataset

Variable	Total (n=74) n (%)	Smokers (n=48) n (%)	Non-smokers (n=26) n (%)	p-value
Sex				**0.013***
Male	53 (71.6)	**39 (81.3)**	**14 (53.9)**	
Female	21 (28.4)	**9 (18.7)**	**12 (46.2)**	
Ethnicity				0.366
Asian	45 (60.8)	27 (60.0)	18 (72.0)	
Hispanic/Latino	4 (5.4)	2 (4.4)	2 (8.0)	
White/Caucasian	21 (28.4)	16 (35.6)	5 (20.0)	
Exposure to CO prior to study?				0.199
None	53(71.6)	32 (66.7)	21 (80.8)	
Yes	21(28.4)	16 (33.3)	5 (19.2)	
Exercise before the study?				**0.047***
None	52(70.3)	**30 (62.5)**	**22 (84.6)**	
Yes	22(29.7)	**18 (37.5)**	**4 (15.38)**	

*Note: Where a significant factor was found, these values are shown in bold; *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

Table 4-4 shows that weight, BMI and baseline exhaled CO were significantly higher for smokers than non-smokers. Table 4-5 shows the distribution of sex, ethnicity and diet, whether the participant was exposed to CO or exercise before the study, separately for smokers and non-smokers. The results showed that a higher

proportion of smokers were male. Some characteristics are not presented in the results due to the small sample size, including, vegetarian (n=2), Black/African American (n=2), and mixed ethnicity (n=2). Moreover, only 5 female non-smokers and 2 smokers agreed to attend the exhaled CO measurement again in period time.

Analysis of the smokers section of the dataset

After comparing non-smokers and smokers, the researcher conducted further investigations within the group of smokers in Table 4-6.

Table 4-6. Basic characteristics overview for smokers and comparison of characteristics between light and heavy smokers by t-test in the exhaled CO experiment dataset

Characteristics	Total (n=48) mean ±SD	Light smokers (n=28) mean ±SD	Heavy smokers (n=20) mean ±SD	p-value
Age (years)	26.6 ±4.5	27.2 ±4.4	25.9 ±4.6	0.302
Height (cm)	174.3 ±8.1	173.4 ±8.8	175.5 ±7.0	0.386
Weight (kg)	72.1 ±13.8	70.9 ±11.2	73.9 ±16.8	0.456
BMI (kg/m2)	23.6 ±3.6	23.5 ±3.2	23.8 ±4.1	0.821
Years of smoking	8.8 ±4.8	8.6 ±4.7	9.0 ±5.0	0.783
Time since last cigarette (hrs ago) (n=47)	22.9 ±53.9	34.3 ±69.4	7.6 ±3.7	0.093†
Cigarettes smoked (daily)	7.1 ±5.6	**3.2 ±2.0**	**12.6 ±4.0**	**<0.001*** **
Cigarettes smoked (weekly)	50.8 ±39.8	**23.1 ±16.6**	**89.6 ±28.6**	**<0.001*** **
Puffs taken per cigarette[a]	11.9 ±4.1	12.4 ±4.3	11.3 ±3.9	0.368
Smoking duration[a] (mins)	3.5 ±1.1	3.6 ±0.8	3.3 ±1.3	0.250
Baseline exhaled CO[a] (ppm)	6.9 ±0.7	**4.8 ±2.6**	**10.0 ±5.8**	**<0.001*** **

*Note: Where a significant factor was found, these values are shown in bold; *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1; [a] result measured from exhaled CO experiment.*

The smokers were then separated into two groups: light smokers and heavy smokers. Some characteristics are not presented in the results due to the small sample size, including, vegetarian (n=1), Black/African American (n=1), Hispanic/Latino (n=2) and mixed ethnicity (n=1). It was clear that light smokers had a lower value of

baseline exhaled CO ppm than heavy smokers. Sex distribution was mostly males with only 8 females in the light smokers' group and one female in the heavy smokers' group (see Appendix 9.2.2, Supplementary Table 9-7 for details).

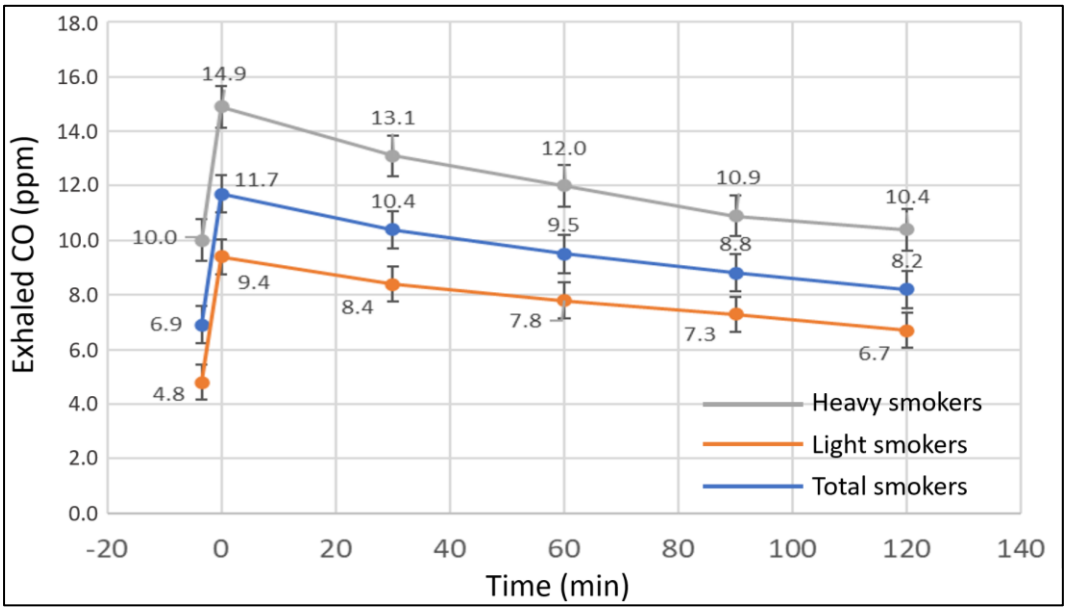

Figure 4-7. Exhaled CO value for smokers at different time points

Figure 4-7 shows the exhaled CO value for total (all) smokers, light smokers and heavy smokers at different time points. As a result, it shows that the decreasing value of CO in heavy smokers was more than the light smokers within 2 hours after smoking, which was around 4.5 ppm in heavy smokers compared to 2.7 ppm in light smokers (see Appendix 9.2.2, Supplementary Table 9-8 for details). However, when calculating the CO half-life, it showed no difference between heavy smokers and light smokers (see Section 4.3.3, Table 4-12).

Factors related to baseline exhaled CO for non-smokers

Here the relationship between baseline exhaled CO and potentially affecting factors in non-smokers is analysed. The variables considered are demographic, physiological and behavioural factors such as age, height, weight, BMI, sex and ethnicity, and, whether the participants had been exposed to CO or exercised before the study. Univariable analysis showed that no variable significantly affected the value of baseline exhaled CO for non-smokers (see Appendix 9.2.2, Supplementary Table 9-9 and Supplementary Table 9-10). The researcher also compared the baseline exhaled CO concentration for females between in period and not in period (n=5) by paired t-test, but no significant difference was found.

Factors related to baseline exhaled CO concentration for all smokers

In this section, the analysis aimed to determine the factors related to baseline exhaled CO of smokers in the exhaled CO experiment dataset. Table 4-7 and Table 4-8 show that sex, smoking status and the number of cigarettes smoked daily and weekly were significantly related to the value of baseline exhaled CO. There is no significant difference of the baseline exhaled CO concentration for females between in period and not in period due to the small sample size (n=2).

Table 4-7. Variation of baseline exhaled CO with demographic and smoking-related factors for smokers analysed with t-test in the exhaled CO experiment dataset

Variable (n=48)	**Baseline exhaled CO (ppm) mean ±SD**	**p-value**
Sex		**0.002****
Male (n=39)	**7.7 ±5.1**	
Female (n=9)	**3.6 ±2.1**	
Sex (only light smokers)		**0.022***
Male (n=20)	**5.5 ±2.7**	
Female (n=8)	**3.0 ±2.3**	
Ethnicity		0.474
Asian (n=27)	7.9 ±5.9	
White/Caucasian (n=16)	5.9 ±3.0	
Smoking status		**<0.001*****
Light smokers (n=28)	**4.8 ±2.6**	
Heavy smokers (n=20)	**10.0 ±5.8**	
CO exposure before the study?		0.094†
None (n=32)	7.8 ±5.6	
Yes (n=16)	5.3 ±2.7	
Exercise before the study?		0.586
None (n=30)	6.6 ±4.2	
Yes (n=18)	7.4 ±6.1	

*Note: Where a significant factor was found, these values are shown in bold; *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

Table 4-8. Univariable linear regression of the relationship between baseline exhaled CO with each demographic, physiological, behavioural and smoking-related factor for smokers in the exhaled CO experiment dataset

Variable (n=48)	**Baseline exhaled CO (ppm)**			
	β [a]	**Beta [b]**	**p-value**	**R^2**
Age (years)	0.180	0.163	0.267	0.027
Height (cm)	0.037	0.061	0.681	0.004
Weight (kg)	0.049	0.136	0.356	0.019
BMI (kg/m2)	0.181	0.132	0.373	0.017
Years of smoking	0.144	0.140	0.342	0.020
Time since last cigarette (hrs ago) (n=47)	-0.025	-0.269	0.067	0.073
Cigarettes smoked (daily)	**0.350**	**0.394**	**0.006****	**0.156**
Cigarettes smoked (weekly)	**0.052**	**0.417**	**0.003****	**0.174**

*Note: Where a significant factor was found, these values are shown in bold; *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1; [b] Beta (standardised coefficient).*

After doing the univariable analysis, multivariable linear regression was used to analyse the factors that affected baseline exhaled CO for smokers. Table 4-9 shows that heavy smokers have a higher baseline exhaled CO concentration when adjusting for sex, years of smoking, time since the last cigarette, and exposure to CO before the study.

Table 4-9. Factors affecting baseline exhaled CO (ppm) for smokers analysed by multivariable linear regression of the exhaled CO experiment dataset

Variable (n=47)	**R^2=0.368, Adjusted R^2=0.291**			
	β [a]	**Beta [b]**	**95% CI [c]**	**p-value**
Smoking status (heavy smoker)	**4.523**	**0.456**	**(1.835, 7.211)**	**0.002***
Sex (female)	-1.736	-0.139	(-5.115, 1.642)	0.305
Exposure to CO (yes)	-1.415	-0.134	(-4.244, 1.414)	0.318
Time since last cigarette (hrs ago)	-0.009	-0.099	(-0.034, 0.015)	0.458
Years of smoking	0.096	0.093	(-0.164, 0.355)	0.461

*Note: ordered by the absolute value of Beta; where a significant factor was found, these values are shown in bold; *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1; [a] β (un-standardised coefficient); [b] Beta (standardised coefficient); [c] CI (confidence interval).*

4.3.2 Results from exhaled CO experiment: CO uptake

The researcher analysed the factors related to CO uptake. In theory, this should relate to the amount of CO a person inhales and how effective their body is at

absorbing that CO into their bloodstream, which could be the CO increases after smoking.

CO increases after smoking (ppm) = CO after smoking (ppm) – CO baseline (ppm)

The following tables give an overview of the results. Note that three participants showed no change in exhaled CO ppm after smoking. The average exhaled CO increase due to smoking was around 4.7 ±2.5 ppm.

Factors related to exhaled CO increased after smoking

In univariable analysis, males had a lower value of exhaled CO increase after smoking than females (4.4 ±2.2 vs 6.3 ±3.0, p=0.037) and exhaled CO increase after smoking is negatively associated with height (β=-0.104, p=0.018) and weight (β=-0.056, p=0.029) (see Appendix 9.2.2, Supplementary Table 9-11 and Supplementary Table 9-12 for details). A multivariable regression was carried out. The results (see Table 4-10) showed that females had a higher CO increase after smoking than males when adjusting for exercise and smoking status.

Table 4-10. Factors affecting CO (ppm) increased after smoking for smokers, as analysed by multivariable linear regression for smokers of the exhaled CO experiment dataset

Variable (n=48)	**R^2=0.185, Adjusted R^2=0.087**			
	β [a]	**Beta [b]**	**95% CI [c]**	**p-value**
Sex (female)	1.885	0.303	(-0.079, 3.848)	0.059†
Exercise (yes)	-0.971	-0.194	(-2.435, 0.492)	0.187
Smoking status (heavy smoker)	0.870	0.176	(-0.586, 2.326)	0.235
Puffs taken per cigarette	0.068	0.114	(-0.114, 0.251)	0.453
BMI (kg/m^2)	-0.068	-0.099	(-0.270, 0.134)	0.501

*Note: ordered by the absolute value of Beta, where there was a significant factor found, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1, [a] β (un-standardised coefficient), [b] Beta (standardised coefficient), [c] CI (confidence interval).*

4.3.3 Results from exhaled CO experiment: CO decreased 2 hours after smoking and exhaled CO half-life

The factors related to CO elimination amounts were analysed. The CO decreases after smoking was calculated as follows:

CO decreased after smoking (ppm) = CO right after smoking (ppm) – CO at 2 hours after smoking (ppm)

The CO half-life was calculated from the half-life equation given in Section 4.2.5. After calculating the results, only one participant showed a higher value of exhaled CO ppm at 120 minutes after smoking rather than immediately after smoking. Considering that the participants were all exposed to the same environmental CO levels during the experiment, the most likely reason for this result is equipment error However, as it was not possible to definitively determine the reason, the researcher decided to exclude the data. Due to this, the data could not be used for calculation of CO half-life. Also, when calculating the exhaled CO half-life, three participants were excluded as their exhaled CO half-life could not be calculated because their CO ppm measured equal to or lower than zero 120 minutes after smoking, which may also be equipment error. Finally, the results show the exhaled CO decrease after smoking was around 3.6 ppm for the smokers, and the average exhaled CO half-life was about 273 minutes (4.6 hours).

Factors related to exhaled CO decreased 2 hours after smoking

Investigating the factors affecting CO decrease after smoking, the univariable test showed that the heavy smokers' CO decreased more than light smokers after smoking (5.6 ±2.8 vs 2.8 ±1.8, p=0.013), and that cigarettes smoked weekly (β= 0.020, p=0.022) had a positive association with the amount of CO decrease after smoking (see Appendix 9.2.2, Supplementary Table 9-13 and Supplementary Table 9-14 for details). Table 4-11 shows that the only factor that affected CO decrease after smoking was smoking status. Heavy smokers recorded a greater amount of CO decrease than light smokers over two hours.

Table 4-11. Factors affecting CO decreased after smoking (ppm) for smokers, as analysed by multivariable linear regression of the exhaled CO experiment dataset

Variable (n=47)	**R^2=0.140, Adjusted R^2= 0.080**			
	β [a]	**Beta [b]**	**95% CI [c]**	**p-value**
Smoking status (Heavy smoker)	**1.892**	**0.392**	**(0.383, 3.087)**	**0.012***
Sex (female)	0.665	0.110	(-1.202, 2.532)	0.476
BMI (kg/m^2)	0.031	0.047	(-0.164, 0.227)	0.747

*Note: ordered by the absolute value of Beta, where a significant factor was found, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1, [a] β (un-standardised coefficient), [b] Beta (standardised coefficient), [c] CI (confidence interval).*

Factors related to exhaled CO half-life

In this section, the analysis aimed to determine the factors related to exhaled CO half-life of smokers in the exhaled CO experiment dataset. Table 4-12 and Table 4-13 show that sex and height were significantly related to the value of exhaled CO half-life.

Table 4-12. Variation of exhaled CO half-life with demographic and smoking-related factors for smokers analysed by t-test in the exhaled CO experiment dataset

Variable (n=45)	CO half-life (min) mean ±SD	p-value
Sex		**0.010***
Male (n=36)	**288.1 ±96.1**	
Female (n=9)	**213.9 ±70.4**	
Ethnicity		0.956
Asian (n=25)	282.8 ±101.8	
White/Caucasian (n=15)	272.7 ±95.4	
Smoking status		0.396
Light smokers (n=25)	262.3 ±90.5	
Heavy smokers (n=20)	287.0 ±22.9	
CO exposure before the study?		0.281
None (n=29)	284.8 ±106.5	
Yes (n=16)	252.4 ±70.2	
Exercise before the study?		0.486
None (n=29)	280.8 ±94.4	
Yes (n=16)	259.7 ±99.3	

*Note: Where a significant factor was found, these values are shown in bold; *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

Table 4-13. Univariable linear regression of the relationship between exhaled CO half-life with each demographic, physiological, behavioural and smoking-related factor for smokers in the exhaled CO experiment dataset

Variable (n=45)	Exhaled CO half-life (min)			
	β	Beta	p-value	R^2
Age (year)	0.145	0.007	0.965	<0.001
Height (cm)	**4.247**	**0.357**	**0.016***	**0.127**
Weight (kg)	1.980	0.292	0.051	0.085
BMI (kg/m2)	4.139	0.159	0.297	0.025
Years of smoking (year)	0.998	0.051	0.741	0.002
Time since last cigarette (hrs ago)	0.056	0.032	0.835	0.001
Cigarettes smoked (daily)	0.569	0.033	0.828	0.001
Cigarettes smoked (weekly)	-0.146	-0.062	0.688	0.004
Puffs taken per cigarette	-4.602	-0.199	0.189	0.040
Smoking duration (min)	2.183	0.025	0.872	0.001

*Note: Where a significant factor was found, these values are shown in bold; *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1; [b] Beta (standardised coefficient).*

The multivariable regression, shown in Table 4-14, shows that females had a shorter exhaled CO half-life than males, although this difference did not reach statistical significance.

Table 4-14. Factors affecting exhaled CO half-life (min) for smokers, as analysed by multivariable linear regression of the exhaled CO experiment dataset

Variable (n=45)	**R^2=0.108, Adjusted R^2=0.043**			
	β [a]	**Beta [b]**	**95% CI [c]**	**p-value**
Sex (female)	-66.879	-0.283	(-143.399, 9.640)	0.085†
Smoking status (Heavy smoker)	2.497	0.030	(-54.454, 65.930)	0.848
BMI (kg/m^2)	2.497	0.096	(-5.460, 10.454)	0.530

*Note: ordered by absolute value of Beta; where a significant factor was found, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1, [a] β (un-standardised coefficient), [b] Beta (standardised coefficient), [c] CI (confidence interval).*

4.4 Discussion and conclusion

Based on the results, some factors are related to CO uptake and elimination in healthy, smoking participants. In this section, a discussion of characteristics of the participants and factors related to CO uptake and elimination will be presented.

4.4.1 Participants: age, sex and ethnicity

The average age of the participants in the study, 27, was younger that of the general population. Only participants aged 18-34 were invited to take part in the study in an attempt to exclude the effects of ageing and diseases as discussed in Section 4.2.3 (Bjartveit and Tverdal, 2005; Maga et al., 2017; Windsor-Shellard et al., 2019; Bowdish, 2019).

The non-smokers group contained 14 males and 12 females and the smokers' group 39 males and 9 females. In general, smoking prevalence varies from country to country but is higher among men than women (Windsor-Shellard et al., 2019; Chinwong et al., 2018) and this was reflected in our study. During the recruitment process the researcher also noted that the majority of females approached declined to join the study and did not want people to know that they smoked. This may be related to the higher social acceptability of smoking for men than women (Parkinson et al., 2009). When comparing the sex difference of light and heavy smokers, the light smokers' group contained 20 males and eight females, and the heavy smokers contained 19 males and only one female. In the UK National Statistics report, the

average daily cigarette consumption of male smokers is also higher than that of female smokers (Allen et al., 2016; Windsor-Shellard et al., 2019).

This study was based at UCL and Goodenough College, both of which are home to many international students. Therefore, the participants included Asian, Black/African-American, Hispanic/Latino, White/Caucasian and mixed ethnicity individuals. The majority of participants were Asian and White.

4.4.2 Influences of demographic, physiological and behavioural factors on baseline CO value, CO uptake and CO elimination

Effects of age, sex and ethnicity on baseline CO value, CO uptake and CO elimination

Age is an important factor that might affect the rate of CO uptake and elimination. Klasner et al. (1998) found that age was a factor that affects CO half-life, as children have a shorter COHb half-life compared to adults. However, in our study, age had no significant effect on exhaled CO half-life, which was similar to findings in other studies (Burney et al., 1982; McNeill et al., 1986; Weaver et al., 2000); it should, however, be noted that it is difficult to draw hard conclusions from these results because the age range in the study was very limited (only those aged 18-34). For baseline COHb, Schimmel et al. (2018) found there was no significant correlation between age and baseline SpCO (COHb in arterial blood), which is similar to our study.

In our study, males had a higher baseline exhaled CO value than females (7.7 ppm vs 3.6 ppm). This may be due to more males being heavy smokers, as heavy smokers tend to have a higher value of COHb (Raub et al., 2000; Prockop and Chichkova, 2007; Maga et al., 2017). In univariable analysis, female smokers had a shorter exhaled CO half-life than male smokers (3.6 hours vs 4.8 hours). Pace et al. (1950) and Zavorsky et al. (2014) also found that females have a shorter COHb half-life than males, which may be due to females having a lower total Hb mass and higher alveolar ventilation. However, the effects of sex were reduced in multivariable analysis, which is similar to the findings form Burney et al. (1982) and Weaver et al. (2000).

Our study showed that ethnicity had no significant influence on baseline exhaled CO, exhaled CO increased after smoking, exhaled CO decreased 2 hours after smoking or exhaled CO half-life. The direct effects of ethnicity on the rate of CO uptake and CO elimination are rarely discussed and could be further explored

(Pan et al., 2020). Later, in Section 5, we discuss the effects of ethnicity on pulmonary function parameters and the relationship between pulmonary function and the rate of CO uptake and elimination.

Effects of BMI, height and weight on baseline CO value, CO uptake and CO elimination

We looked for any effects of BMI but found that BMI had no significant effect on baseline exhaled CO (p=0.373), exhaled CO increased after smoking (p=0.157), exhaled CO decreased 2 hours after smoking (p=0.816) or exhaled CO half-life (p=0.297). However, generally, heavier people have increased blood volume, which may be related to the rate of CO uptake and CO elimination (Coburn et al., 1965; Gosselin et al., 2009). If a person is heavier, the CO uptake increase lower and CO half-life was longer after smoking. Even though higher blood volume may affect the rate of CO uptake and elimination, the effects of Hb concentration and the total Hb mass should also be considered. Moreover, Lenfant (2000 cited in Fröhlich et al., 2016) noted that mammals' lung surface area could be estimated at around one m^2/kg body weight. If the lung surface area is bigger, more gas can transfer into the lungs.

Height is related to pulmonary function, such as lung volumes (Paoletti et al., 1985; Roca et al., 1990; Chhabra et al., 2016). The higher the value of DL_{CO} and lung volumes, the faster the rates of CO uptake and elimination (Forbes et al., 1945; Coburn et al., 1965; Gosselin et al., 2009; Zavorsky et al., 2014). The reason for no significant effects from the height might be that, rather than lung volumes, alveolar ventilation or blood volume may play a more important role in the rate of CO uptake and CO elimination (Coburn et al., 1965; Gosselin et al., 2009; Zavorsky et al., 2014).

Effects of CO exposure and exercise on baseline CO value, CO uptake and CO elimination

Exposure to CO before attending the study showed no significant effects on baseline exhaled CO (p=0.318). However, Zhang et al. (2013) reported that levels of exposure to passive smoking and biomass/coal burning had a positive association with the value of exhaled CO of non-smokers. The majority of participants reported the possibility of CO exposure from heavy traffic as they walked or cycled to the study. Neither smokers nor non-smokers showed a significant difference in baseline exhaled CO compared to those not exposed to CO. A possible reason for this is that the CO amount the participants were exposed to was not enough to make a difference. According to Kirk et al. (1988), the mean CO level in the environment is quite low,

in fact less than 4 ppm in different places, such as the home, office, and travel and leisure situations. Moreover, in the UK report, the mean concentration of CO indoor was around 1.7 ppm, which may not have a significant impact on the participants' exhaled CO concentration (Volans et al., 2007).

In most studies, exercise is shown to have a significant effect on the rate of CO uptake and CO elimination (Forbes et al., 1945; Filley et al., 1954; Zavorsky et al., 2012). In the present study, however, exercise did not affect CO uptake (p=0.187). Of the participants that reported exercise before the study, most reported milder forms, such as walking, and were breathing normally throughout the study, which meant the rate of CO uptake and CO elimination were probably not affected.

4.4.3 Influences of smoking status on baseline CO value, CO uptake and elimination

Smoking-related factors affecting baseline exhaled CO

This study shows that sex, smoking status and number of cigarettes smoked daily and weekly may be related to baseline exhaled CO in the univariable test, while smoking status may be related to baseline exhaled CO in the multivariable regression. Even though years of smoking was not found to affect the baseline exhaled CO in the present study, Deveci et al. (2004) found that exhaled CO not only has a positive association with cigarettes smoked daily but also with period of smoking. The effect of the last time of smoking is controversial: Hawkins et al. (1976) show that COHb (%) is related to the number of cigarettes smoked daily, but not the last time of smoking but for Schimmel et al. (2018), baseline SpCO (%) was related to last time of smoking, not age, years of smoking or a history of daily smoking. There were several potential factors related to the last time of smoking but these particular factors are hard to be controlled for in the study – such as the type of cigarette smoked, the depth of inhalation, whether part or all of the cigarette was smoked and the number of puffs taken (Hawkins et al., 1976).

The baseline exhaled CO values in smokers (6.9 ppm) and non-smokers (1.9 ppm) were similar to the data from Kozienice, a small Polish town, in Maga et al.'s 2017 study, in which the average baseline CO was 6.5 ppm (1.67% COHb) in smokers and 1.07 ppm (0.80% COHb) in non-smokers. However, due to the difference in air quality between big cities and small towns, the average baseline exhaled CO data were much higher for smokers and non-smokers in Krakow, at 12.3

ppm (2.60% COHb) for smokers and 7.02 ppm (1.75% COHb) for non-smokers, and in Warsaw, at 14.4 ppm (2.93% COHb) for smokers and 5.11 ppm (1.45% COHb) for non-smokers (Maga et al., 2017). Generally, the mean of ambient CO concentration is lower than 0.5 ppm from Defra's UKAir between 2015 and 2020 (Defra's UKAir, 2021). Therefore, the ambient CO in our study may not affect the baseline exhaled CO concentration. The lower baseline exhaled CO value of smokers in the study may be related to the lower number of heavy smokers and a longer average time since the last cigarette compared to other studies (Maga et al., 2017; Schimmel et al., 2018). Possible reasons for the longer average time since the last cigarette might be that some of the participants attended the study in the morning and had not yet smoked that day; others stated that they usually smoked only at weekends.

Smoking-related factors affecting CO uptake

Other studies have shown that COHb and exhaled CO values are positively associated with the number of cigarettes smoked (Castleden and Cole, 1975; Hawkins et al., 1976; Vogt et al., 1979; Deveci et al., 2004; Muhammad-Kah et al., 2011; Zhang et al., 2013). Both Vogt et al. (1979) and Zhang et al. (2013) reported that the exhaled CO value is higher for participants that smoke more and inhale more deeply. In our study, the value of exhaled CO showed no difference before and after smoking in a small number of participants: however, some reported that they did not inhale the smoke into their lungs, while others said that they did inhale deeply.

Some studies reported that smokers could lower their CO exposure by reducing the volume of each inhalation, the number of inhalations per cigarette smoked and the tendency to and depth of inhaling (Robinson and Forbes, 1975; Vogt et al., 1979; Weinhold and Stitzer, 1989; Strasser et al., 2007; Muhammad-Kah et al., 2011). In terms of puffs, in general, males tend to have a higher puff volume, longer puff duration and shorter intervals between puffs than females (Muhammad-Kah et al., 2011). Weinhold and Stitzer (1989) reported that puff numbers were related to the CO uptake amount, but these results were not replicated in the present study. All the factors mentioned above may be highly related to smoking habits and are therefore hard to control. Therefore, this might be a reason for the big variation within and between different studies (Castleden and Cole, 1975; Zhang et al., 2013; Maga et al., 2017; Schimmel et al., 2018). Cohen et al. (1971) mentioned that not only smoking habits but also the pulmonary function of individuals affects CO uptake amounts.

This situation should be considered and how to measure the actual amount of CO that enters the body while smoking could be discussed in a future study. For example, there were several factors affecting CO exposure mentioned in the paragraph, such as the volume of each inhalation, the number of inhalations per cigarette smoked, the tendency to and depth of inhaling and pulmonary function. Therefore, for measuring closer data to the actual amount of CO that enters the body, the extra data mentioned above should be collected to adjust when analysing the results in the future study.

Besides smoking habits, some studies show how cigarettes themselves may play a role in CO exposure in smoking because of differences in paper porosity, filters, CO levels, nicotine levels and type (hand-rolled vs factory-made) (Cohen et al., 1971; Robinson and Forbes, 1975; Weinhold and Stitzer, 1989; Strasser et al., 2007; Laugesen et al., 2009). Increasing the paper porosity and lowering the CO level of cigarettes may lower CO exposure for smokers (Robinson and Forbes, 1975; Weinhold and Stitzer, 1989). Lower nicotine levels, however, have been shown to increase CO exposure for smokers due to the increased number of puffs (Strasser et al., 2007). Laugesen et al. (2009) compared the different smoking patterns exhibited when smoking hand-rolled cigarettes and factory-made cigarettes. Their results showed that even though the increased CO ppm was similar in the two types of cigarettes, the CO ppm increase per gram of tobacco burnt was higher in hand-rolled cigarettes than in factory-made ones. Therefore, all participants in this study were given the same cigarette to smoke to avoid any differences in results that might arise from the effects of different cigarettes' properties.

Smoking-related factors affecting CO half-life

In this study, the number of cigarettes smoked weekly did not affect exhaled CO half-life ($p=0.848$), which was similar to the findings in two other studies (Burney et al., 1982; Ozturan et al., 2019). However, it showed that the amount of CO decreased more in heavy smokers than light smokers within 2 hours after smoking (4.5 ppm vs 2.7 ppm). The possible reason for the situation might be the higher peak exhaled CO concentration in heavy smokers compared to light smokers. Therefore, if the exhaled CO half-life was similar in both heavy smokers and light smokers, to decay to the same baseline exhaled CO, the decreasing amount of CO in heavy smokers would be more than in light smokers within the same time period.

Moreover, in Cronenberger et al. (2008), however, found that the median (range) CO half-life was 30.9 hours (7.13-367) in adult smokers. This COHb half-life is much longer than the four hours found by Kao and Nanagas (2004). Possible reasons for this might be the physiological differences between smokers and non-smokers, including decreasing lung function and a higher concentration of COHb in smokers (Nordenberg et al., 1990; Cronenberger et al., 2008; Tantisuwat and Thaveeratitham, 2014). However, in the current study, all participants were young and healthy. Therefore, the effects of smoking may not be as obvious.

4.4.4 Limitations

The exhaled CO experiment involving smokers had several inherent limitations. First, the accuracy of the Smokerlyzer is ±2ppm therefore measurement errors may be present, and the equation describing the relationships between COHb and exhaled CO for the Smokerlyzer was generated from two previous studies (Jarvis et al., 1980, Jarvis et al., 1986). Even though Jarvis et al. (1980) found that impaired lung function and young(er) age may affect the relationship between CO ppm and COHb, the regression of CO ppm and COHb was still valid for normal healthy smokers (r= 0.98, n= 182), which describes the participants in the exhaled CO experiment part of the study.

Second, participants smoked outdoors and did the exhaled CO experiment indoors due to the university's smoking regulations: consequently, there was a delay after smoking of between 1–10 minutes before the participants went on to the next stage of the exhaled CO experiment. Fortunately, the exact times were all recorded in the study, and they were much less than the CO half-life, which was around four hours when breathing outdoor air (Kao and Nanagas, 2004). Also, the CO exposure models showed no significant difference during the first few minutes (Coburn et al., 1965; Gosselin et al., 2009). Therefore, this short delay should not significantly affect the study.

Third, certain aspects of individual personal smoking habits were hard to control for in the study, such as the number of puffs, interval time between puffs and the depth of smoking (Castleden and Cole, 1975; Zhang et al., 2013; Maga et al., 2017; Schimmel et al., 2018): all of these factors have been shown to influence exhaled CO and COHb levels in the body. As we do not distinguish between styles of smoking, there might be variability within smokers. Moreover, when recruiting

participants, many females refused to take part in the study and were unwilling to report their smoking status. This situation resulted in there being more males than females involved in the study. Moreover, we should interpret the results carefully in the light of the narrow age band and small sample size.

Fourth, the researcher only followed and recorded the exhaled CO elimination for up to two hours. Generally, the COHb half-life was around 4 hours (Kao and Nanagas, 2004). Therefore, the exhaled CO half-life in the study was calculated from the COHb half-life equation (Weaver et al., 2000; Ozturan et al., 2019). However, it would be better if the follow-up time could be extended to at least four hours, then the researcher could record the true exhaled CO half-life of the participants and the results of the factors affecting the exhaled CO half-life could be more accurate.

Finally, due to the ethical issue, it is impossible to invite non-smokers to smoke cigarettes and record their CO elimination rates. Therefore, in the study, we could only compare the exhaled CO half-life between light and heavy smokers, despite the almost certain differences in characteristics between smokers and non-smokers. Moreover, in the study, the increased CO ppm in the exhaled CO was limited to that measured after smoking one cigarette, around 5 ppm (1.43% COHb): for some participants this was not enough to cause any increase in exhaled CO and this may also affect the results. Different CO exposure methods could be used in future studies, such as the DL_{CO} test and CO-rebreathing experiment, which are safer and utilise a known dose of CO exposure under clinical and medical staff control.

4.4.5 Conclusion

A total of 74 participants were tested, 48 smokers and 26 non-smokers. The average baseline exhaled CO was around 1.9 ppm for non-smokers and 6.9 ppm for smokers. Also, the average exhaled CO half-life showed no significant difference between the two groups with around 262 min (4.4 hours) for light smokers and 287 min (4.8 hours) for heavy smokers. The results showed that smoking status affected baseline exhaled CO and CO decrease after smoking; no significant factor affected CO increase after smoking and exhaled CO half-life in the study. Even though there were no significant findings of factors affecting CO half-life, it is the first study using exhaled CO concentration from breath CO monitors to calculate the CO half-life and showed similar results as COHb half-life from the blood samples.

Further research should try to recruit more participants and with a wider age band. Also, additional factors related to smoking habits, such as type/brand of cigarettes, interval time between puffs and the depth of smoking could be considered in the future. Furthermore, a version of this exhaled CO experiment was published in the *International Journal of Environmental Research and Public Health* in November 2021 (Pan et al., 2021), please see Appendix 9.3.

5 GATHERING AND ANALYSING THE PRIMARY DATA ON PULMONARY FUNCTION

5.1 Introduction

Pulmonary function tests (PFTs) are routinely carried out by clinical staff in hospitals to understand the pulmonary function of patients (Hughes, 2008; Ranu et al., 2011; Strong, 2014a). Also, pulmonary function, DL_{CO} (the ability of gas to cross the alveolar membrane to the blood in the pulmonary capillaries), plays an important role in CO exposure models and would affect the rate of CO uptake and elimination rate (Coburn et al., 1965; Bruce and Bruce, 2003; Gosselin et al., 2009). Several factors might affect pulmonary function, such as age, gender, height, weight, ethnicity and smoking status (Talaminos Barroso et al., 2018). However, the impacts of these factors varied among the literature (Paoletti et al., 1985; Ip et al., 2007; Chhabra et al., 2016).

Therefore, the overarching aim is to develop a cross-sectional design that uses the PFT data to create a model to produce a more customised predicted value for parameters in order to improve existing CO uptake and elimination models. Other objectives were:

- To investigate the demographic, physiological and behavioural factors affecting DL_{CO}, V_A and K_{CO} in different groups
- To build predictive models of DL_{CO}, V_A and K_{CO} in relatively healthy groups
- To compare the predictive models from the research with models from previous literature.

5.2 Methods

5.2.1 Ethical approval for use of PFT data

Data from standard pulmonary function tests carried out in two hospitals, one in Taiwan (TSGH) and one in the UK (RBH), were gathered. Ethical approval for the study was required because it involved data from human participants. This process was complicated and took far longer than expected due to the unexpected COVID-19 situation. In this study, two separate ethical approval routes needed to be

followed due to the different national bodies governing the processes for each hospital, in addition to the requirements of UCL.

The study had to be approved by the Institutional Review Board of Tri-Service General Hospital in Taiwan (Appendix 9.1.2), the Health Research Authority (HRA) and the Royal Berkshire Hospital (Appendix 9.1.3) in the UK as well as by the UCL Research Ethics Committee (Appendix 9.1.4).

Ethical approval application for Tri-Service General Hospital (TSGH)

For the ethical approval from TSGH, the researcher had to visit Taiwan to discuss all the details of the study (August 2018) to gain an initial agreement to proceed with the ethical application. The researcher then had to prepare all of the documentation. This process included a new project online application, an expedited review form, a proposal, an abstract of the proposal and a case report form (see Table 5-1). On 8th May 2019, the study was given ethical approval confirmation (TSGHIRB No. 1-108-05-066).

Table 5-1. Timeline of ethical approval application for TSGH

Time	Task
TSGH	
May-July 2018	Project proposal
August 2018	Meeting at TSGH
September 2018-January 2019	Prepared all documents
February 2019	Tested the protocols at TSGH
March 2019	Revised the documents
01 April 2019	Submitted the application
April-May 2019	Revised the documents
05 May 2019	Re-submitted the application
08 May 2019	Approval
UCL	
25-26 June 2019	Submission and Approval

In addition to ethical approval from TSGH, ethical approval from UCL was also required to enable the transfer and use of the Taiwanese data. However, as the data was only transferred as secondary, anonymous data, a much lower level of ethics approval was needed and the researcher was able to apply for ethical approval at a departmental level, in this case from the Bartlett School of Environment, Energy and Resources (see Appendix 9.1.4). Documentation for this part of the research was

submitted as a ‘low-risk’ ethical application on 25th June and was rapidly approved on 26th June.

Ethical approval application for Royal Berkshire Hospital, UK (RBH)

For ethical approval from RBH, the researcher was required to visit RBH to discuss all the details of the study (June 2019) to gain an initial agreement to proceed with the ethics application. The researcher then had to prepare all of the documentation. This application included the sponsor process at the UCL level, an ethical application through the Health Research Authority (HRA) and then an ethical approval from RBH (see Table 5-2). Approval from RBH was finally issued on 30th September 2020.

Table 5-2. Timeline of ethical approval application for RBH

Time	Task
UCL	
June 2019-August 2019	Prepared all documents
23 September 2019	Submitted application for UCL sponsorship
10 February 2020	Confirmation of UCL sponsorship
HRA	
25 February 2020	Submitted application to HRA
March 2020	Revised the documents
02 April 2020	Approval from HRA
RBH	
April to July 2020	Delay as only review projects related to COVID-19 were allowed during this period
03 July 2020	Submission to RBH
30 September 2020	Approval from RBH

5.2.2 General recruitment criteria for participants

The recruitment criteria for the participants were discussed with the supervisory team and aimed to reduce confounding factors, thus allowing for a better prediction model to be developed. This work focused on extending existing CO update and elimination models to categories of people that differ from healthy volunteers in terms of their demographic, physiologic and behavioural characteristics, and not because of illness. Therefore, a general recruitment criterion was the identification of hospital attendees who were not diagnosed with a specific disease

and therefore could be considered relatively healthy. Participants younger than 18 were excluded as they are considered being minors.

Table 5-3. Recruitment criteria for participants

Inclusion criteria	Exclusion criteria
Age: over 18 Participated in DL_{CO} or TL_{CO} test Have data recorded from 2014-2019	Patients who did not follow the instructions properly

Definitions used in the study

There were several definitions used in the TSGH and RBH datasets. Diagnosis was defined as the diagnosis recorded in the PFT report in the hospitals. In the study, history of diseases included COPD, hypertension, hyperlipidaemia, anaemia, diabetes mellitus (DM), asthma, cardiovascular diseases and kidney diseases, which were recorded in the medical report of the patients. Other definitions used in the study was shown in Table 5-4 below.

Table 5-4. Definitions used in the study

Variables	Definition and Reference
Anaemia	Hb level of less than 12 g/dL for females and less than 13.5 g/dL for males (Fatemi and Clayton, 2008; Badireddy and Baradhi, 2019)
Cardiovascular diseases	Cardiovascular diseases, including heart diseases, mitral insufficiency (the mitral valve does not close properly when the heart pumps out blood), arrhythmia (a disorder of the rate or rhythm of the heartbeat) and coronary artery diseases (WHO, 2017)
Hb	The reference range is 12.0-16.0 g/dL for women, 13.5-17.5 g/dL for men (Fatemi and Clayton, 2008; Shinkawa et al., 2009; Kawai et al., 2017)
Mixed obstructive and restrictive lung disease	FEV_1/FVC <70% and FVC <80% is defined as mixed obstructive and restrictive lung disease (Vandevoorde et al., 2006; Johnson and Theurer, 2014).
Obesity	BMI equal to or greater than 30 kg/m^2 (WHO, 2020).
Obstructive lung disease	FEV_1/FVC < 70 % and FVC ≥ 80% is defined as obstructive lung disease (Vandevoorde et al., 2006; Ranu et al., 2011; Johnson and Theurer, 2014).
Restrictive lung disease	FEV_1/FVC ≥70 % and FVC <80% is defined as restrictive lung disease (Mannino et al., 2007).
Related renal diseases	Related renal diseases included kidney stones, kidney diseases and cystic diseases of the kidney (Widmaier et al., 2008)

Case Study 1: Tri-Service General Hospital recruitment

For the project, the research team received a one-year anonymised dataset of patients who had had PFTs from June 2017 to May 2018. At TSGH, around 250 patients attend for routine pulmonary function testing each month, giving a total of 3,512 potentially eligible patients (see Figure 5-1). Not all of these patients had the DL_{CO} test and many had pre-existing lung function disorders. The exclusion criteria for the 'relatively healthy' study group were discussed with medical doctors/respiratory physiologists and followed existing references (Barreiro and Perillo, 2004; Stanojevic et al., 2010; Wyka et al., 2011; Johnson and Theurer, 2014) as well as the guidelines set out by the Tri-Service General Hospital and are shown in Table 5-5.

Table 5-5. Exclusion criteria for identification of relatively healthy subjects within hospital population (as mentioned in the literature review section 2.5.1, Figure 2-7)

	Exclusion criteria
1	$FEV_1/FVC<70\%$
2	$FVC<80\%$
3	$TLC < 80\%$
4	$DL_{CO} < 80\%$, $DL_{CO} > 120\%$
5	Lung function diseases, including lung cancer, pneumonia, pneumothorax, emphysema and diseases that strongly affect lung function
6	History of diseases, including COPD, hypertension, hyperlipidaemia, anaemia, diabetes mellitus (DM), asthma, cardiovascular diseases and kidney diseases
7	If the patient attended the DL_{CO} test more than once during the study period, the researcher only kept the last record

However, no history of disease was shown in the TSGH PFT data set, so medical records had to be reviewed manually by the researcher. When reviewing the medical records, the researcher recorded factors related to pulmonary function, such as disease history, co-morbidity, level of Hb and the number of years the participants had smoked. To access this information, the researcher sought and was given permission to access the medical record system and read through the clinic visit notes, emergency room records, examination reports, X-ray reports and admission and discharge summaries. Therefore, patients could be recruited with pulmonary lung function that varied between individuals, but was within normal range for PFT; this group was further divided between those with and without a diagnosis of disease. The data generating process for Tri-Service General Hospital is shown in Figure 5-1.

After preliminary exclusions, three TSGH categories were identified for the research: 1,943 subjects in the overall group, of which 902 were in the TSGH group with normal PFT and 177 in the relatively healthy (i.e. no history of disease) group. All of the subjects in the groups attending TSGH were Asian. The different groups and factors in the groups are shown in Table 5-6.

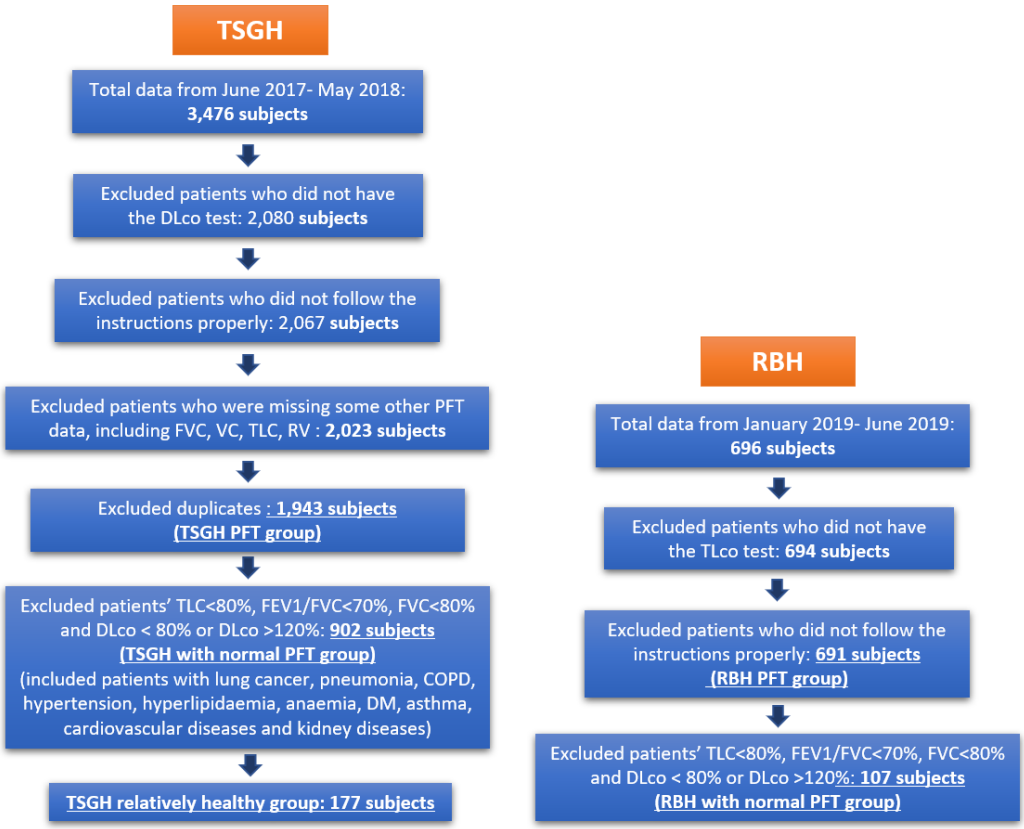

Figure 5-1. Data generating process for TSGH and RBH groups

Table 5-6. Different groups identified among individuals attending the Tri-Service General Hospital

	TSGH group with any PFT	**TSGH group with normal PFT**	**TSGH relatively healthy group**
No. subjects	1,943	902	177
Demographic, physiological and behavioural factors	✓	✓	✓
PFT data	✓	✓ (Normal range)	✓ (Normal range)
Diagnosis	✓	✓	✓
History of disease		✓	None

Case study 2: Royal Berkshire Hospital Recruitment

In the RBH dataset, the researcher sought selected data from patients admitted and tested for TL_{CO} using pulmonary function tests. We used pre-anonymised data of patients from January to June 2019.

The data generating process for the Royal Berkshire hospital is shown in Figure 5-1. After preliminary exclusions, there were 691 subjects in the RBH group with any PFT and further exclusions gave a total of 107 subjects in the RBH group with normal PFT. However, there was no history of diseases recorded in the RBH dataset. The different groups and factors in the groups are shown in Table 5-7.

Table 5-7. Different datasets for Royal Berkshire Hospital

	RBH group with any PFT	**RBH group with normal PFT**
Subjects	691	107
Demographic, physiological and behavioural factors	✓	✓
PFT data	✓	✓ (Normal range)
Diagnosis	✓	✓

5.2.3 Data collection from two hospitals and protocol for PFTs

This section covers data collection from TSGH and RBH hospitals and the protocol for the PFT test.

Data collecting process in two hospitals

All of the PFT data and medical records were gathered from the TSGH and the RBH. When the original data were collected, they were identifiable and held by the relevant hospital. To enable this research, each hospital anonymised its data, then transferred it to UCL to analyse. Figure 5-2 shows the process carried out by both hospitals.

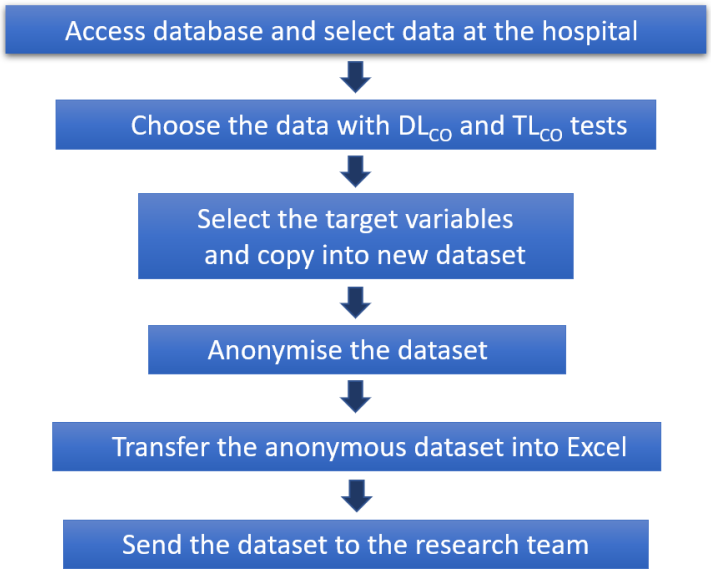

Figure 5-2. Flowchart of the data generating process followed by both hospitals.

The details of the process for collecting data from the two hospitals are given below.

Identification of datasets

- The researcher was permitted access to the pulmonary function test database and selected the relevant dataset. For TSGH, the researcher had access to the dataset from June 2017 to May 2018. For RBH, the researcher had access to the dataset from January 2019 to June 2019.
- From the datasets, the researcher selected only those patients who had taken either the DL_{CO} or the TL_{CO} test and created a new dataset.
- For this new dataset, the researcher selected certain variables including age, height, weight, sex, ethnicity, smoking status, Hb, diagnosis, disease history and PFT data. The variables selected were chosen according to the researcher's background knowledge of factors related to PFT data as discussed in Section 2.5 in the literature review.

Anonymising of patient information

- The datasets were anonymised in both hospitals.
- Only the secondary dataset, containing no identifiable information, was transferred to the UCL research group for analysis.

PFT-DL_{CO} (TLCO) test protocols in the hospitals

The PFTs could only be done by the clinic staff. DL_{CO} protocols were similar at the two hospitals. However, the pulmonary test machines were different: Tri-

Service General Hospital used the Carefusion Vmax Spectra PFT System (Vyaire Medical Inc., Accuracy: ±3%) and the Royal Berkshire Hospital the Masterscreen PFT System (Vyaire Medical Inc., Accuracy: ±2%): both machines are widely used in hospitals and research. The general instructions that the clinic staff gave to patients in both TSGH and RBH followed those suggested in Pellegrino et al. (2005) and Cheung and Cheung (2015):

'Please start with normal breathing. Then, I want you to take a big breath in and blow out empty, and as you do this, I will switch you to the test gas. After blowing out as much as possible, take the strongest, fullest breath that you can, hold it for ten seconds and then blow it out for me'.

The different machines reported different units of DL_{CO} (ml/min/mmHg) and TL_{CO} (mmol/min/kPa). Hence, the researcher converted all TL_{CO} measurements to the same equivalent DL_{CO} to enable comparison and analysis. The conversion factor equation is as follows (Graham et al., 2017):

$$DL_{CO}(\text{ml/min/mmHg}) \times 0.3348 = TL_{CO}(\text{mmol/min/kPa})$$

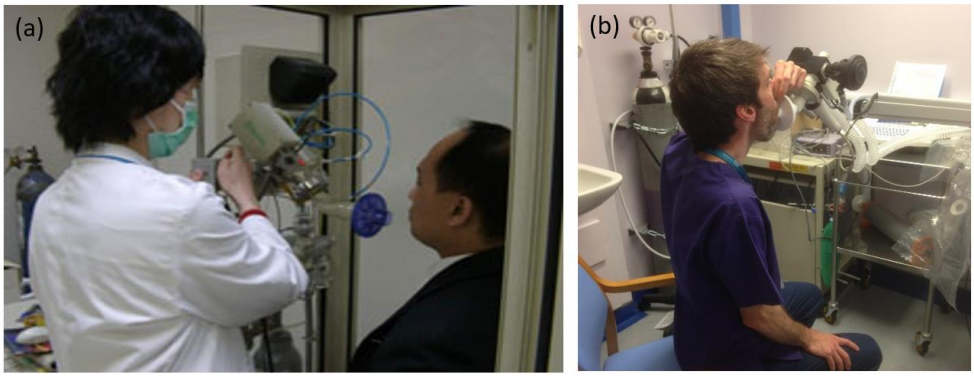

Figure 5-3. Pulmonary test machines in two hospitals: (a) body plethysmography technique used at TSGH and (b) helium dilution technique used at RBH

5.2.4 Data Analysis for PFT data

All data were recorded in Microsoft Excel and analysed using the software outlined in Section 4.2.5. The steps used to analyse the PFT data are presented in Figure 5-4.

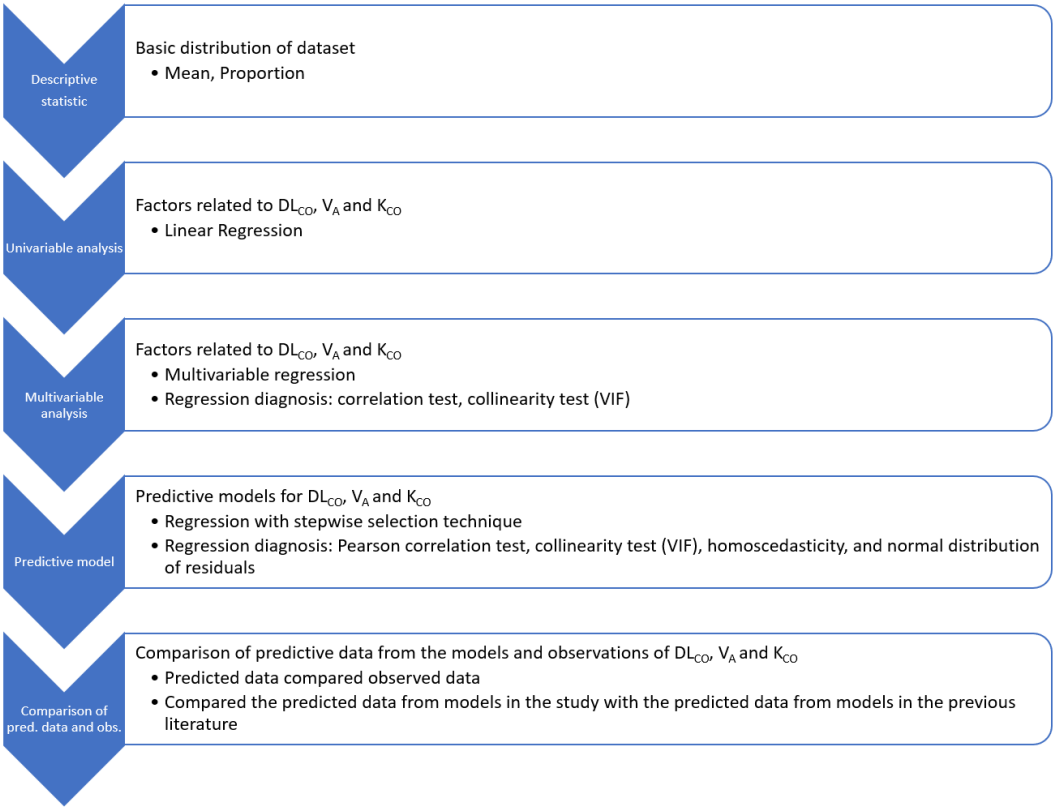

Figure 5-4. Analysis steps for the TSGH and RBH groups

First, the descriptive statistics were used to calculate the mean and proportion of the characteristics of the TSGH and RBH groups.

The univariable analysis was then run to understand the relationship between each variable and DL_{CO}, V_A and K_{CO}. A linear regression was applied to the univariable analysis (Kirkwood and Sterne, 2003) followed by a multivariable regression to investigate the factors related to DL_{CO}, V_A and K_{CO}.

After analysing the factors related to DL_{CO}, V_A and K_{CO}, the predictive models of DL_{CO}, V_A and K_{CO} for the TSGH 'relatively healthy' group and 'RBH group with normal PFT' were developed with demographic, physiological and behavioural factors that have shown a possible relationship with the two, including age, sex, height, weight, Hb and smoking status (Rankin et al., 1961; Anderson and Shephard, 1969; Vázquez-García et al., 2016). Also, the predictive models of DL_{CO}, V_A and K_{CO} were tested with all participants separately by smoking status and sex. Then, using the regression results, the most insignificant factor was excluded step by step. The composition of the final model was based on a combination of the literature, sample size, significant variables, adjusted R^2, SEE (standard error of the estimate), simplicity, ease of use and compliance with the assumptions of regression analysis,

including homoscedasticity and normal distribution of residuals (order of importance in the present study).

The predictive models for DL_{CO}, V_A and K_{CO} were validated by comparing predicted data for and observations of the TSGH and RBH populations in the study. The predicted data from the study were also compared with the data in previous literature.

5.3 Results

This section is separated into several sub-sections. The first gives an overview of the data (Section 5.3.1). The following three give an account of analyses of selected factors related to DL_{CO}, V_A and K_{CO} (Section 5.3.2 to Section 5.3.4). Section 5.3.5 presents the predictive models for DL_{CO}, V_A and K_{CO} based on the data gathered from the TSGH and RBH groups, before a comparison of the predicted data with data from the literature is given in Section 5.3.6.

5.3.1 Overview of the pulmonary function data

The two datasets, from the Tri-Service General Hospital (TSGH), Taiwan, which was Asian population, and the Royal Berkshire Hospital (RBH), UK, which was Caucasian population, are presented separately due to the different protocols and machines used in the PFTs. An overview of the distribution of age, sex, height, weight and smoking status in both groups is shown in Table 5-8.

Table 5-8. Basic characteristics overview of the TSGH and RBH groups

Variable	TSGH group with any PFT Mean± SD/ N (%)	RBH group with any PFT Mean± SD/ N (%)
No. subjects	1,943	691
Age [a] (year)	57.0 ±15.5	65.8 ±14.9
Sex [b]		
Female	769 (40)	340 (49)
Male	1174 (60)	351 (51)
Height [a] (cm)	164.7 ±8.6	169.1 ±9.8
Weight [a] (kg)	65.8 ±13.7	80.9 ±19.3
Smoking status [b]		
Ever smokers	734 (38)	411 (60)
Non-smokers	1209 (62)	275 (40)

Note: [a] Mean ± Standard Deviation, [b] N (%).

Attendees of the Tri-Service General Hospital (TSGH), Taiwan

For all TSGH groups, females comprised around 40% and males around 60%. Approximately 60% were non-smokers while those who had ever smoked at any point made up the remainder. The youngest average age was found in the TSGH 'relatively healthy group'. Average height, weight and BMI were similar in all three TSGH groups. Hb concentration and smoking period data were missing from the TSGH group with any PFT in the study, so are not given.

Table 5-9. Overview of characteristics and PFT parameters for attendees of the Tri-Service General Hospital (TSGH), Taiwan groups

Variable	TSGH group with any PFT	TSGH group with normal PFT	TSGH relatively healthy group
No. subjects	1,943	902	177
Age [a] (year)	57.0 ±15.5	54.1 ±15.0	46.9 ±15.2
Sex [b]			
Female	769 (40)	349 (39)	75 (42)
Male	1174 (60)	553 (61)	102 (58)
Height [a] (cm)	164.7 ±8.6	165.1 ±8.6	166.7 ±9.3
Weight [a] (kg)	65.8 ±13.7	65.5 ±12.6	65.4 ±12.7
BMI [a] (kg/m2)	24.2 ±4.3	23.9 ±3.7	23.3 ±3.4
Hb [a] (g/dL)	-	13.4 ±1.8	14.1 ±1.1
Smoking status [b]			
Current smokers	384 (20)	174 (20)	35 (20)
Ex-smokers	350 (18)	150 (18)	29 (16)
Non-smokers	1209 (62)	555 (62)	113 (64)
Period of smoking [a] (year)	-	27.6 ±13.2	20.9 ±10.3
FVC [a] (L)	2.9 ±0.9	3.3 ±0.8	3.6 ±0.9
FEV_1/FVC [a] (%)	79.8 ±9.6	80.0 ±8.1	81.5 ±7.8
VC [a] (L)	3.0 ±0.8	3.3 ±0.8	3.6 ±0.9
TLC [a] (L)	5.8 ±1.1	6.1 ±1.1	6.3 ±1.2
FRC [a] (L)	3.6 ±0.7	3.7 ±0.8	3.7 ±0.8
RV [a] (L)	2.9 ±0.7	2.8 ±0.6	2.6 ±0.6
RV/TLC [a] (%)	49.4 ±8.9	45.7 ±7.7	42.4 ±7.5
DL_{CO} [a] (mL/min/ mmHg)	17.9 ±5.9	20.3 ±5.0	22.3 ±5.1
DL_{CO} [c] (%)	89.3 ±21.3	98.5 ±10.6	100.6 ±11.1
V_A [a] (L)	4.5 ±0.9	4.9 ±0.9	5.1 ±0.9
V_A [c] (%)	90.3 ±17.6	96.2 ±13.5	96.9 ±12.5
K_{CO} [a] (mL/min/ mmHg/L)	3.9 ±0.9	4.1 ±0.7	4.4 ±0.7
K_{CO} [c] (%)	99.8 ±19.7	103.8 ±14.5	104.8 ±13.1

Note: [a] Mean ± Standard Deviation, [b] N (%), [c] percentage of the measured value compared to the reference value.

However, after exclusions (see Section 5.2.2), a slightly better pulmonary function was evident in all parameters in the TSGH group with normal PFT and the TSGH relatively healthy group in comparison with the TSGH group with any PFT. Generally, if the measured value is 80% to 120% of the reference value, then it is considered a normal value (Wyka et al., 2011), please see DL_{CO} (%), V_A (%) and K_{CO} (%).

After presenting the basic overview of the variables in the different groups, the following figures show the distributions of DL_{CO}, V_A and K_{CO}. The distributions show a similar pattern in the three groups. However, the TSGH relatively healthy group shows a higher average value of DL_{CO}, V_A and K_{CO} than the other two.

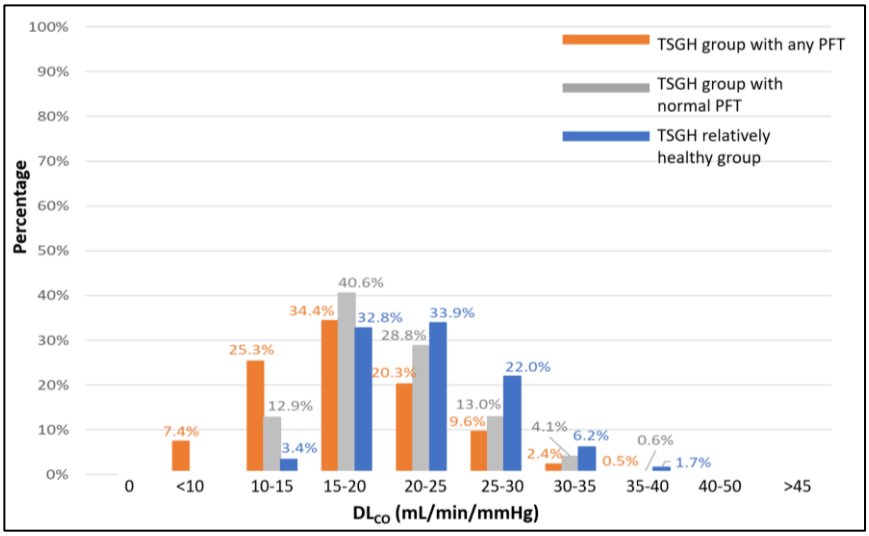

Figure 5-5. Distribution of DL_{CO} in the TSGH groups

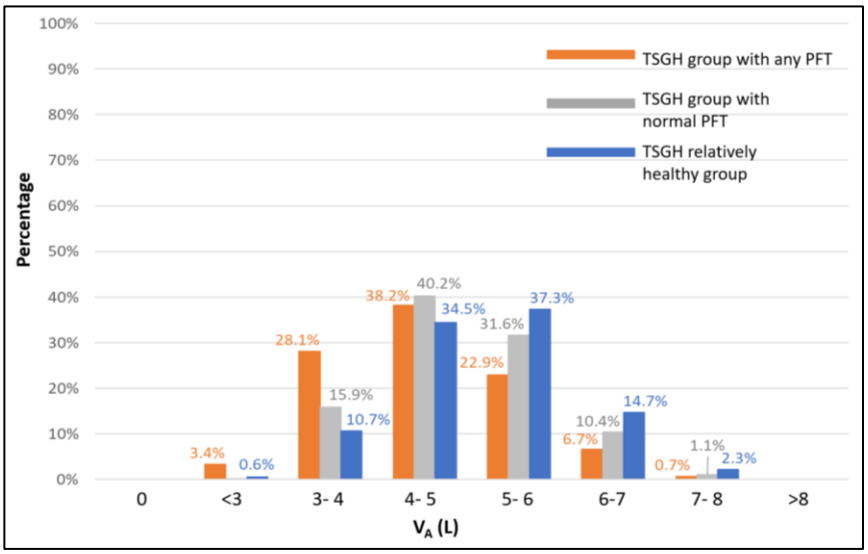

Figure 5-6. Distribution of V_A in the TSGH groups

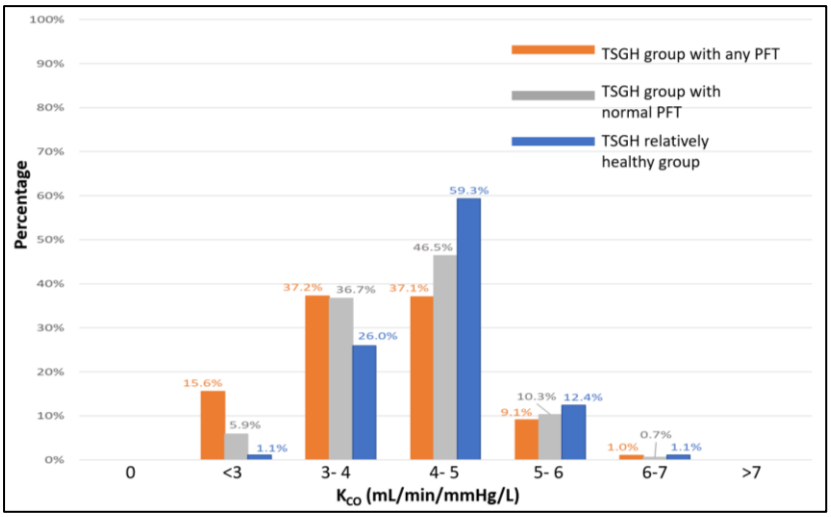

Figure 5-7. Distribution of K_{CO} in the TSGH groups

Attendees of the Royal Berkshire Hospital (RBH), UK

Table 5-10 shows the basic overview of the distribution of demographic, physiological and behavioural factors and PFT parameters in the RBH groups. We can see that the sub-set of those with 'normal' lung function are similar to the whole group in terms of sex and smoking status. Both groups comprised approximately 50/50 males and females. Non-smokers comprised approximately 40% to 50%, and those who had ever smoked were around 50% to 60%.

Table 5-10. Overview of characteristics and PFT parameters for attendees of the Royal Berkshire Hospital (RBH), UK groups

Variable	RBH group with any PFT	RBH group with normal PFT
Number of patients	691	107
Age [a] (year)	65.8 ±14.9	57.4 ±15.8
Sex [b]		
Female	340 (49)	45 (42)
Male	351 (51)	62 (58)
Height [a] (cm)	169.1 ±9.8	172.0 ±10.4
Weight [a] (kg)	80.9 ±19.3	85.9 ±18.6
BMI [a] (kg/m2)	28.3 ±6.6	28.9 ±5.6
Smoking status [b]		
Current smokers	58 (9)	5 (5)
Ex-smokers	353 (51)	47 (44)
Non-smokers	275 (40)	54 (51)
FVC [a] (L)	3.2 ±1.1	4.1 ±1.1
FEV_1/FVC [a] (%)	70.5 ±13.8	73.6 ±6.9
VC [a] (L)	3.3 ±1.1	4.2 ±1.2
TLC [a] (L)	5.3 ±1.5	6.4 ±1.4
FRC [a] (L)	2.7 ±0.9	3.0 ±0.7
RV [a] (L)	2.0 ±0.7	2.2 ±0.5

Variable	RBH group with any PFT	RBH group with normal PFT
RV/TLC [a] (%)	38.6 ±8.6	35.1 ±7.6
DL_{CO} [a] (mL/min/mmHg)	15.6 ±6.2	24.5 ±5.5
DL_{CO} [c] (%)	62.7 ±18.7	89.9 ±7.7
V_A [a] (L)	4.5 ±1.3	5.7 ±1.3
V_A [c] (%)	78.2 ±16.6	93.5 ±10.6
K_{CO} [a] (mL/min/ mmHg/L)	3.5 ±1.0	4.3 ±0.6
K_{CO} [c] (%)	82.9 ±20.1	99.8 ±13.2

Note: [a] Mean ± Standard Deviation, [b] N (%), [c] percentage of the measured value compared to the reference value.

The average age for the RBH group with any PFT was around 65. However, in the subgroup with normal PFT, the average age was around 57. As expected, the values of the pulmonary function parameters were higher in the RBH with normal PFT group. The following figures show the distribution of DL_{CO}, V_A and K_{CO}: the RBH group with normal PFT has higher averages than the RBH group with any PFT.

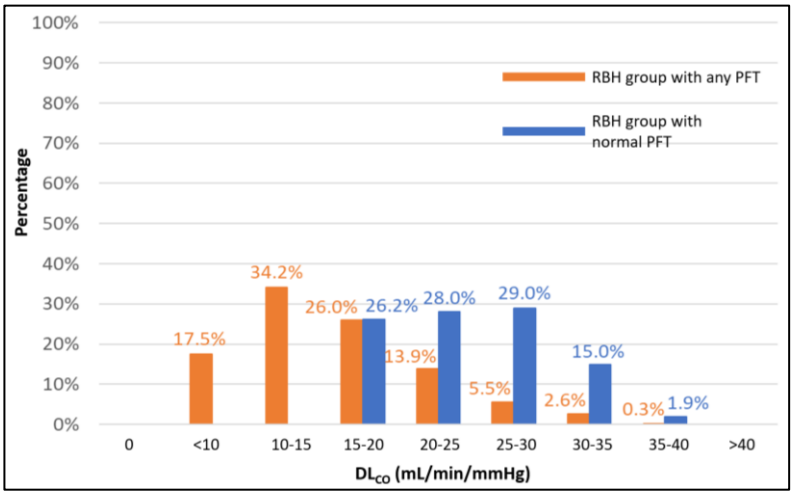

Figure 5-8. Distribution of DL_{CO} in the RBH groups

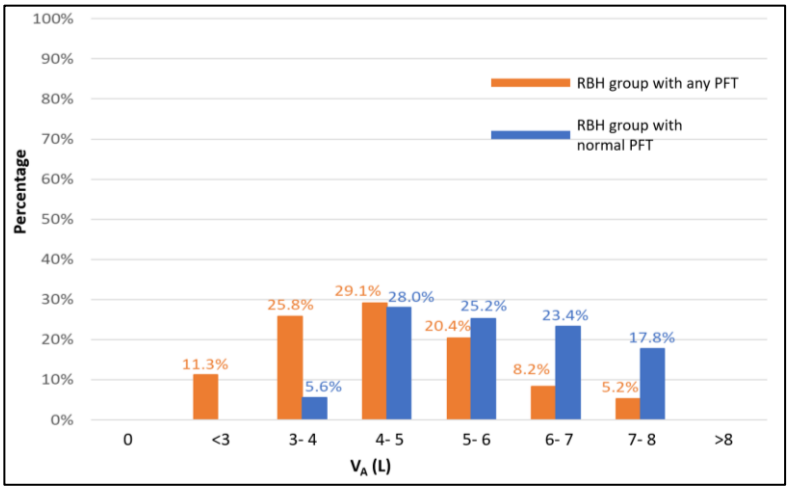

Figure 5-9. Distribution of V_A in the RBH groups

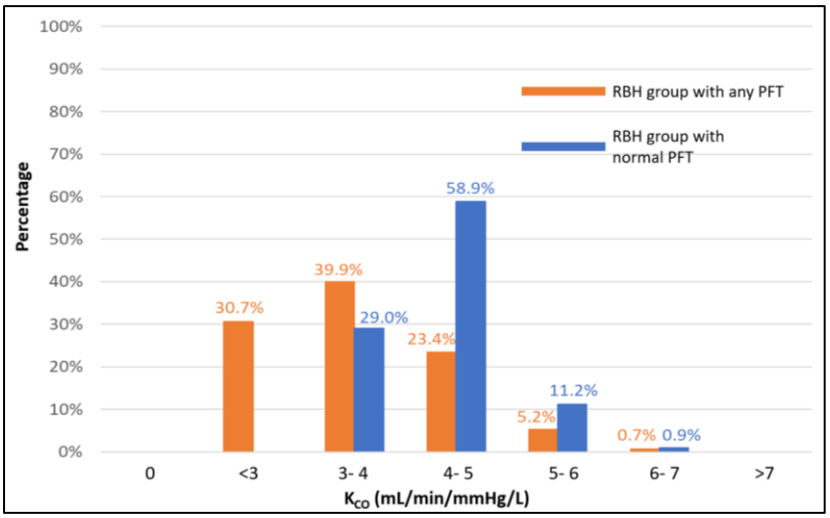

Figure 5-10. Distribution of K_{CO} in the RBH groups

5.3.2 Demographic, physiological and behavioural factors and PFT parameters affecting DL_{CO}

After understanding the basic characteristics and distribution of the variables of different groups, we explored the research question 'Which demographic, physiological and behavioural factors and PFT parameters have an effect on DL_{CO} and to what extent?' The following sub-sections show the univariable analysis and multivariable regression for different groups.

DL_{CO} predictors within the TSGH (Taiwan) group

TSGH group with any PFT

In the TSGH group with any PFT, in univariable analysis, the results showed a difference by sex – males had a higher value of DL_{CO} than females – and also that people with lung illness generally had a lower value of DL_{CO}. Additionally, the value of DL_{CO} had a positive association with height, weight, BMI and FEV_1/FVC and negative association with age, RV/TLC and FRC/TLC (see Table 5-11).

After understanding the distribution of the single variable, a multivariable analysis was conducted to investigate the factors affecting DL_{CO}. Before doing multivariable analysis, a collinearity test was carried out to consider the integration of each factor. In the multivariable regression of DL_{CO}, the variables of K_{CO} and V_A were excluded due to the over-specification. Moreover, due to a high correlation of pulmonary function parameters with each other, some parameters were clustered for analysis, such as FEV_1/FVC, FRC/TLC and RV/TLC. The parameters that were clustered were determined based on the literature (van der Lee et al., 2006; Kaminsky

et al., 2014; Yuan et al., 2014; Kendrick, 2015; Shin et al., 2015; Scarlata et al., 2010). However, FEV_1/FVC had a high correlation with FRC/TLC and RV/TLC, and the lung function parameters were highly correlated with age (Ren et al., 2012; Kendrick, 2015; Thomas et al., 2019). Also, BMI was excluded due to the high correlation with weight (see Appendix 9.2.3, Supplementary Table 9-15 and Supplementary Table 9-16). The same process was applied for all groups in Section 5.3.2 to Section 5.3.4.

Therefore, the final factors included in the multivariable analysis were age, sex, height, weight, smoking status, obesity, restrictive lung disease, obstructive lung disease and mixed obstructive and restrictive lung disease. Table 5-11 shows that any one, or a combination of people being older, have ever smoked, being obese, having restrictive lung disease, obstructive lung disease or mixed obstructive and restrictive lung disease, their DL_{CO} value will be lower. When all other variables are constant, if people are male, taller or heavier, then their DL_{CO} value will be higher.

TSGH group with normal PFT

Univariable analysis revealed that females, non-smokers and people with a history of particular diseases (e.g. anaemia, hypertension, hyperlipidaemia, DM, cardiovascular diseases and kidney disease) had a lower value of DL_{CO}. However, ever smokers showed a higher value of DL_{CO} than non-smokers although this may be due to the fact that the ever smokers group comprised more males than females. The analysis also showed similar results to the TSGH group with any PFT in that DL_{CO} had a positive association with height, weight, BMI, the concentration of Hb and FEV_1/FVC, and negative association with age, the period of smoking, RV/TLC and FRC/TLC (see Table 5-11). In multivariable analysis, after adjusting for possible factors, shows that if people are younger, taller, heavier, non-smokers or have a higher value of Hb, then their DL_{CO} value will be higher.

TSGH relatively healthy group

The univariable analysis results showed similar factors affecting DL_{CO} as in the TSGH group with normal PFT, such as sex and smoking status. Also, age, height, weight, BMI, Hb, FEV_1/FVC, RV/TLC and FRC/TLC are strongly related to DL_{CO}. However, in multivariable regression, shows that if people are male, younger, taller or heavier, they have a higher value of DL_{CO} (see Table 5-11). Although the results showed that Hb concentration significantly affected DL_{CO}, this was not included in

the multivariable regression due to the small sample size (this also applies for the predictive models of V_A and K_{CO} in the next sections).

DL_{CO} predictors within the RBH (UK) group

RBH group with any PFT

In the RBH group with any PFT, the univariable analysis found similar factors affecting DL_{CO}, such as sex, smoking status, lung illness, age, height, weight, BMI, RV/TL and FRC/TLC as with the TSGH group with any PFT (see Table 5-11). After adjusting for possible factors, Table 5-11 shows that if people are older, have ever smoked or have restrictive lung disease or mixed obstructive and restrictive lung disease, their DL_{CO} value will be lower. If people are male, taller or heavier, then their DL_{CO} value will be higher.

RBH group with normal PFT

In univariable analysis, the results showed that sex, age, height, weight, BMI, Hb, RV/TLC and FRC/TLC are strongly linked with DL_{CO}. After adjusting for possible factors, Table 5-11 shows that if people are younger, male or taller, their DL_{CO} value will be higher.

Table 5-11. Factors affecting DL_{CO} analysed by univariable and multivariable linear regression of the TSGH and RBH groups

Variable	Univariable analysis					Multivariable analysis				
	β [a]	95% CI [b]	Beta [c]	p-value	R^2	β [a]	95% CI [b]	Beta [c]	p-value	R^2
TSGH group with any PFT (n^d=1,943; n^f=1,943)										**0.628**
Age (year)	**-0.239**	**(-2.253, -0.226)**	**-0.628**	**<0.001*** **	**0.394**	**-0.198**	**(-0.210, -0.186)**	**-0.520**	**<0.001*** **	
Sex (male)	**3.385**	**(2.870, 3.900)**	**0.281**	**<0.001*** **	**0.078**	**1.494**	**(0.996, 1.992)**	**0.124**	**<0.001*** **	
Height (cm)	**0.344**	**(0.317, 0.370)**	**0.501**	**<0.001*** **	**0.251**	**0.169**	**(0.137, 0.201)**	**0.246**	**<0.001*** **	
Weight (kg)	**0.144**	**(0.126, 0.162)**	**0.334**	**<0.001*** **	**0.111**	**0.075**	**(0.056, 0.093)**	**0.173**	**<0.001*** **	
BMI (kg/m^2)	**0.130**	**(0.069, 0.192)**	**0.094**	**<0.001*** **	**0.009**	-	-	-	-	
Obesity (yes)	0.757	(-0.192, 1.706)	0.036	0.118	0.001	**-1.493**	**(-2.257, -0.728)**	**-0.390**	**<0.001*** **	
Ever smoker	0.201	(-0.340, 0.742)	0.017	0.467	0.0003	**-1.667**	**(-2.036, -1.299)**	**-0.137**	**<0.001*** **	
FEV_1/FVC (%)	**0.140**	**(0.113, 0.167)**	**0.228**	**<0.001*** **	**0.052**	-	-	-	-	
RV/TLC (%)	**-0.417**	**(-0.440, -0.394)**	**-0.627**	**<0.001*** **	**0.394**	-	-	-	-	
FRC/TLC (%)	**-0.267**	**(-0.294, -0.241)**	**-0.411**	**<0.001*** **	**0.169**	-	-	-	-	
Restrictive lung disease (yes)	**-2.887**	**(-3.450, -2.323)**	**-0.222**	**<0.001*** **	**0.049**	**-3.134**	**(-3.506, -2.762)**	**-0.241**	**<0.001*** **	
Obstructive lung disease (yes)	**-2.000**	**(-2.935, -1.065)**	**-0.095**	**<0.001*** **	**0.009**	**-1.716**	**(-2.321, -1.111)**	**-0.081**	**<0.001*** **	
Mixed lung disease [d] (yes)	**-4.809**	**(-5.966, -3.652)**	**-0.182**	**<0.001*** **	**0.033**	**-4.259**	**(-5.010, -3.508)**	**-0.161**	**<0.001*** **	
TSGH group with normal PFT (n^d=902; n^f=291)										**0.778**
Age (year)	**-0.227**	**(-0.243, -0.212)**	**-0.687**	**<0.001*** **	**0.472**	**-0.180**	**(-0.201, -0.158)**	**-0.555**	**<0.001*** **	
Sex (male)	**3.829**	**(3.209, 4.449)**	**0.375**	**<0.001*** **	**0.140**	0.568	(-0.281, 1.416)	0.060	0.189	
Height (cm)	**0.377**	**(0.349, 0.406)**	**0.654**	**<0.001*** **	**0.427**	**0.150**	**(0.096, 0.203)**	**0.273**	**<0.001*** **	
Weight (kg)	**0.216**	**(0.195, 0.238)**	**0.548**	**<0.001*** **	**0.300**	**0.122**	**(0.089, 0.156)**	**0.332**	**<0.001*** **	
BMI (kg/m^2)	**0.324**	**(0.239, 0.409)**	**0.242**	**<0.001*** **	**0.059**	-	-	-	-	
Obesity (yes)	**2.029**	**(0.638, 3.420)**	**0.095**	**0.004****	**0.009**	0.082	(-1.237, 1.400)	0.004	0.903	

Variable	Univariable analysis					Multivariable analysis				
	β [a]	95% CI [b]	Beta [c]	p-value	R^2	β [a]	95% CI [b]	Beta [c]	p-value	R^2
Hb (g/dL, n=374)	**0.966**	**(0.726, 1.205)**	**0.428**	**<0.001*** **	**0.145**	**0.240**	**(0.090, 0.390)**	**0.099**	**0.002** **	
Ever smoker	**0.953**	**(0.286, 1.620)**	**0.093**	**0.005** **	**0.009**	**-0.616**	**(-1.210, -0.022)**	**-0.066**	**0.042***	
Period of smoking (year, n=137)	**-0.199**	**(-0.253, -0.146)**	**-0.535**	**<0.001*** **	**0.286**	-	-	-	-	
FEV_1/FVC (%)	**0.138**	**(0.099, 0.177)**	**0.225**	**<0.001*** **	**0.051**	-	-	-	-	
RV/TLC (%)	**-0.435**	**(-0.466, -0.404)**	**-0.675**	**<0.001*** **	**0.455**	-	-	-	-	
FRC/TLC (%)	**-0.226**	**(-0.262, -0.191)**	**-0.384**	**<0.001*** **	**0.147**	-	-	-	-	
Anaemia	**-2.224**	**(-3.249, -1.198)**	**-0.216**	**<0.001*** **	**0.047**	-	-	-	-	
Hypertension	**-2.348**	**(-3.247, -1.449)**	**-0.203**	**<0.001*** **	**0.041**	-0.046	(-0.721, 0.629)	-0.005	0.894	
Hyperlipidaemia	**-6.641**	**(-2.930, -0.352)**	**-0.101**	**0.013***	**0.010**	-0.467	(-1.381, 0.447)	-0.030	0.316	
Diabetes mellitus	**-1.880**	**(-3.289, -0.472)**	**-0.106**	**0.009** **	**0.011**	0.424	(-0.547, 1.395)	0.027	0.391	
Cardiovascular diseases	**-2.598**	**(-3.893, -1.302)**	**-0.159**	**<0.001*** **	**0.025**	-0.551	(-1.589, 0.488)	-0.034	0.297	
Kidney diseases	**-3.061**	**(-5.803, -0.319)**	**-0.090**	**0.029***	**0.008**	-0.421	(-1.891, 1.049)	-0.017	0.573	
TSGH relatively healthy group (n^d=177; n^f=177)										**0.776**
Age (year)	**-0.200**	**(-0.240, -0.159)**	**-0.590**	**<0.001*** **	**0.348**	**-0.187**	**(-0.212, -0.161)**	**-0.552**	**<0.001*** **	
Sex (male)	**5.938**	**(4.673, 7.202)**	**0.573**	**<0.001*** **	**0.329**	**2.729**	**(1.558, 3.899)**	**0.264**	**<0.001*** **	
Height (cm)	**0.384**	**(0.326, 0.443)**	**0.700**	**<0.001*** **	**0.489**	**0.116**	**(0.040, 0.192)**	**0.211**	**0.003** **	
Weight (kg)	**0.224**	**(0.174, 0.274)**	**0.557**	**<0.001*** **	**0.310**	**0.115**	**(0.069, 0.161)**	**0.286**	**<0.001*** **	
BMI (kg/m^2)	**0.341**	**(0.126, 0.557)**	**0.230**	**0.002** **	**0.053**	-	-	-	-	
Obesity (yes)	0.746	(-3.166, 4.659)	0.028	0.707	0.001	-1.209	(-3.355, 0.936)	-0.046	0.267	
Hb (g/dL, n=53)	**2.349**	**(1.307, 3.391)**	**0.535**	**<0.001*** **	**0.287**	-	-	-	-	
Ever smoker	**1.696**	**(0.129, 3.264)**	**0.159**	**0.034***	**0.025**	-0.387	(-1.199, 0.424)	-0.036	0.348	
Period of smoking (yr, n=18)	-0.115	(-0.335, 0.104)	-0.268	0.282	0.072	-	-	-	-	
FEV_1/FVC (%)	0.070	(-0.027, 0.168)	0.107	0.157	0.011	-	-	-	-	

Variable	Univariable analysis					Multivariable analysis				
	β [a]	95% CI [b]	Beta [c]	p-value	R^2	β [a]	95% CI [b]	Beta [c]	p-value	R^2
RV/TLC (%)	**-0.426**	**(-0.506, -0.347)**	**-0.624**	**<0.001*** **	**0.390**	-	-	-	-	
FRC/TLC (%)	**-0.242**	**(-0.327, -0.158)**	**-0.393**	**<0.001*** **	**0.154**	-	-	-	-	
RBH group with any PFT (n^d=691; n^f=686)										**0.595**
Age (year)	**-0.242**	**(-0.268, -0.217)**	**-0.579**	**<0.001*** **	**0.335**	**-0.209**	**(-0.231, -0.188)**	**-0.501**	**<0.001*** **	
Sex (male)	**3.138**	**(2.238, 4.038)**	**0.252**	**<0.001*** **	**0.064**	**1.552**	**(0.690, 2.414)**	**0.125**	**<0.001*** **	
Height (cm)	**0.286**	**(0.243, 0.328)**	**0.450**	**<0.001*** **	**0.203**	**0.179**	**(0.131, 0.227)**	**0.282**	**<0.001*** **	
Weight (kg)	**0.105**	**(0.082, 0.128)**	**0.327**	**<0.001*** **	**0.107**	**0.036**	**(0.010, 0.063)**	**0.113**	**0.008****	
BMI (kg/m^2)	**0.101**	**(0.031, 0.171)**	**0.107**	**0.005****	**0.012**	-	-	-	-	
Obesity (yes)	**1.003**	**(0.017, 1.989)**	**0.076**	**0.046***	**0.006**	0.153	(-0.876, 1.183)	0.012	0.770	
Ever smoker	**-1.632**	**(-2.577, -0.688)**	**-0.129**	**0.001****	**0.017**	**-1.874**	**(-2.510, -1.238)**	**-0.148**	**<0.001*** **	
FEV_1/FVC (%)	0.031	(-0.003, 0.065)	0.069	0.070†	0.005	-	-	-	-	
RV/TLC (%)	**-0.282**	**(-0.331, -0.232)**	**-0.391**	**<0.001*** **	**0.153**	-	-	-	-	
FRC/TLC (%)	**-0.275**	**(-0.325, -0.225)**	**-0.379**	**<0.001*** **	**0.144**	-	-	-	-	
Restrictive lung disease (yes)	**-3.854**	**(-5.150, -2.559)**	**-0.217**	**<0.001*** **	**0.047**	**-4.800**	**(-5.705, -3.896)**	**-0.271**	**<0.001*** **	
Obstructive lung disease (yes)	0.483	(-0.538, 1.505)	0.035	0.353	0.001	-0.128	(-0.857, 0.602)	-0.009	0.731	
Mixed lung disease [d] (yes)	**-3.887**	**(-5.538, -2.236)**	**-0.173**	**<0.001*** **	**0.030**	**-3.803**	**(-4.951, -2.654)**	**-0.169**	**<0.001*** **	
RBH group with normal PFT (n^d=107; n^f=106)										**0.866**
Age (year)	**-0.212**	**(-0.265, -0.158)**	**-0.609**	**<0.001*** **	**0.370**	**-0.168**	**(-0.195, -0.141)**	**-0.481**	**<0.001*** **	
Sex (male)	**7.100**	**(5.460, 8.741)**	**0.642**	**<0.001*** **	**0.412**	**3.664**	**(2.515, 4.813)**	**0.330**	**<0.001*** **	
Height (cm)	**0.420**	**(0, 359, 0.482)**	**0.798**	**<0.001*** **	**0.637**	**0.211**	**(0.141, 0.281)**	**0.398**	**<0.001*** **	
Weight (kg)	**0.137**	**(0.086, 0.187)**	**0.463**	**<0.001*** **	**0.214**	0.025	(-0.016, 0.065)	0.084	0.230	
BMI (kg/m^2)	0.037	(-0.154, 0.227)	0.037	0.703	0.001	-	-	-	-	
Obesity (yes)	-0.263	(-2.426, 1.899)	-0.024	0.810	0.001	-0.459	(-1.762, 0.844)	-0.041	0.486	

Variable	Univariable analysis					Multivariable analysis				
	β [a]	**95% CI [b]**	**Beta [c]**	**p-value**	**R^2**	**β [a]**	**95% CI [b]**	**Beta [c]**	**p-value**	**R^2**
Ever smoker	0.240	(-1.891, 2.372)	0.022	0.823	0.001	-0.388	(-1.217, 0.442)	-0.035	0.356	
FEV_1/FVC (%)	0.088	(-0.064, 0.240)	0.111	0.255	0.123	-	-	-	-	
RV/TLC (%)	**-0.431**	**(-0.542, -0.319)**	**-0.600**	**<0.001*** **	**0.360**	-	-	-	-	
FRC/TLC (%)	**-0.295**	**(-0.444, -0.147)**	**-0.359**	**<0.001*** **	**0.129**	-	-	-	-	

*Note: ordered by the absolute value of Beta, where a significant factor was found, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1, [a] β (un-standardised coefficient), [b] Beta (standardised coefficient), [c] CI (confidence interval), n^d (sample size for univariable analysis), n^f (sample size for multivariable analysis).*

A summary table of factors affecting DL_{CO} is presented below. Both the TSGH and RBH groups showed a very similar pattern of factors affecting DL_{CO}. Age and height also showed a significant effect through all groups.

Table 5-12. Summary of factors affecting DL_{CO} in the TSGH (Taiwan) and RBH (UK) hospitals

	TSGH group with any PFT	**TSGH group with normal PFT**	**TSGH relatively healthy group**	**RBH group with any PFT**	**RBH group with normal PFT**
Subjects	1943	902	177	691	107
Age	negative	negative	negative	negative	negative
Sex (male)	positive	No effect	positive	positive	positive
Height	positive	positive	positive	positive	positive
Weight	positive	positive	positive	positive	No effect
Hb	-	positive	-	-	-
Ever smoker	negative	negative	No effect	negative	No effect
Obesity	negative	No effect	No effect	No effect	No effect
Restrictive lung disease	negative	-	-	negative	
Obstructive lung disease	negative	-	-	No effect	-
Mixed lung disease [a]	negative	-	-	negative	-

Note: positive=positive relationship with DL_{CO}; negative=negative relationship with DL_{CO}, which means if the independent variable is higher, then the dependent variable is lower, e.g. DL_{CO} declines with age, [a] Mixed obstructive and restrictive lung disease

5.3.3 Demographic, physiological and behavioural factors and PFT parameters affecting V_A

The relationships between V_A and variables were analysed to answer the research question 'Which factors are most closely associated with V_A and to what extent?' The following sub-sections show the variation of V_A with demographic, physiological and behavioural factors and diseases (categorical variables) in five different groups as in Section 5.3.2.

V_A predictors within the TSGH (Taiwan) group

TSGH group with any PFT

In univariable analysis, the results showed that males had a higher V_A value than females and ever smokers had a higher value of V_A than non-smokers, and people with lung illness generally had a lower value. Also, the value of V_A had a positive association with height and weight, and a negative association with age, BMI, RV/TLC and FRC/TLC (see Table 5-14). After showing the distribution of the single variable, Table 5-14 shows that if people are older, heavier, have a restrictive lung disease or mixed obstructive and restrictive lung disease, then their V_A value will be lower. If people are male or taller, their V_A value will be higher.

TSGH group with normal PFT

Univariable analysis showed that females, non-smokers, and people with a history of disease (e.g. hypertension and hyperlipidaemia) had a lower value of V_A. However, ever smokers had a higher value than non-smokers, possibly because there were more males in the ever smokers' group. The analysis also showed similar results to the TSGH group with any PFT in that V_A had a positive association with height, weight and Hb concentration, and a negative association with age, period of smoking, RV/TLC and FRC/TLC. However, in multivariable regression, Table 5-14 shows that if people are younger, male or taller, their V_A value will be higher.

TSGH relatively healthy group

In univariable analysis, the results showed similar factors affecting V_A as in the TSGH group with normal PFT, such as sex, age, height, weight, Hb, smoking status, RV/TLC and FRC/TLC. However, in multivariable regression, Table 5-14 shows that male, younger or taller people had higher V_A values.

V_A predictors within the RBH (UK) group

RBH group with any PFT

Univariable analysis results showed similar factors (sex, age, height, weight, BMI, obesity, lung illness, FEV_1/FVC, RV/TLC and FRC/TLC) affecting V_A as in the TSGH group with any PFT (see Table 5-14). In multivariable analysis, Table 5-14 shows that if people are older, have restrictive lung disease or have mixed obstructive

and restrictive lung disease, then their V_A value is lower. If people are male, taller or have obstructive lung disease, their V_A value is higher.

RBH group with normal PFT

Univariable analysis results showed that sex, age, height, weight, RV/TLC and FRC/TLC affect V_A. In multivariable regression, Table 5-14 shows that if people are younger, male, taller or have a history of smoking, their V_A value was higher.

In summary, all the TSGH and RBH groups showed a very similar pattern. There is a big difference between males and females in that males have a higher V_A. Also, younger and taller people had a higher value of V_A than older and shorter ones.

Table 5-13. Summary of the factors related to V_A in the TSGH (Taiwan) and RBH (UK) hospitals

	TSGH group with any PFT	**TSGH group with normal PFT**	**TSGH relatively healthy group**	**RBH group with any PFT**	**RBH group with normal PFT**
Subjects	1943	902	177	691	107
Age	negative	negative	negative	negative	negative
Sex (male)	positive	positive	positive	positive	positive
Height	positive	positive	positive	positive	positive
Weight	negative	No effect	No effect	No effect	No effect
Ever smoker	No effect	No effect	No effect	No effect	positive
Restrictive lung disease	negative	-	-	negative	-
Obstructive lung disease	No effect	-	-	positive	-
Mixed lung disease [a]	negative	-	-	negative	-

Note: positive=positive relationship with V_A; negative=negative relationship with V_A, which means if the independent variable is higher, then the dependent variable is lower, e.g. V_A declines with age, [a] Mixed obstructive and restrictive lung disease.

Table 5-14. Factors affecting V_A as analysed by univariable and multivariable linear regression of the TSGH and RBH groups

Variable	Univariable analysis					Multivariable analysis				
	β [a]	95% CI [b]	Beta [c]	p-value	R^2	β [a]	95% CI [b]	Beta [c]	p-value	R^2
TSGH group with any PFT (n^d=1,943; n^f=1,943)										**0.651**
Age (year)	**-0.020**	**(-0.023, -0.017)**	**-0.332**	**<0.001*** **	**0.111**	**-0.011**	**(-0.012, -0.009)**	**-0.174**	**<0.001*** **	
Sex (male)	**1.046**	**(0.973, 1.118)**	**0.540**	**<0.001*** **	**0.292**	**0.367**	**(0.289, 0.444)**	**0.189**	**<0.001*** **	
Height (cm)	**0.075**	**(0.071, 0.078)**	**0.678**	**<0.001*** **	**0.459**	**0.062**	**(0.057, 0.067)**	**0.560**	**<0.001*** **	
Weight (kg)	**0.021**	**(0.018, 0.024)**	**0.299**	**<0.001*** **	**0.089**	**-0.006**	**(-0.009, -0.003)**	**-0.082**	**<0.001*** **	
BMI (kg/m^2)	**-0.011**	**(-0.021, -0.001)**	**-0.048**	**0.033***	**0.002**	-	-	-	-	
Obesity (yes)	**-2.280**	**(-4.433, -0.129)**	**-0.082**	**<0.001*** **	**0.007**	-0.074	(-0.192, 0.045)	-0.021	0.225	
Ever smoker	**0.481**	**(0.397, 0.566)**	**0.246**	**<0.001*** **	**0.061**	-0.008	(-0.066, 0.049)	-0.004	0.779	
FEV_1/FVC (%)	-0.002	(-0.006, 0.003)	-0.018	0.419	0.0003	**-**	**-**	**-**	**-**	
RV/TLC (%)	**-0.049**	**(-0.054, -0.045)**	**-0.463**	**<0.001*** **	**0.215**	**-**	**-**		**-**	
FRC/TLC (%)	**-0.025**	**(-0.030, -0.020)**	**-0.239**	**<0.001*** **	**0.057**	**-**	**-**	**-**	**-**	
Restrictive lung disease (yes)	**-0.714**	**(-0.802, -0.627)**	**-0.343**	**<0.001*** **	**0.117**	**-0.719**	**(-0.777, -0.661)**	**-0.345**	**<0.001*** **	
Obstructive lung disease (yes)	**0.307**	**(0.157, 0.458)**	**0.091**	**<0.001*** **	**0.008**	-0.024	(-0.118, 0.070)	-0.007	0.621	
Mixed lung disease [d] (yes)	**-0.453**	**(-0.641, -0.265)**	**-0.107**	**<0.001*** **	**0.011**	**-0.724**	**(-0.841, -0.607)**	**-0.171**	**<0.001*** **	
TSGH group with normal PFT (n^d=902; n^f=291)										**0.708**
Age (year)	**-0.018**	**(-0.022, -0.014)**	**-0.308**	**<0.001*** **	**0.095**	**-0.009**	**(-0.013, -0.004)**	**-0.146**	**0.001**	
Sex (male)	**1.100**	**(1.007, 1.193)**	**0.611**	**<0.001*** **	**0.374**	**0.419**	**(0.237, 0.602)**	**0.237**	**<0.001*** **	
Height (cm)	**0.082**	**(0.078, 0.086)**	**0.804**	**<0.001*** **	**0.647**	**0.062**	**(0.051, 0.074)**	**0.608**	**<0.001*** **	
Weight (kg)	**0.034**	**(0.030, 0.038)**	**0.492**	**<0.001*** **	**0.242**	-0.0004	(-0.008, 0.007)	-0.006	0.914	
BMI (kg/m^2)	**0.018**	**(0.002, 0.033)**	**0.075**	**0.025***	**0.006**	**-**	**-**	**-**	**-**	

Variable	Univariable analysis					Multivariable analysis				
	β [a]	95% CI [b]	Beta [c]	p-value	R^2	β [a]	95% CI [b]	Beta [c]	p-value	R^2
Obesity (yes)	-0.120	(-0.365, 0.126)	-0.032	0.340	0.001	-0.170	(-0.454, 0.114)	-0.047	0.240	
Hb (g/dL, n=374)	**0.131**	**(0.086, 0.176)**	**0.310**	**<0.001*** **	**0.081**	0.001	(-0.031, 0.034)	0.003	0.940	
Ever smoker	**0.542**	**(0.429, 0.654)**	**0.301**	**<0.001*** **	**0.090**	0.098	(-0.030, 0.227)	0.056	0.132	
Period of smoking (year, n=137)	-0.006	(-0.016, 0.004)	**-0.102**	**0.234**	0.011	-	-	-	-	
FEV_1/FVC (%)	-0.005	(-0.012, 0.002)	**-0.046**	**0.164**	0.002	-	-	-	-	
RV/TLC (%)	**-0.052**	**(-0.058, -0.045)**	**-0.457**	**<0.001*** **	**0.209**	-	-	-	-	
FRC/TLC (%)	**-0.021**	**(-0.028, -0.014)**	**-0.203**	**<0.001*** **	**0.041**	-	-	-	-	
Anaemia	-0.026	(-0.216, 0.164)	-0.014	0.789	0.0002	-	-	-	-	
Hypertension	**-0.216**	**(-0.375, -0.057)**	**-0.108**	**0.008****	**0.012**	-0.002	(-0.147, 0.144)	-0.001	0.983	
Hyperlipidaemia	**-0.337**	**(-0.563, -0.111)**	**-0.118**	**0.004****	**0.014**	-0.128	(-0.325, 0.069)	-0.044	0.203	
Diabetes mellitus	-0.087	(-0.335, 0.161)	-0.028	0.490	0.001	-0.079	(-0.289, 0.130)	-0.027	0.457	
Cardiovascular diseases	-0.164	(-0.394, 0.065)	-0.057	0.160	0.003	-0.007	(-0.231, 0.217)	-0.002	0.949	
Kidney diseases	-0.429	(-0.938, 0.079)	-0.068	0.098†	0.005	-0.136	(-0.453, 0.180)	-0.029	0.397	
TSGH relatively healthy group (n^d=177; n^f=177)										**0.722**
Age (year)	**-0.016**	**(-0.025, -0.007)**	**-0.263**	**<0.001*** **	**0.069**	**-0.009**	**(-0.014, -0.004)**	**-0.148**	**0.001****	
Sex (male)	**1.355**	**(1.163, 1.546)**	**0.726**	**<0.001*** **	**0.527**	**0.593**	**(0.358, 0.859)**	**0.318**	**<0.001*** **	
Height (cm)	**0.081**	**(0.073, 0.090)**	**0.818**	**<0.001*** **	**0.670**	**0.060**	**(0.045, 0.075)**	**0.607**	**<0.001*** **	
Weight (kg)	**0.038**	**(0.029, 0.047)**	**0.521**	**<0.001*** **	**0.272**	-0.005	(-0.014, 0.004)	-0.071	0.268	
BMI (kg/m^2)	0.024	(-0.015, 0.064)	0.091	0.228	0.008	-	-	-	-	
Obesity (yes)	-0.181	(-0.887, 0.524)	-0.038	0.612	0.002	0.066	(-0.365, 0.497)	0.014	0.763	
Hb (g/dL, n=53)	**0.543**	**(0.375, 0.712)**	**0.672**	**<0.001*** **	**0.452**	-	-	-	-	
Ever smoker	**0.477**	**(0.200, 0.755)**	**0.249**	**<0.001*** **	**0.062**	-0.052	(-0.215, 0.111)	-0.027	0.533	
Period of smoking (yr, n=18)	-0.012	(-0.053, 0.029)	-0.155	0.540	0.024	-	-	-	-	

Variable	Univariable analysis					Multivariable analysis				
	β [a]	95% CI [b]	Beta [c]	p-value	R^2	β [a]	95% CI [b]	Beta [c]	p-value	R^2
FEV_1/FVC (%)	-0.014	(-0.032, 0.003)	-0.119	0.113	0.014	-	-	-	-	
RV/TLC (%)	**-0.058**	**(-0.074, -0.041)**	**-0.467**	**<0.001*** **	**0.218**	-	-	-	-	
FRC/TLC (%)	**-0.030**	**(-0.046, -0.014)**	**-0.272**	**<0.001*** **	**0.074**	-	-	-	-	
RBH group with any PFT (n^d=691; n^f=686)										**0.741**
Age (year)	**-0.024**	**(-0.031, -0.018)**	**-0.274**	**<0.001*** **	**0.075**	**-0.021**	**(-0.024, -0.017)**	**-0.235**	**<0.001*** **	
Sex (male)	**1.366**	**(1.199, 1.533)**	**0.521**	**<0.001*** **	**0.272**	**0.670**	**(0.554, 0.845)**	**0.267**	**<0.001*** **	
Height (cm)	**0.090**	**(0.083, 0.098)**	**0.676**	**<0.001*** **	**0.457**	**0.066**	**(0.057, 0.074)**	**0.491**	**<0.001*** **	
Weight (kg)	**0.013**	**(0.008, 0.018)**	**0.193**	**<0.001*** **	**0.037**	-0.004	(-0.009, 0.00002)	-0.066	0.051	
BMI (kg/m^2)	**-0.028**	**(-0.043, -0.013)**	**-0.142**	**<0.001*** **	**0.020**	-	-	-	-	
Obesity (yes)	**-0.416**	**(-0.622, -0.211)**	**-0.150**	**<0.001*** **	**0.022**	-0.007	(-0.181, 0.166)	-0.003	0.934	
Ever smoker	0.129	(-0.072, 0.329)	0.048	0.208	0.002	-0.098	(-0.205, 0.009)	-0.037	0.071	
FEV_1/FVC (%)	**-0.021**	**(-0.027, -0.014)**	**-0.216**	**<0.001*** **	**0.047**	-	-	-	-	
RV/TLC (%)	**-0.050**	**(-0.060, -0.039)**	**-0.328**	**<0.001*** **	**0.108**	-	-	-	-	
FRC/TLC (%)	**-0.022**	**(-0.033, -0.011)**	**-0.144**	**<0.001*** **	**0.021**	-	-	-	-	
Restrictive lung disease (yes)	**-1.356**	**(-1.617, -1.096)**	**-0.262**	**<0.001*** **	**0.132**	**-1.452**	**(-1.605, -1.300)**	**-0.390**	**<0.001*** **	
Obstructive lung disease (yes)	**0.877**	**(0.673, 1.082)**	**0.305**	**<0.001*** **	**0.093**	**0.357**	**(0.234, 0.480)**	**0.124**	**<0.001*** **	
Mixed lung disease [d] (yes)	**-0.874**	**(-0.221, -0.527)**	**-0.185**	**<0.001*** **	**0.034**	**-1.009**	**(-1.202, -1.815)**	**-0.213**	**<0.001*** **	
RBH group with normal PFT (n^d=107; n^f=106)										**0.796**
Age (year)	**-0.024**	**(-0.038, -0.009)**	**-0.296**	**0.002****	**0.088**	**-0.008**	**(-0.016, -0.001)**	**-0.104**	**0.035***	
Sex (male)	**1.696**	**(1.333, 2.056)**	**0.671**	**<0.001*** **	**0.450**	**0.372**	**(0.049, 0.695)**	**0.147**	**0.024***	
Height (cm)	**0.104**	**(0.092, 0.116)**	**0.864**	**<0.001*** **	**0.747**	**0.096**	**(0.076, 0.115)**	**0.794**	**<0.001*** **	
Weight (kg)	**0.025**	**(0.013, 0.037)**	**0.373**	**<0.001*** **	**0.139**	-0.008	(-0.019, 0.004)	-0.114	0.186	

Variable	Univariable analysis					Multivariable analysis				
	β [a]	**95% CI [b]**	**Beta [c]**	**p-value**	**R^2**	**β [a]**	**95% CI [b]**	**Beta [c]**	**p-value**	**R^2**
BMI (kg/m^2)	-0.023	(-0.066, 0.020)	-0.103	0.292	0.011	-	-	-	-	
Obesity (yes)	-0.299	(-0.790, 0.193)	-0.117	0.231	0.014	-0.057	(-0.423, 0.309)	-0.022	0.758	
Ever smoker	0.352	(-0.128, 0.833)	0.141	0.149	0.020	**0.372**	**(0.139, 0.605)**	**0.149**	**0.002****	
FEV_1/FVC (%)	-0.025	(-0.060, 0.009)	-0.139	0.152	0.019	-	-	-	-	
RV/TLC (%)	**-0.069**	**(-0.098, -0.041)**	**-0.423**	**<0.001*****	**0.179**	-	-	-	-	
FRC/TLC (%)	**-0.037**	**(-0.073, -0.002)**	**-0.199**	**0.040***	**0.040**	-	-	-	-	

*Note: ordered by the absolute value of Beta, where a significant factor was found, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1, [a] β (un-standardised coefficient), [b] Beta (standardised coefficient), [c] CI (confidence interval), n^d (sample size for univariable analysis), n^f (sample size for multivariable analysis).*

5.3.4 Demographic, physiological and behavioural factors and PFT parameters affecting K_{CO}

In this section, the relationship between K_{CO}, a measure of how well lungs diffuse CO into the bloodstream normalised by lung volume, and various variables is investigated. K_{CO}, normalising DL_{CO} by lung capacity (V_A), gives a better measure of the efficiency of the lung tissue itself in diffusing CO. Although the K_{CO} is derived from DL_{CO} and V_A, it is still worth analysing. K_{CO} has its own clinical implication, which is the index of gas exchange efficiency. It is also an important parameter when diagnosing pulmonary function. Therefore, it is worth exploring and analysing which factor affects the K_{CO} directly.

K_{CO} predictors within the TSGH (Taiwan) group

TSGH group with any PFT

The univariable analysis results showed that age has the greatest effect on reducing K_{CO}. Table 5-16 shows that if people are older, taller, have ever smoked, are obese, have obstructive lung disease, restrictive lung disease or mixed obstructive and restrictive lung disease, their K_{CO} value will be lower. If people are heavier, then their K_{CO} value will be higher.

TSGH group with normal PFT

The univariable analysis, the results were similar to those of the TSGH group with any PFT in that K_{CO} had a positive association with height, weight, BMI, FEV_1/FVC and Hb concentration, and a negative association with age, the period of smoking, RV/TLC and FRC/TLC. Table 5-16 shows that if people are older, male, taller or have a history of smoking, their K_{CO} value will be lower. If people are heavier or have a higher Hb concentration, then their K_{CO} value will be higher.

TSGH relatively healthy group

In univariable analysis, only age, RV/TLC and FRC/TLC showed a negative relationship with K_{CO}; weight, BMI and FEV_1/FVC showed a positive relationship. In multivariable regression (see Table 5-16), age and height had a negative relationship with K_{CO}, while weight showed a positive relationship with K_{CO}.

K_{CO} predictors within the RBH (UK) group

RBH group with any PFT

The results from the univariable analysis were similar to the results of the TSGH group with any PFT. Table 5-16 shows that if people are male, older, taller, have ever smoked or have obstructive disease, their K_{CO} value will be lower. If people are heavier, then their K_{CO} value will be higher.

RBH group with normal PFT

After adjusting for possible factors in the RBH group with normal PFT, Table 5-16 shows that if people are older, taller or have ever smoked, their K_{CO} value will be lower. If people are male or heavier, their K_{CO} value will be higher.

In summary, we can see that similar patterns are found in all the TSGH and RBH groups. Also, age, height and weight showed a significant effect through all groups.

Table 5-15. Summary of the factors related to K_{CO} in the TSGH (Taiwan) and RBH (UK) hospitals

	TSGH group with any PFT	**TSGH group with normal PFT**	**TSGH relatively healthy group**	**RBH group with any PFT**	**RBH group with normal PFT**
Subjects	1943	902	177	691	107
Age	negative	negative	negative	negative	negative
Sex (male)	No effect	negative	No effect	negative	positive
Height	negative	negative	negative	negative	negative
Weight	positive	positive	positive	positive	positive
Hb	-	positive	No effect	-	-
Ever smoker	negative	negative	No effect	negative	negative
Obesity	negative	No effect	No effect	No effect	No effect
Restrictive lung disease	negative	-	-	No effect	-
Obstructive lung disease	negative	-	-	negative	-
Mixed lung disease [a]	negative	-	-	No effect	-

Note: positive=positive relationship with K_{CO}; negative=negative relationship with K_{CO}, which means if the independent variable is higher, then the dependent variable is lower, e.g. K_{CO} declines with age, [a] Mixed obstructive and restrictive lung disease.

Table 5-16. Factors affecting K_{CO} as analysed by univariable and multivariable linear regression of the TSGH and RBH groups

Variable	Univariable analysis					Multivariable analysis				
	β [a]	95% CI [b]	Beta [c]	p-value	R^2	β [a]	95% CI [b]	Beta [c]	p-value	R^2
TSGH group with any PFT (n^d=1,943; n^f=1,943)										**0.450**
Age (year)	**-0.035**	**(-0.037, -0.033)**	**-0.589**	**<0.001*** **	**0.346**	**-0.034**	**(-0.036,-0.031)**	**-0.571**	**<0.001*** **	
Sex (male)	**-0.199**	**(-0.282, -0.117)**	**-0.107**	**<0.001*** **	**0.011**	-0.047	(-0.141, 0.046)	-0.025	0.320	
Height (cm)	**0.009**	**(0.005, 0.014)**	**0.088**	**<0.001*** **	**0.008**	**-0.015**	**(-0.021,-0.009)**	**-0.143**	**<0.001*** **	
Weight (kg)	**0.013**	**(0.010, 0.016)**	**0.194**	**<0.001*** **	**0.038**	**0.021**	**(0.017, 0.024)**	**0.309**	**<0.001*** **	
BMI (kg/m^2)	**0.037**	**(0.028, 0.04)**	**0.175**	**<0.001*** **	**0.031**	-	-	-	-	
Obesity (yes)	**0.417**	**(0.271, 0.562)**	**0.127**	**<0.001*** **	**0.016**	**-0.230**	**(-0.373,-0.086)**	**-0.070**	**0.002****	
Ever smoker	**-0.362**	**(-0.444, -0.280)**	**-0.193**	**<0.001*** **	**0.037**	**-0.327**	**(-0.396,-0.258)**	**-0.174**	**<0.001*** **	
FEV_1/FVC (%)	**0.032**	**(0.029, 0.036)**	**0.342**	**<0.001*** **	**0.117**	-	-	-	-	
RV/TLC (%)	**-0.048**	**(-0.052, -0.044)**	**-0.470**	**<0.001*** **	**0.221**	-	-	-	-	
FRC/TLC (%)	**-0.038**	**(-0.042, -0.034)**	**-0.376**	**<0.001*** **	**0.141**	-	-	-	-	
Restrictive lung disease (yes)	-0.028	(-0.117, 0.061)	-0.014	0.542	0.0002	**-0.088**	**(-0.157,-0.018)**	**-0.044**	**0.014***	
Obstructive lung disease (yes)	**-0.668**	**(-0.810, 0.526)**	**-0.205**	**<0.001*** **	**0.042**	**-0.330**	**(-0.444,-0.217)**	**-0.101**	**<0.001*** **	
Mixed lung disease [d] (yes)	**-0.746**	**(-0.924, -0.567)**	**-0.183**	**<0.001*** **	**0.033**	**-0.398**	**(-0.539,-0.257)**	**-0.072**	**<0.001*** **	
TSGH group with normal PFT (n^d=902; n^f=291)										**0.562**
Age (year)	**-0.030**	**(-0.033, -0.028)**	**-0.630**	**<0.001*** **	**0.397**	**-0.028**	**(-0.033,-0.024)**	**-0.565**	**<0.001*** **	
Sex (male)	**-0.183**	**(-0.279, -0.086)**	**-0.123**	**<0.001*** **	**0.015**	**-0.291**	**(-0.478,-0.104)**	**-0.197**	**0.002****	
Height (cm)	**0.006**	**(0.001, 0.012)**	**0.075**	**0.024***	**0.006**	**-0.021**	**(-0.032,-0.009)**	**-0.240**	**0.001****	
Weight (kg)	**0.015**	**(0.011, 0.018)**	**0.253**	**<0.001*** **	**0.064**	**0.025**	**(0.018, 0.032)**	**0.432**	**<0.001*** **	
BMI (kg/m^2)	**0.052**	**(0.039, 0.064)**	**0.264**	**<0.001*** **	**0.070**	-	-	-	-	
Obesity (yes)	**0.517**	**(0.316, 0.718)**	**0.166**	**<0.001*** **	**0.028**	0.203	(-0.087, 0.493)	0.067	0.169	

Variable	Univariable analysis					Multivariable analysis				
	β [a]	95% CI [b]	Beta [c]	p-value	R^2	β [a]	95% CI [b]	Beta [c]	p-value	R^2
Hb (g/dL, n=374)	**0.084**	**(0.044, 0.123)**	**0.238**	**<0.001*** **	**0.045**	**0.044**	**(0.011, 0.077)**	**0.116**	**0.009** **	
Ever smoker	**-0.255**	**(-0.352, -0.159)**	**-0.171**	**<0.001*** **	**0.029**	**-0.171**	**(-0.302,-0.040)**	**-0.116**	**0.011***	
Period of smoking (year, n=137)	**-0.034**	**(-0.042, -0.026)**	**-0.586**	**<0.001*** **	**0.344**	-	-	-	-	
FEV_1/FVC (%)	**0.031**	**(0.026, 0.037)**	**0.351**	**<0.001*** **	**0.123**	-	-	-		
RV/TLC (%)	**-0.044**	**(-0.049, -0.038)**	**-0.465**	**<0.001*** **	**0.216**	-	-	-	-	
FRC/TLC (%)	**-0.028**	**(-0.033, -0.023)**	**-0.328**	**<0.001*** **	**0.108**	-	-	-	-	
Anaemia	**-0.425**	**(-0.583, -0.267)**	**-0.265**	**<0.001*** **	**0.070**	-	-	-	-	
Hypertension	**-0.285**	**(-0.416, -0.155)**	**-0.172**	**<0.001*** **	**0.029**	-0.007	(-0.156, 0.141)	-0.005	0.924	
Hyperlipidaemia	-0.032	(-0.219, 0.155)	-0.014	0.737	0.0002	0.019	(-0.182, 0.220)	0.008	0.854	
Diabetes mellitus	**-0.311**	**(-0.514, -0.108)**	**-0.122**	**0.003** **	**0.015**	0.133	(-0.080, 0.347)	0.055	0.221	
Cardiovascular diseases	**-0.384**	**(-0.570, -0.197)**	**-0.163**	**<0.001*** **	**0.027**	-0.122	(-0.350, 0.107)	-0.049	0.296	
Kidney diseases	-0.208	(-0.603, 0.187)	0.301	-0.043	0.002	0.098	(-0.225, 0.422)	0.025	0.551	
TSGH relatively healthy group (n^d=177; n^f=177)										**0.455**
Age (year)	**-0.025**	**(-0.031, -0.020)**	**-0.577**	**<0.001*** **	**0.333**	**-0.029**	**(-0.034,-0.024)**	**-0.655**	**<0.001*** **	
Sex (male)	-0.003	(-0.205, 0.198)	-0.002	0.976	<0.001	0.005	(-0.234, 0.243)	0.003	0.970	
Height (cm)	0.006	(-0.005, 0.017)	0.084	0.265	0.007	**-0.029**	**(-0.044,-0.013)**	**-0.398**	**<0.001*** **	
Weight (kg)	**0.012**	**(0.004, 0.019)**	**0.225**	**0.003** **	**0.051**	**0.028**	**(0.018, 0.037)**	**0.523**	**<0.001*** **	
BMI (kg/m^2)	**0.048**	**(0.019, 0.076)**	**0.245**	**0.001** **	**0.060**	-	-	-	-	
Obesity (yes)	0.314	(-0.194, 0.823)	0.092	0.224	0.008	-0.291	(-0.728, 0.147)	-0.085	0.191	
Hb (g/dL, n=53)	-0.021	(-0.184, 0.142)	-0.036	0.799	0.001	-	-	-	-	
Ever smoker	-0.064	(-0.271, 0.143)	-0.046	0.543	0.002	-0.017	(-0.182, 0.149)	-0.012	0.841	
Period of smoking (yr, n=18)	-0.013	(-0.032, 0.007)	-0.330	0.180	0.109	-	-	-	-	
FEV_1/FVC (%)	**0.026**	**(0.014, 0.039)**	**0.307**	**<0.001*** **	**0.094**	-	-	-	-	

Variable	Univariable analysis					Multivariable analysis				
	β [a]	95% CI [b]	Beta [c]	p-value	R^2	β [a]	95% CI [b]	Beta [c]	p-value	R^2
RV/TLC (%)	**-0.035**	**(-0.047, -0.023)**	**-0.393**	**<0.001*** **	**0.154**	-	-	-	-	
FRC/TLC (%)	**-0.022**	**(-0.033, -0.010)**	**-0.269**	**<0.001*** **	**0.072**	-	-	-	-	
RBH group with any PFT (n^d=691; n^f=686)										**0.404**
Age (year)	**-0.034**	**(-0.038, -0.029)**	**-0.512**	**<0.001*** **	**0.262**	**-0.029**	**(-0.033,-0.025)**	**-0.446**	**<0.001*** **	
Sex (male)	**-0.391**	**(-0.534, -0.248)**	**-0.200**	**<0.001*** **	**0.040**	**-0.236**	**(-0.400,-0.072)**	**-0.121**	**0.005****	
Height (cm)	**-0.008**	**(-0.015, -0.0003)**	**-0.078**	**0.041***	**0.006**	**-0.013**	**(-0.022,-0.004)**	**-0.130**	**0.005****	
Weight (kg)	**0.013**	**(0.010, 0.017)**	**0.266**	**<0.001*** **	**0.071**	**0.014**	**(0.008, 0.019)**	**0.267**	**<0.001*** **	
BMI (kg/m^2)	**0.047**	**(0.036, 0.057)**	**0.315**	**<0.001*** **	**0.099**	-	-	-	-	
Obesity (yes)	**0.549**	**(0.399, 0.698)**	**0.264**	**<0.001*** **	**0.070**	-0.0002	(-0.196, 0.196)	-0.0001	0.998	
Ever smoker	**-0.495**	**(-0.640, -0.350)**	**-0.248**	**<0.001*** **	**0.061**	**-0.385**	**(-0.507,-0.264)**	**-0.193**	**<0.001*** **	
FEV_1/FVC (%)	**0.020**	**(0.015, 0.025)**	**0.284**	**<0.001*** **	**0.081**	-	-	-	-	
RV/TLC (%)	**-0.020**	**(-0.028, -0.011)**	**-0.173**	**<0.001*** **	**0.030**	-	-	-	-	
FRC/TLC (%)	**-0.040**	**(-0.048, -0.032)**	**-0.353**	**<0.001*** **	**0.125**	-	-	-	-	
Restrictive lung disease (yes)	**0.256**	**(0.049, 0.464)**	**0.092**	**0.016***	**0.009**	0.169	(-0.003, 0.341)	0.061	0.054	
Obstructive lung disease (yes)	**-0.468**	**(-0.625, -0.311)**	**-0.218**	**<0.001*** **	**0.047**	**-0.166**	**(-0.305,-0.027)**	**-0.077**	**0.020***	
Mixed lung disease [d] (yes)	-0.219	(-0.482, 0.044)	-0.062	0.103	0.004	-0.031	(-0.249, 0.188)	0.009	0.784	
RBH group with normal PFT (n^d=107; n^f=106)										**0.796**
Age (year)	**-0.020**	**(-0.026, -0.013)**	**-0.512**	**<0.001*** **	**0.262**	**-0.023**	**(-0.029,-0.016)**	**-0.590**	**<0.001*** **	
Sex (male)	-0.019	(-0.254, 0.216)	-0.016	0.874	0.0002	**0.303**	**(0.035, 0.569)**	**0.248**	**0.027***	
Height (cm)	-0.002	(-0.013, 0.009)	-0.037	0.709	0.001	**-0.030**	**(-0.046,-0.014)**	**-0.519**	**<0.001*** **	
Weight (kg)	0.006	(-0.0003, 0.012)	0.181	0.062†	0.033	**0.010**	**(0.0004, 0.019)**	**0.303**	**0.042***	

Variable	Univariable analysis					Multivariable analysis				
	β [a]	**95% CI [b]**	**Beta [c]**	**p-value**	**R^2**	**β [a]**	**95% CI [b]**	**Beta [c]**	**p-value**	**R^2**
BMI (kg/m^2)	**0.025**	**(0.004, 0.045)**	**0.227**	**0.019***	**0.051**	**-**	**-**	**-**	**-**	
Obesity (yes)	0.184	(-0.051, 0.419)	0.149	0.124	0.022	-0.027	(-0.330, 0.275)	-0.022	0.858	
Ever smoker	-0.182	(-0.413, 0.049)	-0.151	0.122	0.023	**-0.298**	**(-0.491,-0.105)**	**-0.248**	**0.003****	
FEV_1/FVC (%)	**0.032**	**(0.016, 0.048)**	**0.368**	**<0.001*****	**0.135**	**-**	**-**	**-**	**-**	
RV/TLC (%)	**-0.024**	**(-0.038, -0.009)**	**-0.299**	**0.002****	**0.089**	**-**	**-**	**-**	**-**	
FRC/TLC (%)	**-0.022**	**(-0.039, -0.005)**	**-0.245**	**0.011***	**0.060**	**-**	**-**	**-**	**-**	

*Note: ordered by the absolute value of Beta, where a significant factor was found, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1, [a] β (un-standardised coefficient), [b] Beta (standardised coefficient), [c] CI (confidence interval), n^d (sample size for univariable analysis), n^f (sample size for multivariable analysis).*

5.3.5 Predictive models for DL_{CO}, V_A and K_{CO} in the TSGH relatively healthy group and RBH group with normal PFT

After determining the factors related to DL_{CO}, V_A and K_{CO} in the TSGH and RBH groups, the predictive models for DL_{CO}, V_A and K_{CO} were explored based on simple demographic, physiological and behavioural factors. Each model was considered with R^2, SEE, significant parameters, the sample size and then assessed for clinical and biological plausibility.

History of disease and pulmonary function parameters were excluded as the model were focused on the relatively healthy group and only use simple demographic, physiological and behavioural variables to predict DL_{CO}, V_A and K_{CO}. The importance of the models is to estimate the diffusion coefficient (DL_{CO}) for CO poisoned patients. This value could then be used to backcast to determine the concentration and duration of CO exposure. It might also be used to calculate a treatment regime to purge CO from their bodies more quickly. The aim would be to use simple demographic, physiological and behavioural variables, which are easy to obtain when patients arrive at the hospitals.

Certain variables were excluded from the model, such as the period of smoking (high correlation with smoking status and also due to missing data) and BMI (high correlation to weight).

Predictive models for DL_{CO}

Predictive models were run for DL_{CO} for all participants, including smokers/non-smokers and females/males.

TSGH relatively healthy group

The variables included sex, age, height, weight, Hb and smoking status (ever smokers or non-smokers), which were based on the literature (Talaminos Barroso et al., 2018). Then, multivariate linear regression models were constructed using a stepwise selection technique for all participants in the TSGH relatively healthy group (see Table 5-17).

After analysing Regression A, B and C, their adjusted R^2s were 0.833, 0.768 and 0.768 respectively. The final model, Regression C, was selected by adjusted R^2, SEE and significant parameters, and considering the sample size. The final model used for all participants in TSGH relatively group was,

$$DLco = 2.269 + 2.695 \times Sex(female: 0, male: 1) - 0.186 \times Age(yr) + 0.122 \times Height(cm) + 0.104 \times weight(kg)$$

Table 5-17. Predictive models for DL_{CO} of all participants in the TSGH relatively healthy group

	Regression A	**Regression B**	**Regression C**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**
Sex (male)	**2.426*** **(0.165, 4.686)**	**2.729*** **(1.558, 3.900)**	**2.695*** **(1.526, 3.864)**
Age	**-0.185*** **(-0.229, -0.141)**	**-0.185*** **(-0.211, -0.159)**	**-0.186*** **(-0.212, -0.160)**
Height	**0.160** **(0.052, 0.268)**	**0.129** **(0.056, 0.202)**	**0.122** **(0.051, 0.193)**
Weight	0.061† (-0.001, 0.123)	**0.103*** **(0.063, 0.143)**	**0.104*** **(0.064, 0.144)**
Hb	0.075 (-0.809, 0.959)	excluded	excluded
Smoking status	-1.291† (-2.695, 0.112)	-0.395 (-1.207, 0.417)	excluded
Intercept	-1.817 (-21.901, 0.112)	1.295 (-9.772, 12.361)	2.269 (-8.612, 13.150)
N	53	177	177
SEE	2.038	2.469	2.468
R^2	0.853	0.775	0.774
Adjusted R^2	0.833	0.768	0.768

*Note: *Where a significant difference was found in DL_{CO} value, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*
Regression A included variables: sex, age, height, weight, Hb and smoking status
Regression B included variables: sex, age, height, weight and smoking status
Regression C included variables: sex, age, height and weight

Please see Table 5-17 as an example. The same processes were then applied for different factors (V_A and K_{CO}) and subsets (smokers and non-smokers, males and females) as shown below (see Appendix 9.2.3 for details).

The predictive model of DL_{CO} for all participants, non-smokers and smokers, females and males in the TSGH relatively healthy group is presented in Table 5-18. The predictive models for non-smokers and smokers, males and females in TSGH relatively healthy group are shown below:

For non-smokers: $$DLco = -2.612 + 2.760 \times \text{Sex}(\text{female}: 0, \text{male}: 1) - 0.168 \times Age(yr) + 0.149 \times Height(cm) + 0.099 \times Weight(kg)$$

For ever smokers: $$DLco = 21.626 + 3.499 \times \text{Sex}(\text{female}: 0, \text{male}: 1) - 0.246 \times Age(yr) + 0.155 \times Weight(kg)$$

For females: $\text{DLco} = -3.089 - 0.121 \times \text{Age(yr)} + 0.131 \times \text{Height(cm)} + 0.119 \times \text{weight(kg)}$

For males: $\text{DLco} = 6.607 - 0.216 \times \text{Age(yr)} + 0.119 \times \text{Height(cm)} + 0.107 \times \text{weight(kg)}$

RBH group with normal PFT

Table 5-18 shows the predictive models of DL_{CO} for each group in the RBH group with normal PFT and the equations below show the predictive models for non-smokers and smokers, males and females in the RBH group with normal PFT,

The final model used for all participants: $DLco = -9.213 + 3.537 \times Gender(female: 0, male: 1) - 0.168 \times Age(yr) + 0.240 \times Height(cm)$

For non-smokers: $DLco = -15.446 + 3.087 \times \text{Sex(female: 0, male: 1)} - 0.149 \times Age(yr) + 0.272 \times Height(cm)$

For ever smokers: $DLco = -1.366 + 4.201 \times \text{Sex(female: 0, male: 1)} - 0.189 \times Age(yr) + 0.183 \times Height(cm) + 0.029 \times Weight(kg)$

For females: $\text{DLco} = -10.651 - 0.142 \times \text{Age(yr)} + 0.240 \times \text{Height(cm)}$

For males: $\text{DLco} = -4.963 - 0.189 \times \text{Age(yr)} + 0.243 \times \text{Height(cm)}$

After obtaining the predictive model for DL_{CO}, the DL_{CO} values for different sexes, ages, and smoking status were calculated by using the predictive models for ever smokers and non-smokers (see Table 5-19). To show the effects of age, three age points were included: 20, 40 and 60. Height and weight were set as 174 cm and 71 kg for males, and 159 cm and 54 kg for females, the values of the 50% percentiles for height and weight in the TSGH relatively healthy group. The results showed that in both groups, even though the ever smokers have a higher value of DL_{CO} than non-smokers at 20 years of age, their DL_{CO} decreased more than non-smokers at 60. DL_{CO} showed a higher value in the RBH group than in the TSGH group through all the different sexes, ages and smoking status.

Table 5-18. Predictive models for DL_{CO} in TSGH relatively healthy group and RBH group with normal PFT

	All participants	**All participants**		**All participants**	
		Non-smokers	**Smokers**	**Females**	**Males**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
TSGH relatively healthy group					
Sex (male)	**2.695*** (1.526, 3.864)**	**2.760*** (1.414, 4.106)**	**3.499* (1.688, 5.329)**	-	-
Age	**-0.186*** (-0.212, -0.160)**	**-0.168*** (-0.198, -0.139)**	**-0.246*** (-0.293, -0.198)**	**-0.121*** (-0.159, -0.083)**	**-0.216*** (-0.249, -0.182)**
Height	**0.122** (0.051, 0.193)**	**0.149* (0.063, 0.236)**	excluded	**0.131* (0.033, 0.230)**	**0.119* (0.024, 0.215)**
Weight	**0.104*** (0.064, 0.144)**	**0.099*** (0.051, 0.146)**	**0.155** (0.091, 0.218)**	**0.119*** (0.067, 0.172)**	**0.107*** (0.051, 0.163)**
Hb	excluded	excluded	excluded	excluded	excluded
Smoking status	excluded	-	-	excluded	excluded
Intercept	2.269 (-8.612, 13.150)	-2.612 (-15.838, 10.614)	21.626 (17.548, 25.704)	-3.089 (-18.188, 12.009)	6.607 (-9.109, 22.324)
N	177	113	64	75	102
SEE	2.468	2.370	2.607	2.042	2.628
R^2	0.774	0.795	0.737	0.648	0.701
Adjusted R^2	0.768	0.787	0.724	0.633	0.692
RBH group with normal PFT					
Sex (male)	**3.537*** (2.420, 4.654)**	**3.087*** (1.513, 4.661)**	**4.201*** (2.518, 5.884)**	-	-
Age	**-0.168*** (-0.195, -0.141)**	**-0.149*** (-0.185, -0.112)**	**-0.189*** (-0.231, -0.147)**	**-0.142*** (-0.172, -0.113)**	**-0.189*** (-0.231, -0.148)**
Height	**0.240*** (0.185, 0.295)**	**0.272*** (0.063, 0.236)**	**0.183*** (0.094, 0.272)**	**0.240*** (0.176, 0.308)**	**0.243*** (0.160, 0.325)**

	All participants	All participants		All participants	
		Non-smokers	Smokers	Females	Males
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Weight	excluded	excluded	0.029† (-0.005, 0.063)	excluded	excluded
Smoking status	excluded	-	-	excluded	excluded
Intercept	-9.213† (-18.859, 0.433)	-15.466* (-29.384, -1.547)	-1.366 (-15.857, 13.125)	-10.651† (-21.918, 0.615)	-4.963 (-20.490, 10.563)
N	106	54	52	45	62
SEE	2.058	1.973	2.131	1.498	2.353
R^2	0.863	0.895	0.839	0.851	0.737
Adjusted R^2	0.859	0.889	0.825	0.844	0.728

*Note: Where a significant difference was found in DL_{CO} value, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

Table 5-19. DL_{CO} for different smoking status, sexes and ages

Smoking status	Sex	Age (year)	DL_{CO} (mL/min/mmHg)	
			TSGH	RBH
Non-smoker	male	20	29.7	31.9
		40	26.3	28.9
		60	23.0	25.9
	female	20	23.1	24.8
		40	19.7	21.8
		60	16.4	18.9
Smoker	male	20	31.2	32.9
		40	26.3	29.1
		60	21.4	25.3
	female	20	25.1	25.5
		40	20.2	21.7
		60	15.2	18.0

Predictive models for V_A

After building the predictive models for DL_{CO}, the predictive models for V_A were run with the same processes as above.

TSGH relatively healthy group

The predictive models of V_A for each group in the TSGH relatively group are presented in Table 5-21 and below.

For all participants: $V_A = -3.917 + 0.576 \times Gender(female: 0, male: 1) - 0.010 \times Age(yr) + 0.055 \times Height(cm)$

For non-smokers: $V_A = -3.564 + 0.642 \times \text{Sex}(\text{female}: 0, \text{male}: 1) - 0.009 \times Age(yr) + 0.052 \times Height(cm)$

For ever smokers: $V_A = -4.769 + 0.449 \times \text{Sex}(\text{female}: 0, \text{male}: 1) - 0.012 \times Age(yr) + 0.061 \times Height(cm)$

For females: $V_A = -4.477 + 0.056 \times \text{Height(cm)}$

For males: $V_A = -3.776 - 0.012 \times \text{Age(yr)} + 0.058 \times \text{Height(cm)}$

RBH group with normal PFT

The predictive models of V_A for each group in the RBH group with normal PFT are presented in Table 5-21 and below.

The final model for all participants in the RBH group with normal PFT is as follows: $V_A = -10.163 + 0.369 \times Gender(female: 0, male: 1) - 0.008 \times Age(yr) + 0.097 \times Height(cm) - 0.009 \times Weight(kg) + 0.374 \times smoking\ status(nonsmoker: 0, ever\ smoker: 1)$

For non-smokers: $V_A = -11.925 + 0.107 \times Height(cm) - 0.011 \times Weight(kg)$

For ever smokers: $V_A = -9.842 + 0.629 \times \text{Sex(female: 0, male: 1)} - 0.014 \times Age(yr) + 0.094 \times Height(cm)$

For females: $V_A = -4.888 - 0.013 \times \text{Age(yr)} + 0.063 \times \text{Height(cm)}$

For males: $V_A = -14.988 + 0.125 \times Height(cm) - 0.013 \times Weight(kg) + 0.653 \times Smoking\ status(nonsmoker: 0, ever\ smoker: 1)$

Having obtained the predictive model for V_A, V_A values were calculated by using the predictive models for ever smokers and non-smokers for different sexes, ages, and smoking status as shown in Table 5-20. Age, height and weight were set as described earlier in the section. The results showed that the RBH group had a slightly higher V_A value than the TSGH group.

Table 5-20. V_A for different smoking status, sexes and ages

Smoking status	Sex	Age (year)	V_A (L)	
			TSGH	RBH
Non-smoker	male	20	5.9	5.9
		40	5.7	5.9
		60	5.6	5.9
	female	20	4.5	4.5
		40	4.3	4.5
		60	4.2	4.5
Smoker	male	20	6.0	6.8
		40	5.8	6.5
		60	5.5	6.3
	female	20	4.7	4.8
		40	4.5	4.5
		60	4.2	4.3

Table 5-21. Predictive models for V_A in TSGH relatively healthy group and RBH group with normal PFT

	All participants	**All participants**		**All participants**	
		Non-smokers	**Smokers**	**Females**	**Males**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
TSGH relatively healthy group					
Sex (male)	**0.576*** (0.343, 0.808)**	**0.642*** (0.374, 0.910)**	0.449† (-0.038, 0.936)	-	-
Age	**-0.010*** (-0.015, -0.005)**	**-0.009** (-0.014, -0.003)**	**-0.012* (-0.022, -0.001)**	excluded	**-0.012** (-0.019, -0.005)**
Height	**0.055*** (0.042, 0.068)**	**0.052*** (0.037, 0.068)**	**0.061*** (0.036, 0.084)**	**0.056*** (0.040, 0.072)**	**0.058*** (0.040, 0.076)**
Weight	excluded	excluded	excluded	excluded	excluded
Hb	excluded	excluded	excluded	excluded	excluded
Smoking status	excluded	-	-	excluded	excluded
Intercept	-3.917*** (-5.998, -1.936)	-3.564** (-6.153, -0.974)	-4.769* (-8.695, -0.842)	-4.477*** (-7.035, -1.919)	-3.776* (-6.938, -0.613)
N	177	113	64	75	102
SEE	0.494	0.478	0.530	0.414	0.544
R^2	0.720	0.720	0.677	0.393	0.421
Adjusted R^2	0.715	0.712	0.661	0.385	0.409
RBH group with normal PFT					
Sex (male)	**0.369* (0.048, 0.690)**	excluded	**0.629** (0.184, 1.074)**	-	-
Age	**-0.008* (-0.016, -0.008)**	excluded	**-0.014* (-0.025, -0.003)**	**-0.013* (-0.022, 0.003)**	excluded
Height	**0.097*** (0.080, 0.114)**	**0.107*** (0.088, 0.126)**	**0.094*** (0.072, 0.116)**	**0.063*** (0.042, 0.084)**	**0.125*** (0.102, 0.148)**

	All participants	All participants		All participants	
		Non-smokers	Smokers	Females	Males
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Weight	**-0.009*** **(-0.016, -0.002)**	-0.011† (-0.023, 0.001)	excluded	excluded	**-0.013*** **(-0.024, -0.002)**
Smoking status	**0.374**** **(0.142, 0.605)**	-	-	excluded	**0.653***** **(0.346, 0.960)**
Intercept	-10.163 (-13.015, -7.312)	-11.925*** (-14.654, -9.196)	-9.842*** (-13.650, -6.034)	-4.888*** (-8.612, -1.164)	-14.988*** (-18.704, -11.271)
N	106	54	52	45	61
SEE	0.580	0.573	0.566	0.495	0.583
R^2	0.796	0.781	0.820	0.603	0.704
Adjusted R^2	0.786	0.772	0.809	0.584	0.689

*Note: *Where a significant difference was found in V_A value, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

Predictive models for K_{CO}

After building the predictive models for V_A, the same process was performed with predictive models for K_{CO}.

TSGH relatively healthy group

The predictive models of K_{CO} for each group in the TSGH relatively group are presented in Table 5-23 and below.

For all participants: $K_{CO} = 8.351 - 0.029 \times Age(yr) - 0.026 \times Height(cm) + 0.025 \times Weight(kg)$

For non-smokers: $K_{CO} = 7.168 - 0.026 \times Age(yr) - 0.017 \times Height(cm) + 0.021 \times Weight(kg)$

For ever smokers: $K_{CO} = 10.990 - 0.036 \times Age(yr) - 0.044 \times Height(cm) + 0.036 \times Weight(kg)$

For females: $K_{CO} = 7.485 - 0.024 \times \text{Age(yr)} - 0.023 \times \text{Height(cm)} + 0.028 \times \text{Weight(kg)}$

For males: $K_{CO} = 8.851 - 0.031 \times \text{Age(yr)} - 0.027 \times \text{Height(cm)} + 0.023 \times \text{Weight(kg)}$

RBH group with normal PFT

The predictive models of K_{CO} for each group in the RBH group with normal PFT were presented in Table 5-23 and below.

For all participants: $K_{CO} = 9.887 + 0.301 \times Gender(female: 0, male: 1) - 0.023 \times Age(yr) - 0.029 \times Height(cm) + 0.009 \times Weight(kg) - 0.297 \times smoking\ status(nonsmoker: 0, ever\ smoker: 1)$

For non-smokers: $K_{CO} = 8.594 + 0.449 \times \text{Sex(female: 0, male: 1)} - 0.023 \times Age(yr) - 0.018 \times Height(cm)$

For ever smokers: $K_{CO} = 9.413 - 0.021 \times Age(yr) - 0.028 \times Height(cm) + 0.010 \times Weight(kg)$

For females: $K_{CO} = 5.347 - 0.017 \times \text{Age(yr)}$

For males: $K_{CO} = 12.553 - 0.025 \times \text{Age(yr)} - 0.043 \times \text{Height(cm)} + 0.012 \times \text{Weight(kg)} - 0.485 \times \text{Smoking status(nonsmoker: 0, ever smoker: 1)}$

Having obtained the predictive model for K_{CO}, Table 5-22 shows the K_{CO} values using the predictive models for ever smokers and non-smokers for different sexes, ages, and smoking status. The results showed that smokers' K_{CO} value decreased more than non-smokers as they aged.

Table 5-22. K_{CO} for different smoking status, sexes and ages

Smoking status	Sex	Age (year)	K_{CO} (mL/min/mmHg/L)	
			TSGH	RBH
Non-smoker	male	20	5.2	5.5
		40	4.7	5.0
		60	4.2	4.5
	female	20	5.1	5.3
		40	4.6	4.8
		60	4.0	4.4
Smoker	male	20	5.2	4.8
		40	4.5	4.4
		60	3.8	4.0
	female	20	5.2	5.1
		40	4.5	4.7
		60	3.8	4.2

Table 5-23. Predictive models for K_{CO} in TSGH relatively healthy group and RBH group with normal PFT

	All participants	**All participants**		**All participants**	
		Non-smokers	**Smokers**	**Females**	**Males**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
TSGH relatively healthy group					
Sex (male)	excluded	excluded	excluded	-	-
Age	**-0.029*** (-0.034, -0.024)**	**-0.026*** (-0.033, -0.020)**	**-0.036*** (-0.046, -0.027)**	**-0.024*** (-0.034, -0.014)**	**-0.031*** (-0.037, -0.024)**
Height	**-0.026*** (-0.037, -0.014)**	**-0.017* (-0.033, -0.002)**	**-0.044*** (-0.063, -0.024)**	**-0.023† (-0.049, 0.004)**	**-0.027* (-0.045, -0.010)**
Weight	**0.025*** (0.017, 0.033)**	**0.021*** (0.010, 0.031)**	**0.036*** (0.022, 0.050)**	**0.028*** (0.014, 0.042)**	**0.023*** (0.013, 0.033)**
Hb	excluded	excluded	excluded	excluded	excluded
Smoking status	excluded	-	-	excluded	excluded
Intercept	8.351 (6.718, 9.985)	7.168*** (4.966, 9.370)	10.990*** (8.132, 13.849)	7.485 (3.456, 0.042)	8.851 (6.027, 11.674)
N	177	113	64	75	102
SEE	0.501	0.519	0.459	0.545	0.472
R^2	0.450	0.438	0.520	0.364	0.522
Adjusted R^2	0.440	0.422	0.496	0.337	0.507
RBH group with normal PFT					
Sex (male)	**0.301* (0.036, 0.566)**	**0.449* (0.062, 0.836)**	excluded	-	-
Age	**-0.023*** (-0.029, -0.016)**	**-0.023*** (-0.031, -0.014)**	**-0.021*** (-0.030, -0.012)**	**-0.017** (-0.027, -0.008)**	**-0.025*** (-0.032, -0.017)**
Height	**-0.029*** (-0.044, -0.015)**	-0.018† (-0.037, -0.001)	**-0.028*** (-0.043, -0.014)**	excluded	**-0.043*** (-0.060, -0.026)**

	All participants	All participants		All participants	
		Non-smokers	Smokers	Females	Males
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Weight	**0.009** **(0.003, 0.015)**	excluded	**0.010*** **(0.002, 0.017)**	excluded	**0.012**** **(0.004, 0.019)**
Smoking status	**-0.297**** **(-0.489, -0.106)**	-	-	excluded	**-0.485***** **(-0.707, 0.262)**
Intercept	9.887 (7.531, 12.243)	8.594*** (1.787, 12.257)	9.413*** (6.948, 11.878)	5.347*** (4.772, 5.961)	12.553 (9.602, 15.504)
N	106	54	52	45	61
SEE	0.480	0.486	0.470	0.521	0.422
R^2	0.400	0.371	0.433	0.237	0.567
Adjusted R^2	0.370	0.333	0.398	0.220	0.536

*Note: *Where a significant difference was found in K_{CO} value, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

5.3.6 Comparison of the predictive models of DL_{CO}, V_A and K_{CO} with the literature

Our study shows that sex, age, height, weight and smoking status are the main factors included in the predictive model of DL_{CO}, V_A and K_{CO}. However, the predictive models found in the literature differ (Paoletti et al., 1985; Park et al., 1986; Roca et al., 1990; Ip et al., 2007; Chhabra et al., 2016). Therefore, to compare the models, the researcher used the mean value of the age, height and weight in this study for the comparison. The data from the TSGH relatively healthy group and the RBH group with normal PFT are shown in Table 5-24. The description of the literature for comparison is shown in Table 5-25. The comparison of the different predictive models of DL_{CO}, V_A and K_{CO} are shown in Table 5-26.

Table 5-24. Observed mean value of DL_{CO}, V_A and K_{CO} from TSGH relatively healthy group and RBH group with normal PFT

	TSGH		**RBH**	
	Males	**Females**	**Males**	**Females**
Age (years)	47.0	46.7	57.1	57.8
Height (cm)	172.7	158.5	178.1	163.8
Weight (kg)	71.6	56.8	91.1	78.6
DL_{CO} (mL/min/mmHg)	24.8	18.8	27.5	20.4
V_A (L)	5.7	4.3	6.4	4.7
K_{CO} (mL/min/mmHg/L)	4.3	4.4	4.3	4.3

Table 5-25. Details of literature for comparison of DL_{CO}, V_A and K_{CO}

No	Study	Sample size	Age	Country	Ethnicity
A1	The present study (TSGH)	177	18- 86	Taiwan	Asian
A2	The present study (RBH)	107	19- 88	UK	Caucasian
B	Chhabra et al., 2016	357	18- 71	India	Asian
C	Ip et al., 2007	568	18- 80	Hong Kong	Asian
D	Paoletti et al., 1985	712	M: 18- 65; F: 19- 65	Italy	Caucasian
E	Park et al., 1986	90	20- 69	South Korea	Asian
F	Roca et al., 1990	361	20- 70	Spain	Caucasian

Table 5-26. Comparison of the predictive model of DL_{CO} (STPD), V_A (BTPS) and K_{CO} by using the mean values from the study

Study	Sex	Model								Pred. Mean	Obs. Mean	Dif.
		A	H	W	BSA	AH	A^2	Evsm.	Const.			
Predictive model of DL_{CO} (STPD)												
A1 (TSGH)	M	-0.216	0.119	0.107					6.607	24.7	24.8	-0.1
	F	-0.121	0.131	0.119					-3.089	18.8	18.8	0.0
TSGH Ref.	M	-0.238			15.500				6.800	24.3	24.8	-0.5
	F	-0.117			15.500				0.500	19.4	18.8	0.6
B (Asian)	M	-0.624	0.318				0.006		-7.813	30.0	24.8	5.2
	F	-0.099	0.449						-44.150	22.4	18.8	3.6
C (Asian)	M	-0.196	0.419						-33.912	29.3	24.8	4.5
	F	-0.936	-0.060			0.006			33.061	20.5	18.8	1.7
E (Asian)	M	-0.216	0.350						23.168	27.2	24.8	2.4
	F	-0.153	0.249						-11.662	20.7	18.8	1.9
A2 (RBH)	M	-0.189	0.243						-4.963	27.5	27.5	0.0
	F	-0.142	0.240						-10.651	20.5	20.4	0.1
RBH* Ref.	M	-0.066	0.111						-6.030	29.8	27.5	2.3
	F	-0.049	0.082						-2.740	23.5	20.4	3.1
D (Cau.)	M	-0.194	0.441						-31.382	36.1	27.5	8.6
	F	-0.068	0.157						5.077	26.9	20.4	6.5
F (Cau.)	M	-0.196	0.367						-21.898	32.3	27.5	4.8
	F	-0.123	0.137	0.092					1.888	24.4	20.4	4.0
Predictive model of V_A (BTPS)												
A1 (TSGH)	M	-0.012	0.058						-3.776	5.7	5.7	0.0
	F		0.056						-4.447	4.4	4.3	0.1
TSGH Ref.	M		0.080						-7.230	6.5	5.7	0.8
	F		0.066						-5.940	4.5	4.3	0.2
B (Asian)	M		0.087	-0.019					-8.152	5.5	5.7	-0.2
	F		0.068						-6.893	3.9	4.3	-0.4
A2	M		0.125	-0.013				0.653	-14.988	6.1	6.4	-0.3

Study	Sex	Model								Pred. Mean	Obs. Mean	Dif.
		A	H	W	BSA	AH	A^2	Evsm.	Const.			
(RBH)	F	-0.013	0.063						-4.888	4.7	4.7	0.0
RBH	M		0.080						-7.230	7.0	6.4	0.6
Ref.	F		0.066						-5.940	5.8	4.7	1.1
F	M		0.095	-0.016					-9.052	6.3	6.4	-0.1
(Cau.)	F		0.050						-3.555	4.6	4.7	-0.1
Predictive model of K_{CO}												
A1	M	-0.031	-0.027	0.023					8.851	4.4	4.3	0.1
(TSGH)	F	-0.024	-0.023	0.028					7.485	4.3	4.4	-0.1
TSGH	M	-0.030							5.560	4.2	4.3	-0.1
Ref.	F	-0.030							5.560	4.2	4.4	-0.2
B	M	-0.037							7.315	5.6	4.3	1.3
(Asian)	F	No significant predictor found.										
C	M	-0.025		0.011					5.622	5.2	4.3	0.9
(Asian)	F	-0.155	0.072	0.014		0.001			16.184	5.0	4.4	0.6
E	M	-0.026							5.529	4.3	4.3	0.0
(Asian)	F	-0.023							5.658	4.6	4.4	0.2
A2	M	-0.025	-0.043	0.012				-0.485	12.553	4.6	4.3	0.3
(RBH)	F	-0.017							5.347	4.4	4.3	0.1
RBH	M	Calculate from DL_{CO} and V_A								4.3	4.3	0.0
Ref.	F									4.1	4.3	0.2
D	M	-0.023	-0.001						6.060	4.5	4.3	0.2
(Cau.)	F	-0.017	-0.025						9.771	4.7	4.3	0.4
F	M	0.034	-0.032	0.019					10.958	5.1	4.3	0.8
(Cau.)	F	-0.017	-0.025						9.771	5.5	4.3	1.2

*Cau.: Caucasian, Evsm., ever smoker (ever smoker is a binary term in which ever smoker is 1 and never smoker is 0), A: age in years, H: height in cm, W: weight in kg, BSA: body surface area in m^2, Const.: constant, Pred. Mean: the predicted value of DL_{CO} (mL/min/mmHg), V_A (L) and K_{CO} (mL/min/mmHg/ L) by using means, Dif.: pred. mean minus obs. mean, Ref.: reference equation from the hospital, * the calculation is based on TL_{CO}*

Table 5-26 shows that most predictive models of DL_{CO} included only age and height. However, in our TSGH study, we found that weight also plays an important role when predicting DL_{CO}, similar to Roca et al.'s (1990) findings; age, height and weight may be predictors for V_A. The predictive mean of our present study was similar to other results in the literature (Roca et al., 1990; Chhabra et al., 2016); age, height and weight may be related to K_{CO}. The predictive value of K_{CO} in the TSGH component of the present study is similar to that in Park et al.'s (1986) study and that of the RBH component to Paoletti et al.'s (1985) study. The RBH data found that smoking status was a factor in the predictive model for V_A and K_{CO}: if a person has a history of smoking, his V_A is higher and K_{CO} is lower than people with no history of smoking.

For predictions of DL_{CO}, V_A and K_{CO}, the model performed better when the difference between predicted (mean) and observed (mean) is smaller. The results showed that in both components – TSGH (Asian participants) and RBH (Caucasian participants) – the difference between predicted and observed values were smaller than in other literature. Overall, it shows that the predicted value of DL_{CO} from the four studies from Asia (Hong Kong, India, Korea and Taiwan) (Park et al., 1986; Ip et al., 2007; Chhabra et al., 2016), was lower than that of those from the UK, Roca et al.'s study (Barcelona) and Paoletti et al.'s study (Italy) (Paoletti et al., 1985; Roca et al., 1990).

5.4 Discussion and conclusion

Based on the results, the researcher found several factors related to DL_{CO}, V_A and K_{CO}. However, some factors were found not to be related, even though they influenced DL_{CO}, V_A and K_{CO} in other studies. In this section, the characteristics of the groups analysed in the study are discussed to determine the representativeness of the study. Factors related to DL_{CO}, V_A and K_{CO} are also compared to results from other literature.

DL_{CO} is the product of V_A and K_{CO}. In general, V_A decreases may due to alveolar damage or loss, reduced alveolar expansion, inefficient distribution of inspired air or airflow obstruction (Hughes and Pride, 2012). K_{CO} decreases may due to anaemia, reduced capillary volume and flow, damage to the barrier between alveolars and capillaries, or damage to microvasculature (Hughes and Pride, 2012).

5.4.1 Characteristics of the TSGH and RBH groups

In this study, the TSGH groups comprised approximately 60% males and 40% females. More males than females participated in the TSGH group because the TSGH is a military hospital. Even though the hospital also serves civilians, the number of military patients is enough to skew the numbers away from the general population distribution of around 47.6% males and 52.4% females (Department of Civil Affairs (Taipei), 2019). The RBH groups showed that males and females were distributed similarly to the UK sex distribution (Office for National Statistics (UK), 2019).

The proportion of current smokers was around 20% for all TSGH groups, somewhat higher than that of the general population at around 14.5% (Health promotion administration (Taiwan), 2019). In the RBH groups, the percentage of current smokers was around 8% in the RBH group with any PFT and 5% in the RBH group with normal PFT, much lower than the reported 14.1% of the general population (Public Health England, 2019). However, the report also shows that the current smoking rate was decreasing with age from 16.0% of 18-24 year olds to only 7.8% of over-65s (Public Health England, 2019). In our study, the average age of the RBH groups was 57 and 65 respectively, which might lower their current smoking rate.

Moreover, the difference in the processes for ethical applications between Taiwan and the UK are worth discussing. First, the regulation is different. In the UK, when using personal data, the General Data Protection Regulation on data protection and privacy in the European Union and the European Economic Area in EU law should be followed. Therefore, besides the ethical application itself, the researcher should apply for data protection. However, in Taiwan, there is no need for a separate process of data protection. Secondly, there are 3 different parties that the researcher needed to apply for obtaining ethical approval in the UK, including UCL, NHS and Royal Berkshire Hospital. However, in Taiwan, the researcher only needed to apply for ethical approval from the Tri-Service General Hospital. Thirdly, the documents needed for the application were different.

The ethical application in the UK is very time-consuming and needed great effort. It may delay the research plan. However, it did provide a lot more details for protecting the data, patients' rights and researchers' rights compared to the ethical application in Taiwan.

5.4.2 Demographic, physiological and behavioural factors which may affect the values of DL_{CO}, V_A and K_{CO}

Effects of age, sex and height on DL_{CO}, V_A and K_{CO}

Age, sex and height have been shown to influence DL_{CO} in the literature (Talaminos Barroso et al., 2018) and this was confirmed in our study. If a person is older or shorter, the value of DL_{CO} declines due to gas exchange decline based on lung size, alveolar surface area and reduction in blood volume (Hepper et al., 1960; Talaminos Barroso et al., 2018; Bowdish, 2019). Paoletti et al. (1985) also found a negative relationship between age and $DL_{CO,}$ based on participants aged 20-65. However, they found the relationship between DL_{CO} and participants aged 8-19 was positive, and DL_{CO} increased slightly with age when corrected for V_A (Paoletti et al., 1985; Schaefer, 2019). Our study excluded participants younger than 18 for ethical reasons, so we did not have data from not yet mature lungs. The difference before and after the age of 20 in Paoletti et al.'s study might be attributed to lung function development. Usually, the lungs mature around at 20-25, after which lung function declines (Weibel and Gomez, 1962; Bowdish, 2019).

Sex showed a significant relationship to DL_{CO} in most of the predictive models in Section 5.3. Usually, males tend to have a higher value of DL_{CO} than females (Talaminos Barroso et al., 2018). However, as shown in Table 5-12, sex did not have a significant effect on DL_{CO} in TSGH group with normal PFT. This group contains the history of diseases. In our study, there were more males with history of diseases (including hypertension, DM and cardiovascular diseases) compared to females, and people with history of diseases tend to have a lower value of DL_{CO}, please see the discussion (Section 5.4.3). Therefore, it may affect the results. In most of the literature, researchers reported the model of DL_{CO} by separating participants by sex to get a more accurate prediction of DL_{CO} value. The reason for this is the basic physiological and anatomical differences between males and females, such as the size of lungs and airway tubes, the number of bronchi and the alveolar surface area (Paoletti et al., 1985; Stanojevic et al., 2008; Townsend et al., 2012; Talaminos Barroso et al., 2018).

In the predictive models, age, sex and height were also shown to influences V_A (Table 5-21). Taller people have a higher value of V_A (Hepper et al., 1960; Forrest, 1970). For the value of K_{CO}, the effects between sexes were controversial in the study, which is similar to the literature (Kendrick and Laszlo, 1990; Zahir et al., 2010;

Talaminos Barroso et al., 2018). In our study, height and age also showed a negative relationship with K_{CO} (Table 5-23). The K_{CO} decreased due to the loss of lung elasticity causing by ageing and lower perfusion of the lungs in the upright position for taller people because of gravity (Hughes and Pride, 2012).

Effects of weight, BMI and obesity on DL_{CO}, V_A and K_{CO}

Weight and BMI have also been reported to affect DL_{CO} (Talaminos Barroso et al., 2018). Our study found that heavier people tend to have a higher value of DL_{CO} (Table 5-12), which is similar to Blakemore et al.'s (1957) findings. Lenfant (2000 cited in Fröhlich et al., 2016) also reported that weight is strongly related to the lung surface area – if the lung surface area is bigger, more gas can transfer into the lung. However, weight did not show a significant effect on DL_{CO} in RBH group with normal PFT (Table 5-12). Also, our study shows that if people have a higher BMI, they may have either a higher value of DL_{CO} or no change in their DL_{CO}. The effects of BMI, however, remain controversial in the literature (Salome et al., 2009; Dixon and Peters, 2018). Therefore, even though weight and BMI are sometimes correlated, weight is reported to have more influence than BMI, as shown in our and other studies (Paoletti et al., 1985; Vázquez-García et al., 2016). In our study, weight was shown to affect K_{CO} in the TSGH and RBH groups, which was similar to other studies (Roca et al., 1990; Ip et al., 2007).

The effects of obesity on DL_{CO} are also controversial. In our study, no significant relationship was shown between obesity and DL_{CO} in multivariable regression in the TSGH group with normal PFT, TSGH relatively healthy group and RBH groups, which is similar to several other studies (Sharp et al., 1964; Salome et al., 2009). However, some studies have found that obese people may have a lower DL_{CO} due to alveolar volume decline or structural changes caused by increased lipid deposition (Enache et al., 2011), similar to the TSGH group with any PFT; however, as our data did not include body fat and lipid deposition information, we are unable to draw any hard conclusions. Other studies showed an increase in DL_{CO} in severely obese patients due to pulmonary blood volume rise (Rubinstein et al., 1990; Saydain et al., 2004; Dixon and Peters, 2018). Even though the effects of weight, BMI and obesity on DL_{CO} remain controversial, as Littleton (Littleton, 2012) stated, the distribution of fat may be more important than the BMI, just as the abdominal fat mass located on the thoracic cage may reduce the V_A (Zavorsky et al., 2008).

Therefore, in our study, as the data supplied only contained the BMI value without body fat information, we may not be able to identify the real effects of obesity on DL_{CO}.

Effects of Hb on DL_{CO}, V_A and K_{CO}

In our study, the concentration of Hb was shown to have a relationship with DL_{CO} and K_{CO} in the TSGH group with normal PFT (Table 5-12 and Table 5-15). If a person has a higher concentration of Hb, he/she may have a higher value of DL_{CO} due to the high affinity between CO and haem in the blood (Dolan, 1985; Cotes et al., 2006). This finding is similar to the results from other studies (Rankin et al., 1961; Herbert et al., 1965; Crapo et al., 1995). However, after the researcher excluded anaemic participants and ran the multivariable regression for the factors that affect the DL_{CO} value in the TSGH relatively healthy group (Table 5-18), the results showed that Hb might not be the most significant factor to consider in relatively healthy group, similar to Knudson et al.'s (1987) study. The possible reason for lowering the effects of Hb to DL_{CO} was to exclude the extreme value (anaemia) of Hb for analysing. Studies also stated that if a person shows an extreme Hb value, then the concentration of Hb should be considered (Mohsenifar et al., 1982; Marrades et al., 1997). Another reason may be the free CO in the blood: even though the amount of free CO is limited, it also plays a role in CO poisoning.

Moreover, the menstrual cycle also has an influence on DL_{CO} in females. The value of Hb is at its peak before menstruation, after which the DL_{CO} value starts to drop in line with the blood volume loss due to the period, which may also relate to the concentration of Hb in the body (Sansores et al., 1995; Farha et al., 2007; Talaminos Barroso et al., 2018). Therefore, an adjustment for the concentration of Hb in the DL_{CO} test may be needed (Marrades et al., 1997).

Effects of smoking on DL_{CO}, V_A and K_{CO}

In the univariable test, smoking status was shown to influence DL_{CO}. People with a history of ever smoking had a higher value of DL_{CO} than non-smokers in TSGH relatively healthy group (Table 5-11). Two possible reasons for this might be that the smokers' group included more males and taller individuals than the non-smokers' group. When running the multivariable regression, smoking status showed a negative relationship with DL_{CO} and K_{CO}, similar to other studies that show that smoking may cause a lower value of DL_{CO} (Frans et al., 1975; Miller et al., 1983;

Popović-Grle et al., 1992; Sansores et al., 1992). Also, Sill found smoking may have a negative impact on DL_{CO} even in the population of relatively healthy young adults (Sill, 2016). Moreover, Thompson et al. (2008) found ex-smokers may be a predictor for DL_{CO} and ex-smokers may have a lower value of DL_{CO}. There are several possible reasons for the decline in DL_{CO} and K_{CO}, such as change in the volume of red blood cells in the capillaries, COHb concentration, alveolar-capillary diffusion and anatomical lesions (Frans et al., 1975; Yang, 1993; Graham et al., 2002; Najeeb, 2010; Boyer et al., 2015). Simply speaking, the chemicals in tobacco smoke would have adverse impacts on the lungs, such as destruction of the lung parenchyma and the lung vessels, and cause the gas exchange efficiency (K_{CO}) to decrease (Boyer et al., 2015). Additionally, several clinical conditions may cause a decrease in DL_{CO}, including emphysema, COPD or pulmonary fibrosis (Harvey et al., 2015; Nakazawa et al., 2018). The predictive model for RBH group with normal PFT showed that ever smoking may be a factor that affects V_A and K_{CO}. Even though we found the DL_{CO} showed normal value, underlying factors such as emphysema may increase V_A and decrease K_{CO} (Frans et al., 1997). These underlying factors should be further checked with chest x-rays and CT scans.

Moreover, a relationship between the period of smoking and DL_{CO} was found in the univariable test in TSGH group with normal PFT (Table 5-11). The longer the smoking period, the lower the person's value of DL_{CO}. Van Ganse et al. (1972) found that the lifetime number of packs smoked may affect DL_{CO} and K_{CO}. However, in our study, the lifetime number of packs smoked was not recorded.

When comparing smokers and non-smokers in our predictive model for DL_{CO} and K_{CO}, the values of DL_{CO} and K_{CO} decreased more in smokers than in non-smokers (Table 5-19 and Table 5-22). Storebø et al. (2016) also found that DL_{CO} declined more rapidly over a nine-year period commensurate with higher age, baseline current smoking, more pack years, heavier weight, and lower FEV_1 in a community sample.

Effects of ethnicity on DL_{CO}, V_A and K_{CO}

Our study showed that Caucasians (RBH dataset) had a higher value of DL_{CO} than Asians (TSGH dataset) at the same sex, age, height and weight (see Section 5.3.5 and 5.3.6). This may be related to the difference in ethnicity. The American Thoracic Society (ATS) and the European Respiratory Society (ERS) have suggested that the reference values for lung functions should take different ethnicities into

account (Stanojevic et al., 2010; Stanojevic et al., 2017). However, the value of the K_{CO} did not show a big difference among different ethnicities in Section 5.3.5 which was similar to Korotzer et al.'s (2000) findings. According to Yang (1992), the difference in DL_{CO} values between Chinese people and Caucasians may be more related to the differences in the lung volume than to ethnic variations in the inherent characteristics of the alveolar-capillary membrane; Chhabra et al. (2016) agree. Another study also found that the values of FVC, FEV_1 and V_A were lower in Asians than Europeans, but the values of DL_{CO} and K_{CO} were similar (Korotzer et al., 2000).

However, when making a comparison of the predictive models of DL_{CO} in the present study with the literature, there is a big difference in the predicted DL_{CO} value even in the same ethnicity. The results might suggest poor predictability of approach beyond specific dataset used. Therefore, when building the predictive models for DL_{CO} researchers should not only take ethnicity into account as ATS and ERS suggested but also have a big and representative dataset based on the target population.

5.4.3 PFT factors and diseases may affect the values of DL_{CO}, V_A and K_{CO}

Effects of FEV_1/FVC, RV/TLC and FRC/TLC on DL_{CO}, V_A and K_{CO}

The parameters of pulmonary function may also be related to each other. Their functions were to indicate and quantify the ability of lungs and determine if participants had any potential lung disorders (Hughes, 2008; Ranu et al., 2011; Strong, 2014d). Our study, after clustering the PFT parameters, showed that FEV_1/FVC, RV/TLC and FRC/TLC might be related to DL_{CO}, V_A and K_{CO}. However, all these factors are like DL_{CO}, V_A and K_{CO}, in that they are all also highly correlated with age (Ren et al., 2012; Kendrick, 2015; Htun et al., 2018; Thomas et al., 2019).

FEV_1/FVC showed a positive relationship with DL_{CO} in univariable regression in two groups (Table 5-11). In general, lower FEV_1/FVC may indicate obstructive lung diseases and lower values of DL_{CO} (Crapo et al., 1995; Saydain et al., 2004). The RV/TLC and FRC/TLC are indicators of gas trapping or obstructive ventilation (Kendrick, 2015; Shin et al., 2015) and are higher in heavy smokers (Elbehairy et al., 2017). In our study, RV/TLC and FRC/TLC showed a negative relationship with DL_{CO}, V_A and K_{CO} in univariable regression in all of the groups (Table 5-11, Table 5-14 and Table 5-16).

Effects of lung function disease on DL_{CO}, V_A and K_{CO}

In the present study, obstructive lung diseases are negatively associated with K_{CO} (Table 5-11, Table 5-14 and Table 5-16). However, their effects on DL_{CO} and V_A are as in no general consensus yet. People who suffer from obstructive lung diseases, such as COPD, asthma, emphysema (damage to the air sacs in the lungs) and chronic bronchitis may have a hard time exhaling all the air from the lungs; for them, exhalation is slower and more difficult than normal. Obstructive lung diseases may be caused by damage to the lungs or a narrowing of the airways inside the lungs. After exhaling fully, such patients still have an abnormally large amount of air left in the lungs (Hughes, 2008; Ranu et al., 2011; Leader, 2019).

The reason for being unable to identify any specific effects of obstructive lung diseases on DL_{CO}, V_A and K_{CO} might be the different pathophysiology of each disease. For example, even though COPD, asthma and emphysema are all considered examples of obstructive lung diseases, the individual diseases have different effects on DL_{CO}, V_A and K_{CO}: usually, those with asthma have a normal or increased DL_{CO}, those with COPD may have a lower DL_{CO} and those with emphysema may have a lower DL_{CO} and K_{CO} (Frans et al., 1997; Saydain et al., 2004; Magnussen et al., 2017). The lower value of DL_{CO} in COPD patients may be due to loss of lung units or poor mixing of inspired air (Hughes and Pride, 2012). The increased or normal value of DL_{CO} in patients with asthma may be due to the increased pulmonary capillary blood volume or extravasation of red blood cells into the alveolus (Weitzman and Wilson, 1974; Stewart, 1988; Hughes and Pride, 2012). The lower values of DL_{CO} and K_{CO} in emphysema may be due to the lung damage (Frans et al., 1997). Therefore, when investigating the effects of lung disease on DL_{CO}, V_A and K_{CO}, we should look carefully at the details of each specific disease to avoid mixing different pathophysiologies and results as the causes of different effects on lung function parameters. Therefore, the specific pathophysiology of each lung disease should be researched more first. Then, depending on the different pathophysiology, the individuals with abnormal PFT should be separated into different groups and analysed and discussed separately.

In the present study, restricted lung disease and mixed obstructive and restrictive lung disease had a negative relationship with V_A and K_{CO} (Table 5-14 and Table 5-16). People who suffer from restrictive lung diseases, such as pneumonia, pulmonary fibrosis, sarcoidosis (an autoimmune disease), etc., may have difficulties

in fully expanding their lungs; for them, the inhalation is not complete. This can occur when tissue in the chest wall becomes stiffened, or due to weakened muscles or damaged nerves (Hughes, 2008, Ranu et al., 2011, Leader, 2019).

Wémeau-Stervinou et al. (2012) have shown that idiopathic interstitial pneumonia, for example, might alter DL_{CO} due to the changes in the gas exchange area, the thickness of the alveolar-capillary membrane and the ventilation/perfusion relationship in the lung. Mo et al.'s (2020) study recruited discharged cases of COVID-19 and found their DL_{CO} and K_{CO} had decreased based on the degree of severity. COVID-19 patients with pneumonia had a lower DL_{CO} and K_{CO} value than those with milder forms of the illness.

Effects of history of disease on DL_{CO}, V_A and K_{CO}

In our study, hypertension, hyperlipidaemia, DM, cardiovascular diseases and kidney disease showed no significant effects on DL_{CO}, V_A or K_{CO} in the multivariable regression (Table 5-11, Table 5-14 and Table 5-16), which is similar to the findings of Saydain et al. (2004) and Partridge et al. (1979). The effects of hypertension, DM, cardiovascular diseases and kidney disease on DL_{CO}, V_A and K_{CO} as found in the literature are discussed below.

Saydain et al. (2004) also found that hypertension did not affect DL_{CO}, V_A and K_{CO}. The effects of hyperlipidaemia on DL_{CO}, however, remain contentious (Partridge et al., 1979). Enzi et al. (1976) found that patients with hyperlipidaemia had a significantly lower value of DL_{CO} due to hyperlipoproteinemia or fat micro embolism.

Research results on the effects of DM on lung function are contradictory. DM has been shown to affect DL_{CO} in that people with DM may have a lower value of DL_{CO} which may be due to a lower pulmonary capillary blood volume and lung capillary damage; the lower value of V_A may be similarly responsible for the lower TLC that DM causes due to the chest wall stiffness increasing (Boulbou et al., 2003). However, Guvener et al. (2003) found that the lower value of DL_{CO} had only a mild effect clinically.

In terms of cardiovascular diseases, people with chronic heart failure have been found to have a lower value of DL_{CO} and V_A due to the loss of lung tissue for gas exchange (Agostoni et al., 2006). For kidney disease, some studies showed that people with chronic renal failure, especially if on dialysis, may have a lower DL_{CO}:

this may be due to fluid retention (related to oedema) or disorder in the tissue, resulting in restrictions and inhibited CO diffusion (Bush and Gabriel, 1991; Batubara et al., 2017).

Thus, the literature shows that hyperlipidaemia, DM, cardiovascular diseases and kidney disease may affect DL_{CO}, V_A and K_{CO}, which is similar to our univariable analysis results (Table 5-11, Table 5-14 and Table 5-16). These effects were not seen in our multivariable analysis, but this may be because other factors, such as age, play a more important role than these diseases. Another reason might be that only the normal PFT value was included in the TSGH group with normal PFT, which is less than the extremely low values of DL_{CO}, V_A and K_{CO} caused by severe disease. More accurate results could be obtained if the severity of disease was also considered when investigating the effects of disease on DL_{CO}, V_A and K_{CO}.

5.4.4 Limitations

The limitations to the pulmonary function test were as follows. First, the PFT machines used in the TSGH and RBH were different. This may result in a slightly different outcome when calculating PFT parameters (O'Donnell et al., 2010). Fortunately, it may not affect the outcomes since the data from TSGH and RBH were analysed separately in the study. Also, Stanojevic et al. (2017) found that even though there were some differences in DL_{CO} data between different types of PFT equipment used in different centres, it did not have a big effect since these differences were generally within the range of physiological variability.

Second, the researcher did not have menstruation timing information for all female participants or body surface area for all participants as this information was not recorded in the PFTs, both of which may potentially affect DL_{CO}. Moreover, not every participant who had a DL_{CO} test had previously received a blood test, leading to incomplete Hb data for the participants.

Third, BMI was the only factor used to define obesity, but body fat is also an important factor that should also be considered. Body fat was not, however, recorded in the study. Also, smoking habits were based on patients' self-reported answers. Therefore, it may be more accurate for the report to be done using an exhaled CO test or by double-checking with participants' family members. Also, there may be a recall bias about the period of smoking, since some participants were older and might

not have recalled the time of smoking accurately. Also, years of smoking and packs of smoking should also be considered when asking the smoking status.

Fourth, after excluding the PFT data which has been noted that 'the patients did not follow the instruction properly', the quality of data increased. However, there are still some data that looked implausible, especially, if it is a negative value for PFT data. There are several ways to deal with implausible values. In our study, when there was an implausible value, the researcher would check the original data in the PFT report or medical records to correct. However, if there was no original data recorded in the PFT report or medical records, the value would be deleted.

Fifth, even though the inclusion/exclusion criteria of the PFT tests was established based on the standards from the literature (Wang et al., 1997; Stanojevic et al., 2010; Johnson and Theurer, 2014), some healthy participants with lower PFT tests may have been excluded. However, the false exclusion rate should be low since a lower value of PFT parameters usually indicates some lung problems.

Sixth, all the diagnosis was based on text from the PFT report in the hospitals. The researcher could not access the ICD code for each patient. However, the PFT reports were under qualified clinic staff supervision. Therefore, there should not be a significant difference between diagnosis on the reports and ICD code.

Seventh, most of the history of disease and diagnosis information was missing in the RBH dataset. Therefore, it was hard to differentiate between effects of lung illness and that of other diseases. Fortunately, in the RBH group with normal PFT, most lung illnesses were excluded and only relatively healthy participants remained in the group. Also, we could see that the history of diseases did not show a significant effect in the TSGH group with normal PFT. The basic demographic, physiological and behavioural factors explained major variations of DL_{CO}, V_A and K_{CO} in the regression, therefore, the effects of diseases should be limited.

Last, the data was only collected from medical records in the two hospitals taking part in the study. Therefore, if the participants had attended other medical facilities, the researcher did not have access to those data (which may or may not have affected the results). However, this could also be a strength when comparing results to other predictive models from previous studies as, in most of these, the participants' health status information came from self-reporting questionnaires or surveys, not from their actual medical records (Paoletti et al., 1985; Ip et al., 2007;

Chhabra et al., 2016): some participants may not be sure about or aware of certain medical conditions and so may have inadvertently self-reported as healthy.

5.4.5 Conclusion

Sections 5.3.2 to 5.3.4 revealed that the factors affecting DL_{CO}, V_A and K_{CO} showed a similar pattern in the TSGH (Taiwan) and RBH (UK) groups with any PFT when individuals of similar sex, age, height, smoking status and disease status are compared. Once age, sex, height, smoking status and disease status are adjusted for, the difference in predicted DL_{CO}, V_A and K_{CO} between TSGH (Asian) and RBH (Caucasian) groups is reduced. Sections 5.3.5 showed the different predictive models for DL_{CO}, V_A and K_{CO} based on all participants, groups of male and female and groups of non-smokers and ever smokers. In the present study, the final predictive models for DL_{CO}, V_A and K_{CO} would be the ones that were based on different sex groups and were used to compare with models from other literature in Section 5.3.6. The reasons might be the basic anatomy and physiology are different between females and males. Also, most of the predictive models for DL_{CO}, V_A and K_{CO} were based on different sexes in previous literature. In the predictive models, smoking status did not seem to be a significant factor for the DL_{CO}, V_A and K_{CO}. However, the results did show that the value of DL_{CO} and K_{CO} in smokers decreased more rapidly than in non-smokers. Therefore, the effects of smoking status on DL_{CO} and K_{CO} should be considered in the older group.

Section 5.3.6 shows that the predicted DL_{CO} in the present study was slightly lower than it was in the literature (Paoletti et al., 1985; Park et al., 1986; Roca et al., 1990; Ip et al., 2007; Chhabra et al., 2016), while the predicted data of K_{CO} was close to the data from other literature (Paoletti et al., 1985; Park et al., 1986; Roca et al., 1990; Ip et al., 2007; Chhabra et al., 2016). The remaining difference may be attributed to factors not measured in the present study or the difference might be affected by poor predictability of the approach beyond the specific dataset that was used. Therefore, for further studies, a representative dataset based on the target population should be considered when building the predictive models for pulmonary function parameters.

Also, more factors that might affect the parameters of pulmonary function could be explored, such as body fat, the location of fat in the body, menstruation timing information and body surface area. Moreover, future studies could be based not only

on relatively healthy populations but also on individuals with abnormal PFT. Therefore, the findings could be expanded to individuals with abnormal PFT and give the information of the specific pathophysiology effects on pulmonary function parameters.

6 UPDATED EXISTING CO UPTAKE AND ELIMINATION MODEL AND SIMULATION IN DIFFERENT CO EXPOSURE SCENARIOS

6.1 Introduction

CFK models have been widely used to predict the rate of CO uptake and elimination (Coburn et al., 1965; Gosselin et al., 2009). However, they were mainly based on healthy, young, male and white population. In this section, the predicted values for COHb from the CFK (1965) and modified CFK (2009) models with estimated DL_{CO} from the study were compared and validated with a wide range of measured data from the literature (Stewart et al., 1970), and that gathered during our CO-rebreathing experiment (Section 3) and our exhaled CO experiment (Section 4) (Coburn et al., 1965; Gosselin et al., 2009). The CFK models with estimated DL_{CO} were then used to predict the CO uptake and elimination for different individuals in various CO exposure scenarios.

The objectives were:

- To investigate whether the predictions of the CFK (1965) model and modified CFK (2009) model with the estimated DL_{CO} fit the data from the literature, the CO-rebreathing experiment and the exhaled CO data from the research among students
- To simulate the CFK (1965) model and modified CFK (2009) model with estimated DL_{CO} for different individuals in different CO exposure scenarios.

6.2 Methods

6.2.1 Replicating the CFK (1965) model and modified CFK (2009) model

The study developed a set of procedures to replicate the CFK (1965) and modified CFK (2009) models and to test the data against the literature (Coburn et al., 1965; Stewart et al., 1970; Peterson and Stewart, 1975; Gosselin et al., 2009).

The process and results of replicating the models are provided below.

CFK (1965) model simulation process and results

The CFK (1965) model consists of several equations (see, Section 2.3.2 and Appendix 9.2.1). The overall process for this was as follows: first, the researcher

completed a simple unit check of the CFK (1965) model for each parameter (see Appendix 9.2.1, Supplementary Table 9-1 for details).

Model for the CFK model

$$\frac{\frac{[COHb]\overline{Pco2}}{[O2Hb]M} - Vco\left[\frac{1}{DLco} + \frac{PB - PH2O}{V_{AR}}\right] - PIco}{\frac{[COHb]0\overline{Pco2}}{[O2Hb]M} - Vco\left[\frac{1}{DLco} + \frac{PB - PH2O}{V_{AR}}\right] - PIco} = e^{-\frac{t\overline{Pco2}}{MV_{BL[O2Hb]}\left(\frac{1}{DLco} + \frac{713}{V_{AR}}\right)}}$$

The second, and longest, part required replicating the CFK model in an Excel sheet. This required breaking up the CFK model into smaller portions, and then coding each of these sub-algorithms into cells in Excel.

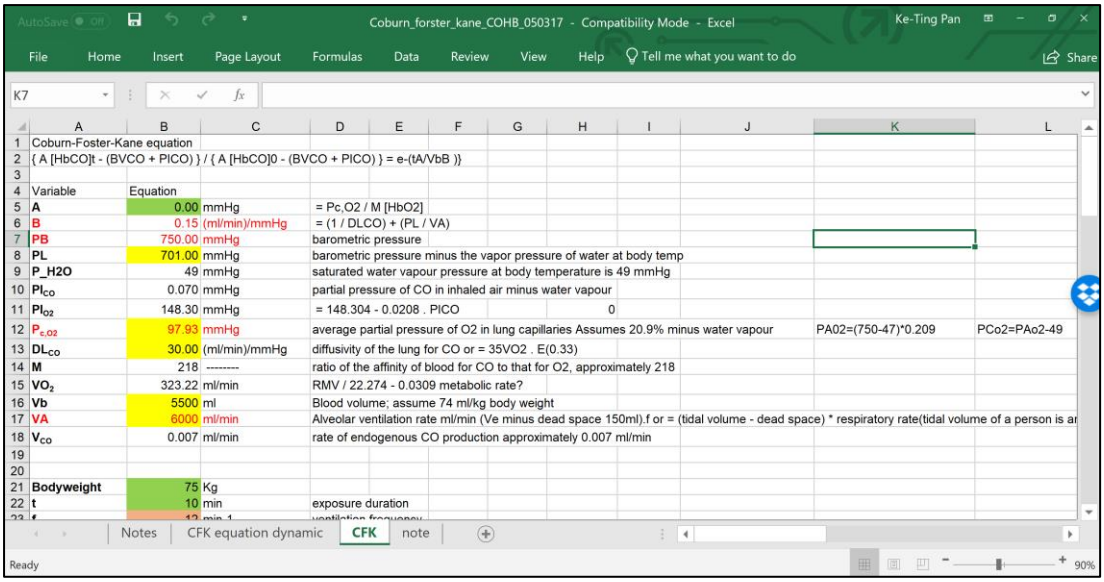

Figure 6-1. Screenshot of the Excel version of the CFK model created by the researcher

Third, the researcher validated the Excel version of the CFK model with the data from Stewart et al.'s (1970) study (see Appendix 9.2.1, Supplementary Table 9-2 for details).

As no individual values were reported in Stewart et al.'s study, the average physiological parameters for 30-year-old men at rest were used in the simulations (Stewart et al., 1970). The average height and weight were determined to be 174.4 cm and 70 kg by Demirjian (1980 cited in Gosselin et al., 2009) (see Appendix 9.2.1, for details of the calculations and values of parameters).

Table 6-1 presents a comparison between the experimental data of COHb as reported in Stewart et al.'s (1970) study and simulated data from the original CFK model by using linear regression. The slope between the simulated values and the experimental data was 0.947, which is close to 1, within a small confidence interval

(0.913–0.981). Also, the R^2 is 98.9%, and this is a good indication of the validity of the model.

Table 6-1. Linear regressions (y = m · x) between the COHb values simulated by the CFK (1965) model (x) and the experimental data (y) from Stewart et al. (1970)

	Estimated slope (m)	95% CI	SEE	R^2	Points
CFK model	0.947	(0.913, 0.981)	0.252	0.989	37

Modified (2009) CFK model simulation process and results

The modified CFK (2009) model (Gosselin et al., 2009) was reviewed, and a similar process to that for the CFK (1965) model was carried out. The CFK (2009) model also consists of several equations (see Section 2.3.3 and Appendix 9.2.1).

First, the researcher completed a unit check of the main modified (2009) CFK model (see Appendix 9.2.1, Supplementary Table 9-4 for details).

CO amount in the lungs for the modified CFK model

$$\frac{dA_{CO}(t)}{dt} = Q_{ALV}\left[C_{EXT}(t) - \frac{A_{CO}(t)}{V_{ALV}}\right] - DL_{CO}[\frac{A_{CO}(t)}{V_{ALV}} \times R \times T - \frac{B_{CO}(t) \times P_{O2}}{M(B_{COHb}^{Max} - B_{CO}(t))}]$$

CO amount in the blood for the modified CFK model

$$\frac{dB_{CO}(t)}{dt} = DL_{CO}\left[\frac{A_{CO}(t)}{V_{ALV}} \times R \times T - \frac{B_{CO}(t) \times P_{O2}}{M\left(B_{COHb}^{Max} - B_{CO}(t)\right)}\right] - k_{HbS} \times B_{CO}(t) + k_{CO2} \times B_{CO}(t) + Endo$$

CO amount in tissue for the modified CFK model

$$\frac{dS_{CO}(t)}{dt} = k_{HbS} \times B_{CO}(t) - k_{Sf} \times S_{CO}(t) - k_{CO2} \times S_{CO}(t)$$

Python V 2.2.2 using the Jupyter V 1.1.1 version was used to simulate the modified CFK model, and all predicted data output was recorded in the Excel file. The Python coding used in the study was developed based on teaching material from an on-line course at Brigham Young University (Hedengren, 2018).

When replicating the model, all of the data from the parameters were calculated directly from equations derived from the literature (Paoletti et al., 1985; ICRP, 1994; Gosselin et al., 2009) (see Appendix 9.2.1, for details of the parameters and calculations).

After simulating the modified CFK model, Table 6-2 shows that the slope between simulated values and experimental data was 0.923 within a small confidence interval (0.884–0.963) and high R^2 of 98.4%.

Table 6-2. Linear regressions ($y = m \cdot x$) between the COHb values simulated by the modified CFK (2009) model (x) and the experimental data (y) from Stewart et al. (1970)

	Estimated slope (m)	**95% CI**	**SEE**	**R^2**	**Points**
Modified CFK model	0.923	(0.884, 0.963)	0.301	0.984	37

Following this successful replication, the researcher was able to use these two models to investigate further aspects of CO exposure, as detailed in the following sections.

6.2.2 Comparing predicted data from CFK based models with measured data

First, the predicted data was simulated from four different models (see Section 5.3.5 for details),

1) the CFK (1965) model with original DL_{CO}
2) the modified CFK (2009) model with original DL_{CO}
3) the CFK (1965) model with estimated DL_{CO} from the predictive model of TSGH and RBH groups
4) the modified CFK (2009) model with estimated DL_{CO} from the predictive model of TSGH and RBH groups (see Section 5.3.5 for details).

The predicted data were then compared with the three sets of measured data, from the literature, the CO-rebreathing experiment (Section 3) and the exhaled CO readings (Section 4). Then, a Pearson correlation test was carried out to measure the correlation between the predicted data from CFK models and the measured data from the three datasets. A Pearson correlation coefficient of more than 0.7 would indicate a high correlation. Also, a validity test (linear regression) was run. If the slope of the regression between the measured and predicted data is close to one and has a higher

value of adjusted R^2 than other models, it means the predicted data provides a closer approximation to the measured data (Gosselin et al., 2009).

6.2.3 Simulation of CO uptake and elimination in different scenarios using the CFK and modified CFK models

The CFK (1965) model with estimated DL_{CO} and the modified CFK (2009) model with estimated DL_{CO} were used to simulate COHb for different individuals in different scenarios. Although the total amount of CO exposure was the same in both models, the scenarios included exposing (different) participants to CO at a high level within a short period and at a low level over a longer period. The different CO uptake and CO elimination scenarios for different individuals in different CO exposure scenarios are shown in Table 6-3. The researcher used three different baseline CO values for non-smokers, light smokers and heavy smokers. The initial COHb was assumed based on results from the exhaled CO experiment in Section 4 (see Table 6-4).

Table 6-3. Different simulation of CO uptake and CO elimination scenarios

	Simulated subject	**Exposure scenarios**	
CFK (1965) model with estimated DL_{CO}	Male vs female Young vs old Tall vs short Smoker vs non-smoker	Scenario A	100 ppm for 500 minutes
Modified CFK (2009) model with estimated DL_{CO}		Scenario B	10,000 ppm for 5 minutes

Table 6-4. Baseline CO value for simulation

	Non-smokers	**Smokers**	**Light smokers**	**Heavy smokers**
Cigarettes per day	None	0-20	<10	≥ 10
Exhaled CO (ppm)	1.92	6.90	4.80	10.00

6.3 Results

In this section, both CO uptake and CO elimination were predicted using the CFK (1965) model and the modified CFK (2009) model from the literature (Coburn et al., 1965; Gosselin et al., 2009). The predicted data were compared to measured data from the literature, the CO-rebreathing method and the exhaled CO experiment. These models were then used to simulate the rate of CO uptake and elimination in different scenarios.

6.3.1 Comparing the CFK models with the measured data

We compared the measured data from the literature (Stewart et al., 1970), data from our CO-rebreathing experiment and from the exhaled CO experiment with the predicted data from the CFK (1965) and the modified CFK (2009) models.

Comparison of measured data from literature (Stewart et al., 1970) with predicted data from the CFK models

The existing CFK models, with and without updated $DL_{CO,}$ were used to compare with data from Stewart et al.'s (1970) study (see Appendix 9.2.1, Supplementary Table 9-2 for details) (Coburn et al., 1965; Gosselin et al., 2009). For simulation, the average age was assumed as 30-year-old men at rest. Following Demirjian (1980 cited in Gosselin et al., 2009), the average height and weight were determined to be 174.4 cm and 70 kg. The final parameters used in the simulations are shown in Table 6-5 and Supplementary Table 9-54 in Appendix 9.2.4. All of the parameter values were derived from the literature and presented in BTPS (body temperature, pressure, water vapour saturated) condition (Coburn et al., 1965; Peterson and Stewart, 1975; Gosselin et al., 2009).

Table 6-5. parameters used in the CO models

Parameters	Original DL_{CO}		DL_{CO} from the study	
	CFK (1965)	Modified CFK (2009)	TSGH	RBH
Initial COHb (%)	0.7	0.7	0.7	0.7
DL_{CO} (ml/min)/mmHg	27.9	48.1	33.9	37.0

The CFK (1965) model and modified CFK (2009) model were used to predict the COHb values following a subject's exposure to 50 ppm CO for one hour, three hours, eight hours and 24 hours.

One hour at 50ppm

First, exposure of 50 ppm for one hour was predicted by the models, and the results compared to the data from Stewart et al.'s (1970) study. Figure 6-2 shows that the peak of measured data was 2.1% in Stewart et al.'s (1970) study while other peaks for the CFK (1965) were 1.74% (with original DL_{CO} from CFK (1965) model), 1.79% (with DL_{CO} calculated from TSGH relatively healthy group) and 1.80% (with DL_{CO} calculated from RBH group with normal PFT). The modified CFK (2009) models were 1.73% (with original DL_{CO} from CFK (2009) model), 1.67% (with DL_{CO}

calculated from TSGH relatively healthy group) and 1.68% (with DL_{CO} calculated from RBH group with normal PFT). The same comparison steps were repeated in the prediction of COHb values for 3, 8 and 24 hours CO exposure below. It shows that the predicted data from the CFK (1965) model with estimated DL_{CO} had a higher value than the predicted data from the CFK (1965) model when predicting exposure to 50 ppm CO for one hour. When comparing the predicted data from the modified CFK (2009) model and the modified CFK (2009) model with estimated DL_{CO}, the modified CFK (2009) model predicted higher values than the modified CFK (2009) model with estimated DL_{CO}.

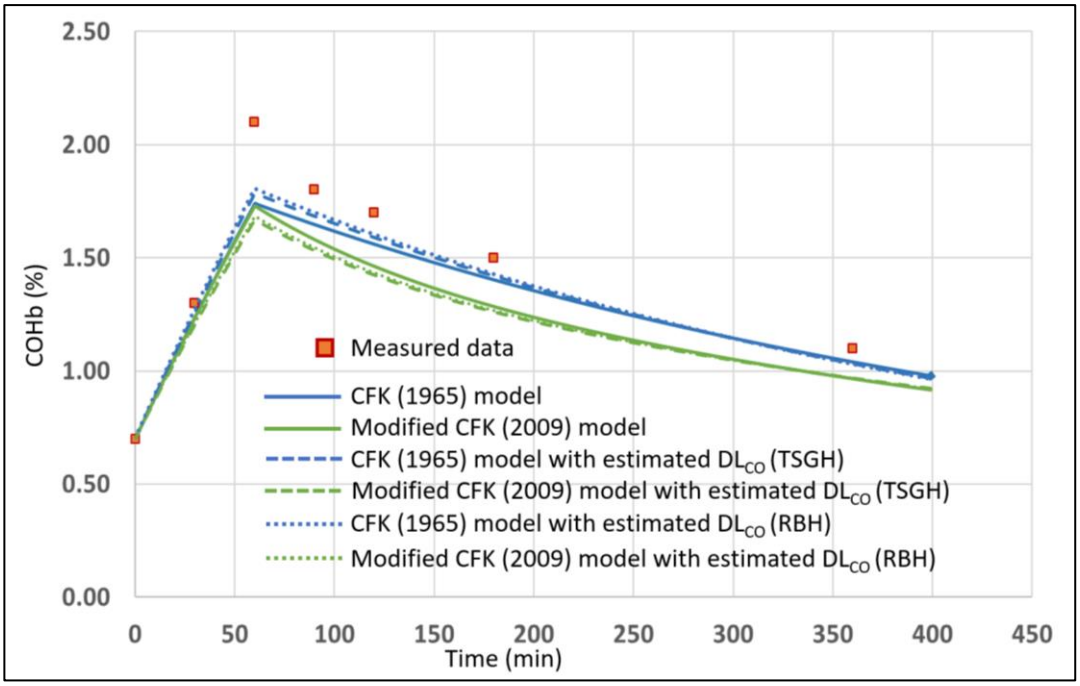

Figure 6-2. Measured COHb (%) data and predicted COHb (%) data for exposure to 50 ppm CO for one hour

Three hours at 50ppm

As seen in Figure 6-3, the peak of measured data was 3.80% in in Stewart et al.'s (1970) study while other peaks for the CFK (1965) were 3.44% (original), 3.54% (TSGH) and 3.58% (RBH); modified CFK (2009) models were 3.34% (original), 3.21% (TSGH) and 3.25% (RBH). When comparing the simulated data from the CFK (1965) model and the CFK (1965) model with estimated DL_{CO}, the predicted data from the CFK (1965) model with estimated DL_{CO} had slightly higher values than the CFK (1965) model. However, the modified CFK (2009) model with estimated DL_{CO} had lower predicted data than the modified CFK (2009) model.

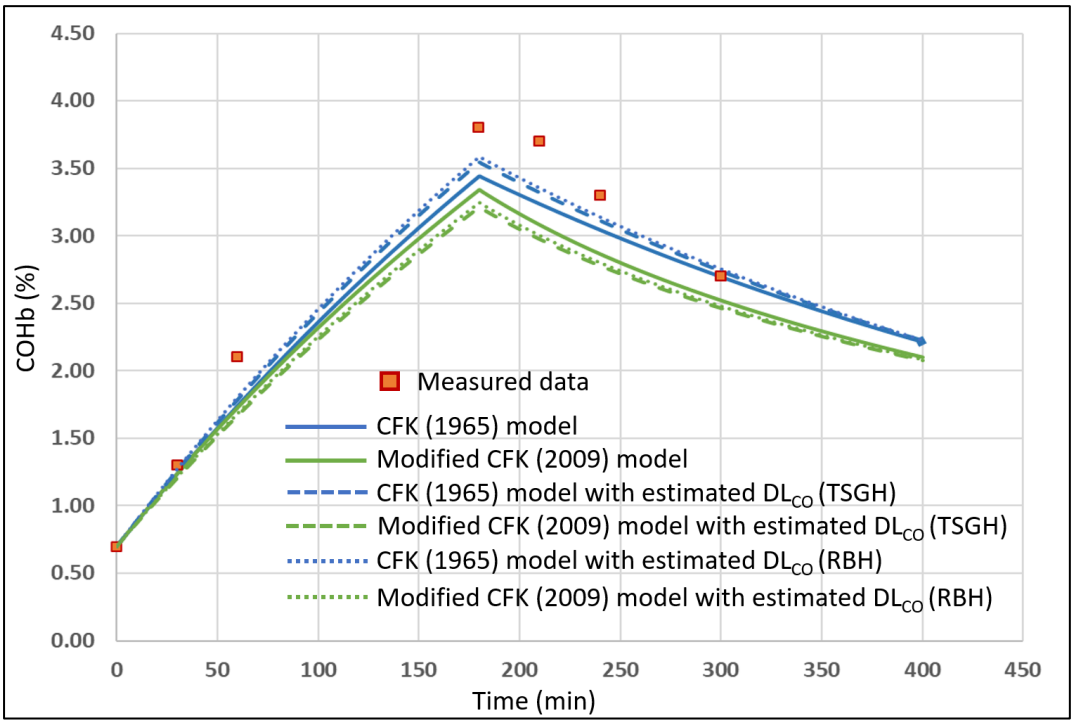

Figure 6-3. Measured COHb (%) data and predicted COHb (%) data of exposure to 50 ppm CO for three hours

Eight hours at 50ppm

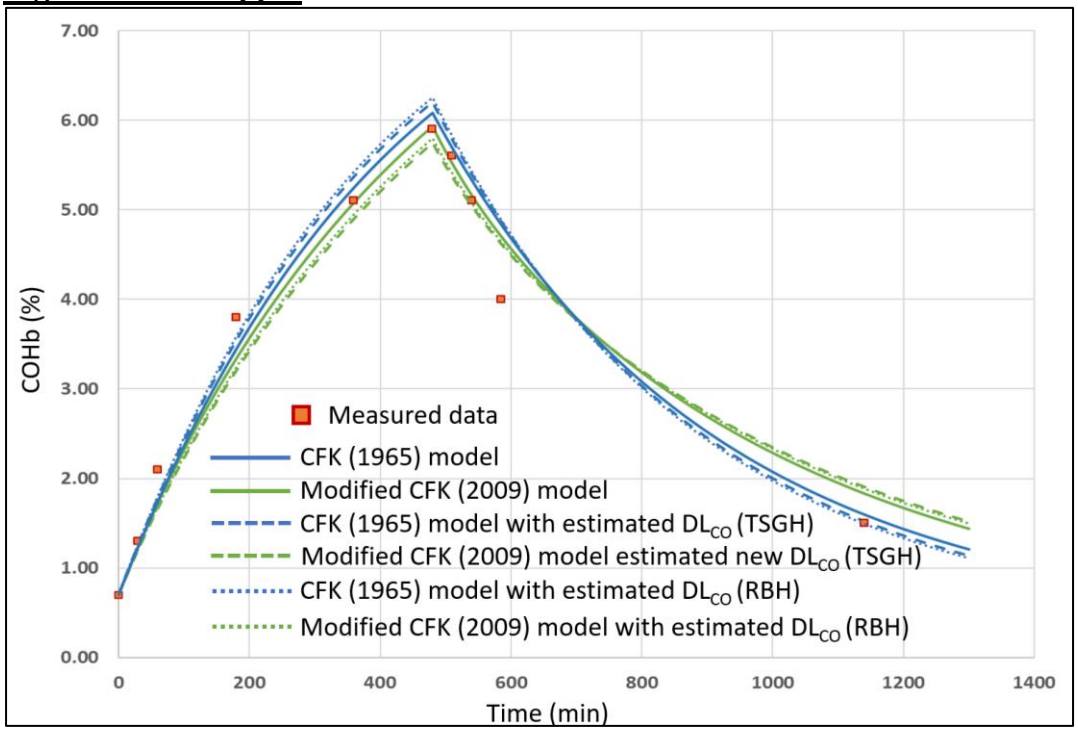

Figure 6-4. Measured COHb (%) data and predicted COHb (%) data of exposure to 50 ppm CO for eight hours

Figure 6-4 shows the peak of measured data was 5.90% in in Stewart et al.'s (1970) study while other peaks for the CFK (1965) were 6.08% (original), 6.20% (TSGH) and 6.25% (RBH); modified CFK (2009) models were 5.93% (original),

5.75% (TSGH) and 5.80% (RBH). It shows that the predicted data from the CFK (1965) model with estimated DL_{CO} had a slightly higher value than the predicted data from the CFK (1965) model when simulating exposure of 50 ppm CO for eight hours. Moreover, the modified CFK (2009) model and the modified CFK (2009) model with estimated DL_{CO} had similar peak values when simulating exposure of 50 ppm CO for eight hours.

Twenty-four hours at 50ppm

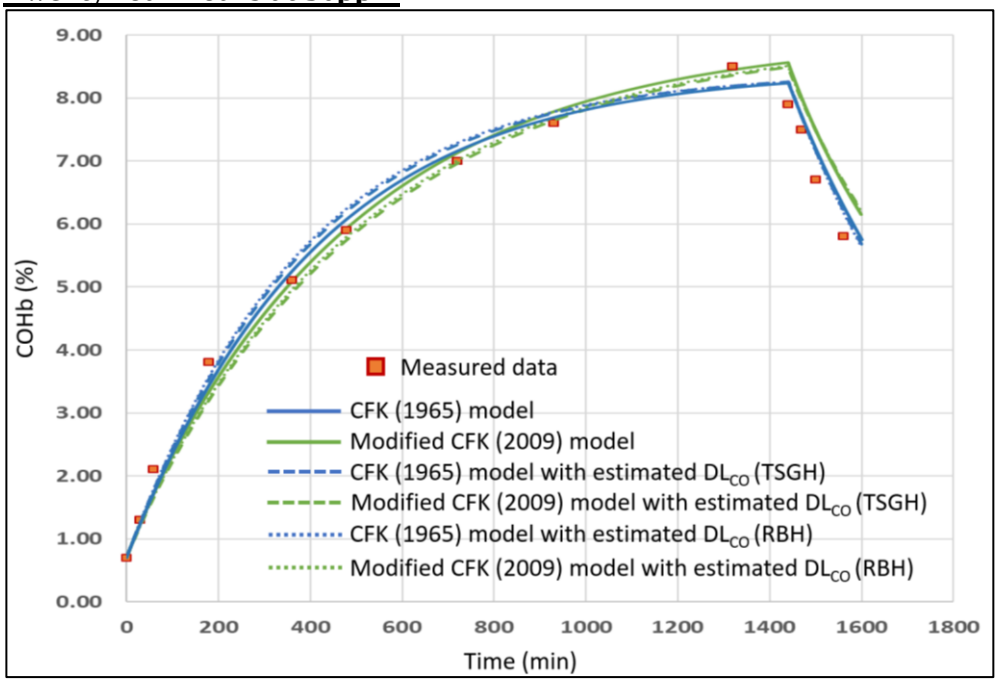

Figure 6-5. Measured COHb (%) data and predicted COHb (%) data of exposure to 50 ppm CO for 24 hours

Figure 6-5 shows the peak of measured data was 7.90% in Stewart et al.'s (1970) study while other peaks for the CFK (1965) were 8.24% (original), 8.26% (TSGH) and 8.26% (RBH); modified CFK (2009) models were 8.56% (original), 8.49% (TSGH) and 8.51% (RBH). It shows that the CFK (1965) model with estimated DL_{CO} had higher simulated data than the CFK (1965) model during CO uptake, while the modified CFK (2009) model had higher simulated data than the modified CFK (2009) model with estimated DL_{CO} during CO uptake.

The predicted data from the CFK models and the measured data from Stewart et al. (1970) was compared. The correlation test is shown in Table 6-6. The correlation coefficient shows a high correlation between all simulated data and experimental data from the literature, between 0.990 and 0.995. The validity test using regression is shown in Table 6-7 and Figure 6-6. All simulations showed a similar slope, which was close to one within a range of 0.923 to 0.947. Therefore, it

showed that the CFK models with the estimated DL_{CO} values have a similar prediction of CO uptake and elimination rate to Stewart et al.'s (1970) study.

Table 6-6. Correlation test between the COHb values simulated by the CFK models and the experimental data from Stewart et al. (1970)

	Measured data
CFK (1965) model	0.995
CFK (1965) model with estimated DL_{CO} (TSGH)	0.995
CFK (1965) model with estimated DL_{CO} (RBH)	0.995
Modified CFK (2009) model	0.992
Modified CFK (2009) model with estimated DL_{CO} (TSGH)	0.990
Modified CFK (2009) model with estimated DL_{CO} (RBH)	0.991

Table 6-7. Linear regressions ($y = m \cdot x$) between the COHb values simulated by the CFK models (x) and the experimental data (y) from Stewart et al. (1970)

	Estimated slope (m)	**95% CI**	**Adjusted R^2**	**Points**
CFK (1965) model	0.947	(0.913, 0.981)	0.989	37
CFK (1965) model with estimated DL_{CO} (TSGH)	0.942	(0.911, 0.974)	0.990	37
CFK (1965) model with estimated DL_{CO} (RBH)	0.940	(0.909, 0.971)	0.991	37
Modified CFK (2009) model	0.923	(0.884, 0.963)	0.984	37
Modified CFK (2009) model with estimated DL_{CO} (TSGH)	0.932	(0.886, 0.977)	0.980	37
Modified CFK (2009) model with estimated DL_{CO} (RBH)	0.929	(0.885, 0.973)	0.981	37

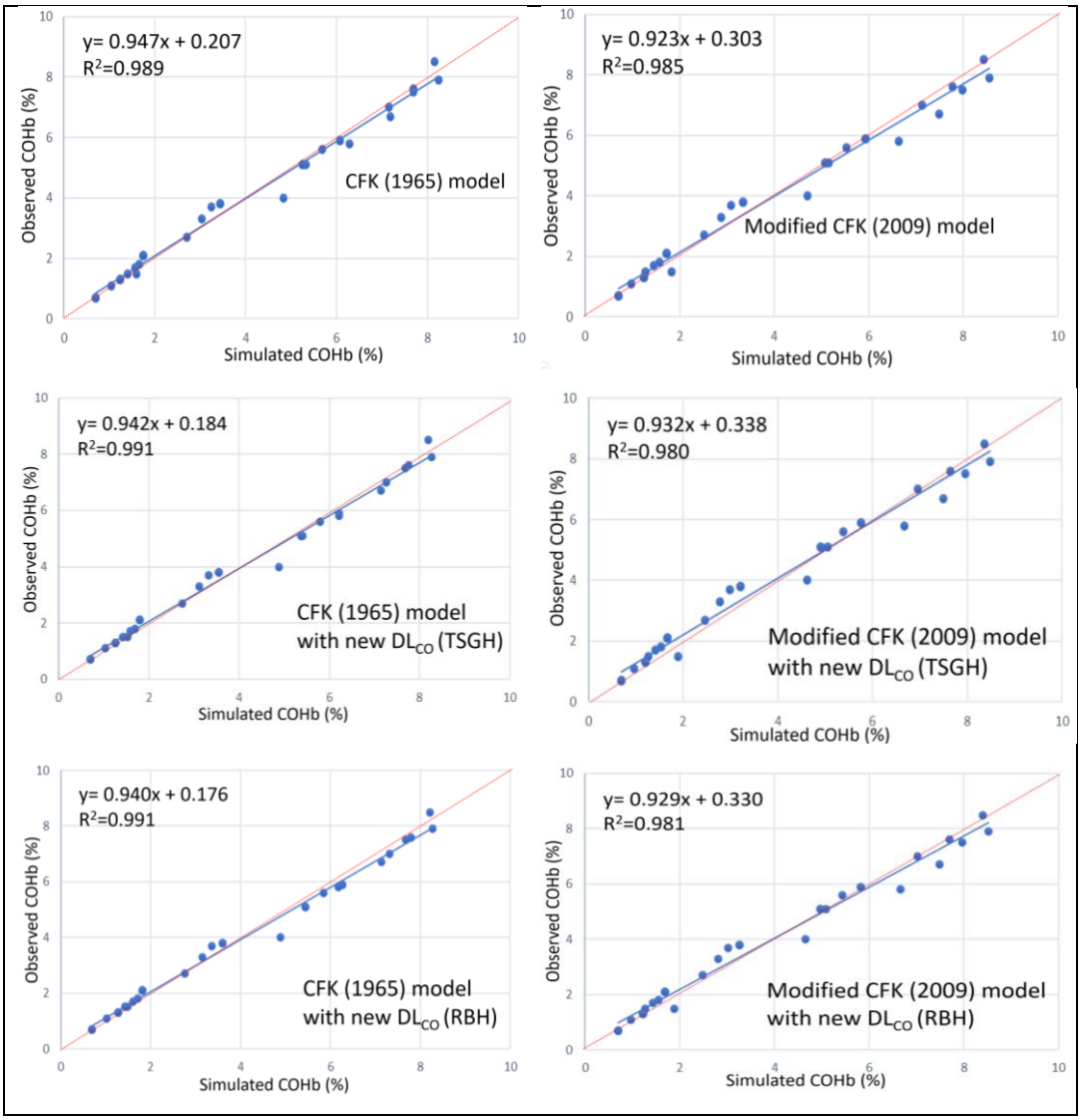

Figure 6-6. The slope between the predicted data from the CFK models and the measured data from Stewart et al. (1970), red reference line, y=x

Comparison of CO-rebreathing data with predicted data from the CFK models

The measured data is taken from the CO-rebreathing experiment (Section 3). Most parameters are taken from the CFK and modified CFK models (Coburn et al., 1965; Peterson and Stewart, 1975; Gosselin et al., 2009). The details of parameters for simulation are shown in Appendix 9.2.4, Supplementary Table 9-55. For each participant who attended the CO-rebreathing experiment in Southampton Hospital, four different simulations (CFK models and modified CFK models) were used to predict the COHb data and then compare it with the measured COHb data. The following figures show the measured data and predicted data from the models for Subjects A, B and C.

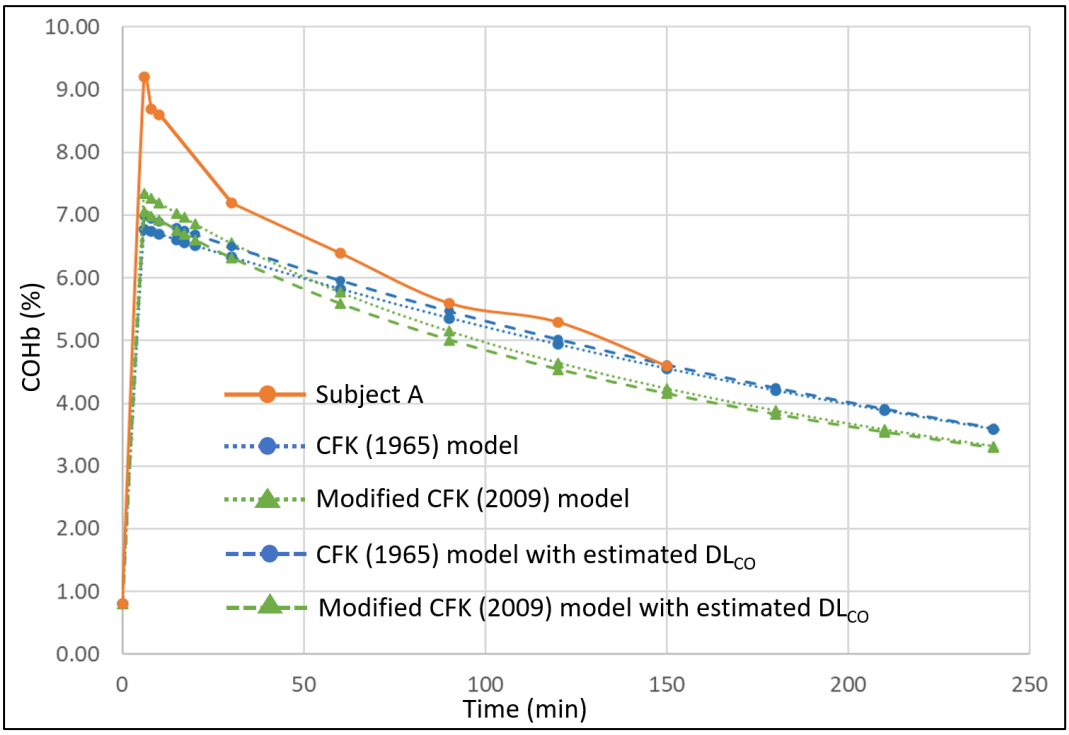

Figure 6-7. Measured COHb (%) data and predicted COHb (%) data of Subject A

Figure 6-7 shows Subject A's measured and predicted data of CO uptake and elimination. The measured COHb at six minutes was 9.2%; however, the predicted data was 7.19% for the CFK (1965) model, 6.78% for the modified CFK (2009) model, 6.98% for the CFK (1965) model with estimated DL_{CO} and 7.06% for the modified CFK (2009) model with estimated DL_{CO}. Post-peak, measured COHb at 150 minutes was 4.6% compared with the predicted data, which was 4.55% for the CFK (1965) model, 4.24% for the modified CFK (2009) model, 4.62% for the CFK (1965) model with estimated DL_{CO} and 4.16% for the modified CFK (2009) model with estimated DL_{CO}. Overall, the predicted data of Subject A in all CO models was lower than the measured data, with a difference of approximately 2% COHb around the peak.

Figure 6-8 shows Subject B's measured data and predicted data of CO uptake and elimination. The measured COHb at eight minutes was 6.40%; however, the predicted data was 4.55% for the CFK (1965) model, 4.97% for the modified CFK (2009) model, 4.67% for the CFK (1965) model with estimated DL_{CO} and 4.70% for the modified CFK (2009) model with estimated DL_{CO}. Post-peak, measured COHb at 210 minutes was 3.8%, higher than the simulated data, which was 3.12% for the CFK (1965) model, 2.93% for the modified CFK (2009) model, 3.16% for the CFK (1965) model with estimated DL_{CO} and 2.85% for the modified CFK (2009) model

with estimated DL_{CO}. The predicted data of Subject B in all CO models was lower than the measured data from the CO-rebreathing method.

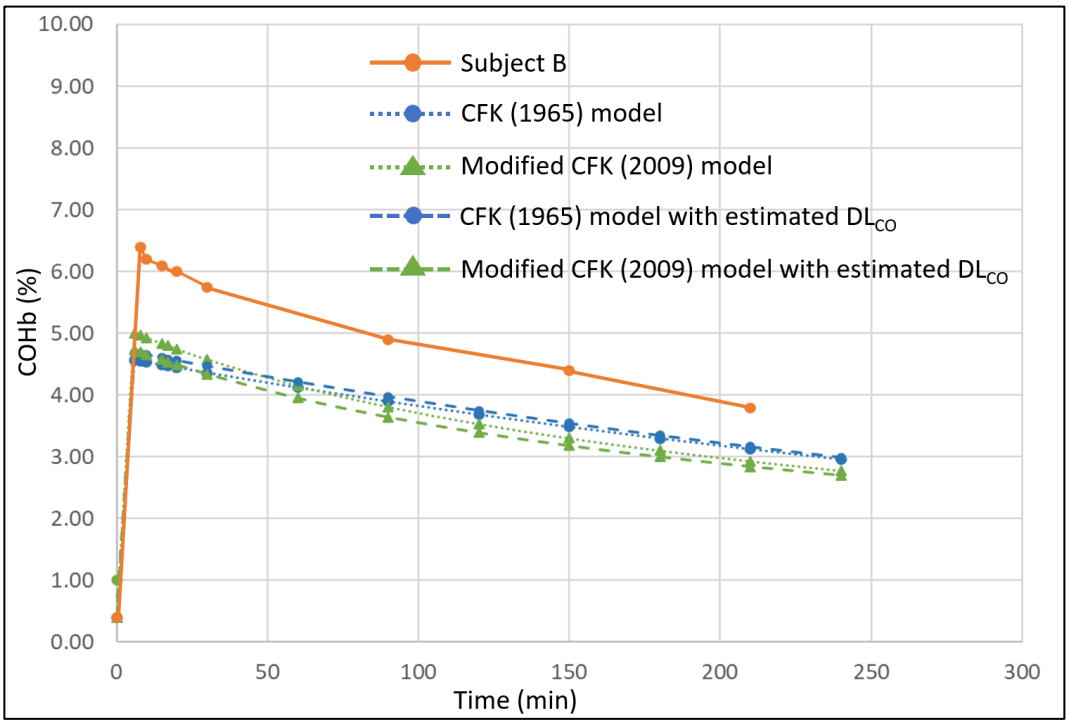

Figure 6-8. Measured COHb (%) data and predicted COHb (%) data of Subject B

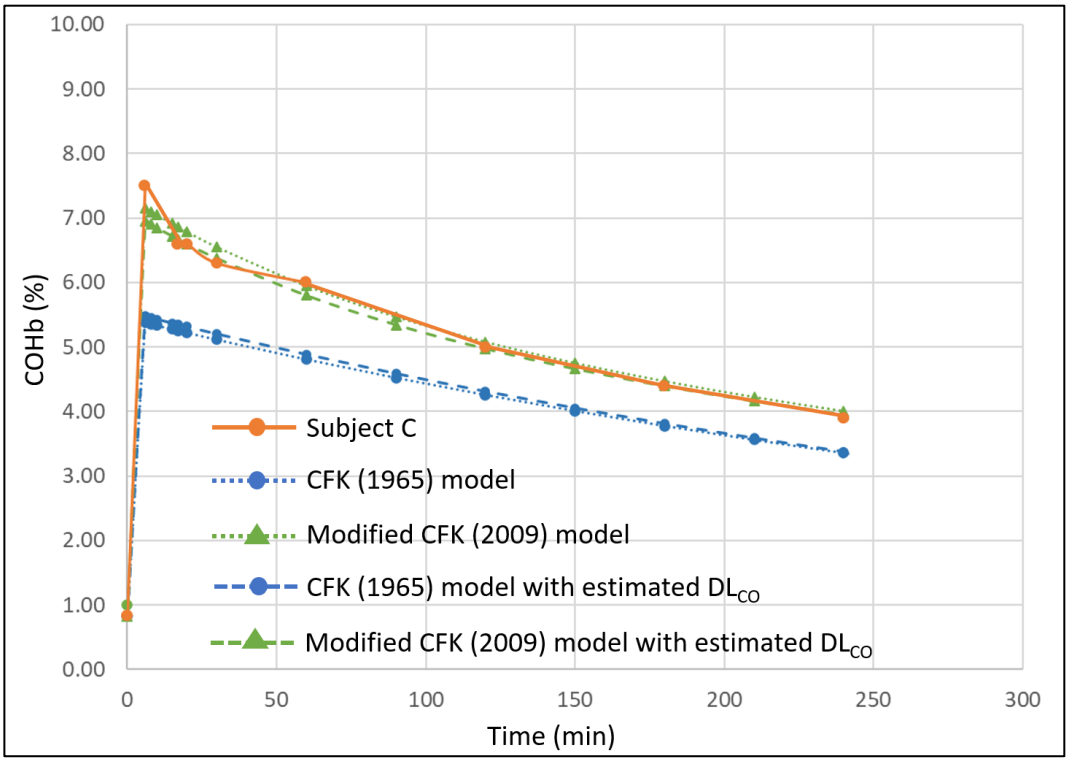

Figure 6-9. Measured COHb (%) data and predicted COHb (%) data of Subject C

Figure 6-9 shows Subject C's measured data and predicted data of CO uptake and elimination. The measured COHb at six minutes was 7.5%; however, the

simulated data was 5.38% for the CFK (1965) model, 7.16% for the modified CFK (2009) model, 5.35% for the CFK (1965) model with estimated DL_{CO} and 6.79% for the modified CFK (2009) model with estimated DL_{CO}. Post-peak, measured COHb at 240 minutes was 3.9%, compared with the predicted data of 3.36% for the CFK (1965) model, 4.01% for the modified CFK (2009) model, 3.35% for the CFK (1965) model with estimated DL_{CO} and 3.91% for the modified CFK (2009) model with estimated DL_{CO}. Moreover, a better prediction from modified CFK models than CFK models was shown in Subject C in the figure.

After comparing the predicted and measured data for each participant, the predicted data from the CFK models and the measured data from the CO-rebreathing experiment were also compared. The correlation test is shown in Table 6-8. The correlation coefficient showed a high correlation for all simulated data and experimental data from the CO-rebreathing experiment, ranging from 0.952 to 0.974.

Table 6-8. Correlation test between the COHb values simulated by the CFK models and the experimental data from the CO-rebreathing experiment

	CFK (1965) model	**CFK (1965) model with estimated DL_{CO}**	**Modified (2009) CFK model**	**Modified CFK (2009) model with estimated DL_{CO}**
Measured data	0.971	0.974	0.958	0.952

Table 6-9. Linear regressions ($y = m \cdot x$) between the COHb values simulated by the CFK models (x) and the experimental data (y) from the CO-rebreathing experiment

	Estimated slope (m)	**95% CI**	**Adjusted R^2**	**Points**
CFK (1965) model	1.279	(1.148, 1.409)	0.940	27
CFK (1965) model with estimated DL_{CO}	1.271	(1.150, 1.392)	0.947	27
Modified CFK (2009) model	1.078	(0.945, 1.211)	0.915	27
Modified CFK (2009) model with estimated DL_{CO}	1.108	(0.962, 1.254)	0.903	27

The validity test, using regression, is shown in Table 6-9 and Figure 6-10. All models showed a similar prediction with the regression slopes ranging from 1.078 to 1.279, which were close to 1. However, only the slopes of modified CFK models

contained 1, suggesting that modified CFK models might have a better prediction than CFK models in this CO-rebreathing dataset.

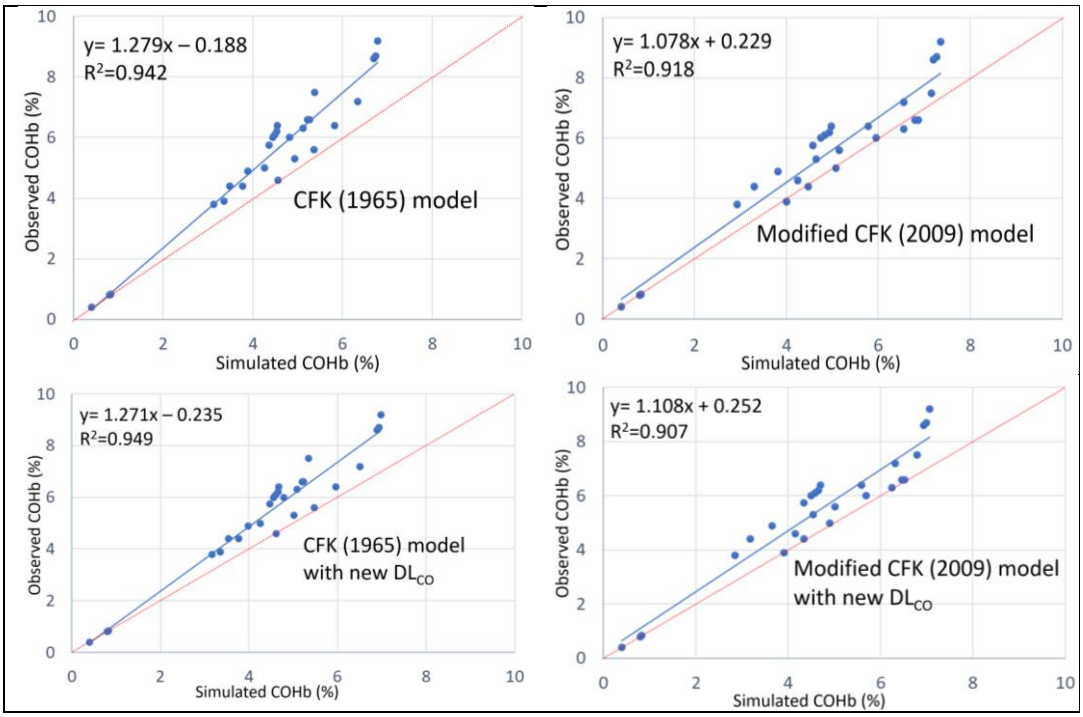

Figure 6-10. The slope between the predicted data from the CFK models and the measured data from the CO-rebreathing experiment, red reference line, y=x

Comparison of the exhaled CO data of smokers with predicted data from the CFK models

In the exhaled CO experiment, the exhaled CO of 48 smokers (27 Asian, 16 Caucasian) was recorded before and after smoking. The COHb was calculated using the following equation taken from Jarvis et al.'s (1986) study:

$$\text{COHb}(\%) = 0.63 + 0.16 \times [\text{value(ppm) of Micro Smokerlyzer}]$$

The calculation and parameter details are given in Section 6.2.1 and Appendix 9.2.4, Supplementary Table 9-56. WHO reported that each smoking period is generally equivalent to a CO exposure of 400-500 ppm over six minutes, according to the International Programme on Chemical Safety (WHO, 1999). However, the average duration of each smoking period was around 3.5 minutes; therefore, CO exposure was assumed to be an average of 450 ppm for four minutes in the study. CO uptake and elimination were calculated for each person. The tables below show the measured and predicted data from different models for Asian and Caucasian smokers.

Figure 6-11, Table 6-10 and Table 6-11 compare the predicted data with measured data from Asian smokers. The correlation test showed that all correlation coefficient had a high correlation for all simulated data and experimental data from the exhaled CO experiment (0.933-0.936) and the CFK models with estimated DL_{CO} had a slightly higher coefficient than the CFK models. All regression slopes of the CFK models were close to 1 (1.010- 1.045), which means an acceptable prediction.

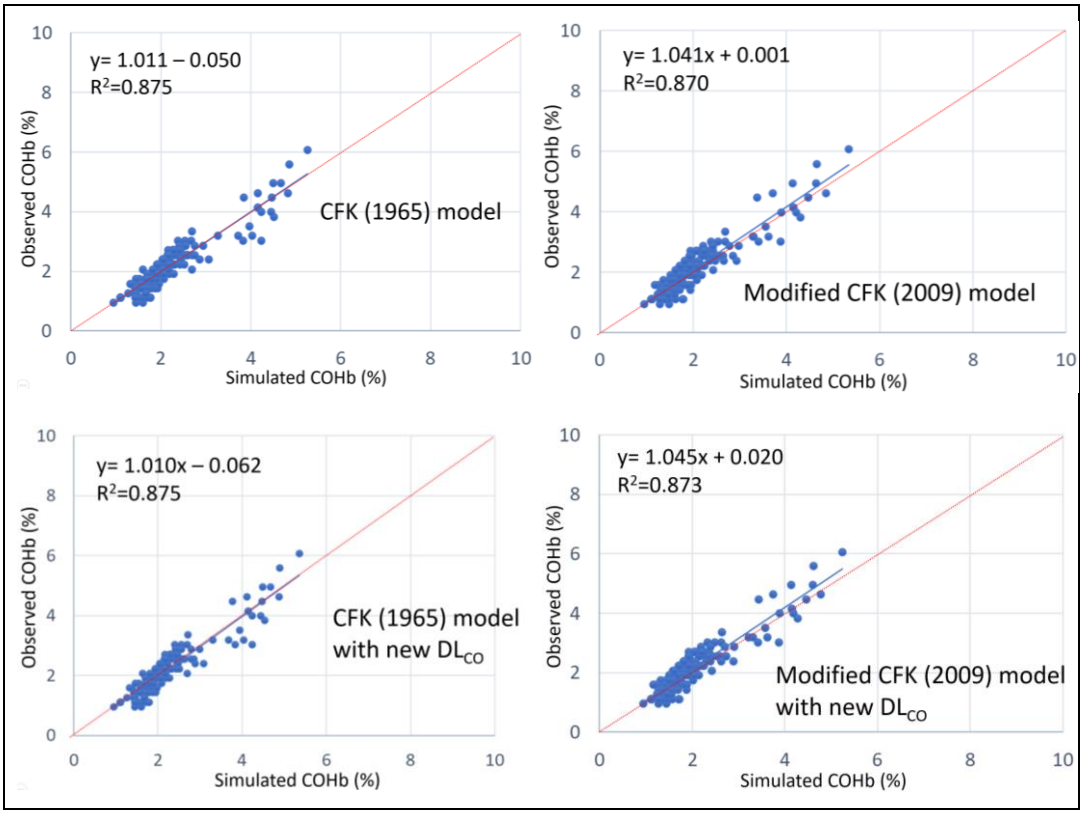

Figure 6-11. The slope between the predicted data from the CFK models and the measured data of Asian smokers from the exhaled CO experiment, red reference line, y=x

Table 6-10. Correlation test between the COHb values simulated by the CFK models and the measured data of Asian smokers from the exhaled CO experiment

	CFK (1965) model	**CFK (1965) model with estimated DL_{CO}**	**Modified (2009) CFK model**	**Modified CFK (2009) model with estimated DL_{CO}**
Measured data	0.935	0.936	0.933	0.934

Table 6-11. Linear regressions (y = m · x) between the COHb values simulated by the CFK models (x) and the experimental data (y) from the exhaled CO experiment of Asian smokers

	Estimated slope (m)	95% CI	Adjusted R^2	Points
CFK (1965) model	1.011	(0.952, 1.071)	0.874	162
CFK (1965) model with estimated DL_{CO}	1.010	(0.951, 1.070)	0.874	162
Modified CFK (2009) model	1.041	(0.979, 1.104)	0.870	162
Modified CFK (2009) model with estimated DL_{CO}	1.045	(0.983, 1.108)	0.872	162

Figure 6-12, Table 6-12 and Table 6-13 compare the predicted data with measured data from Caucasian smokers. The correlation test showed that all correlation coefficient had a high correlation for all simulated data and experimental data from the exhaled CO experiment (0.915-0.925) and the modified CFK (2009) model with estimated DL_{CO} had a higher coefficient than the modified (2009) CFK model. In the regression test, all the slopes were close to 1 (m, 0.990- 1.015), which means an acceptable prediction.

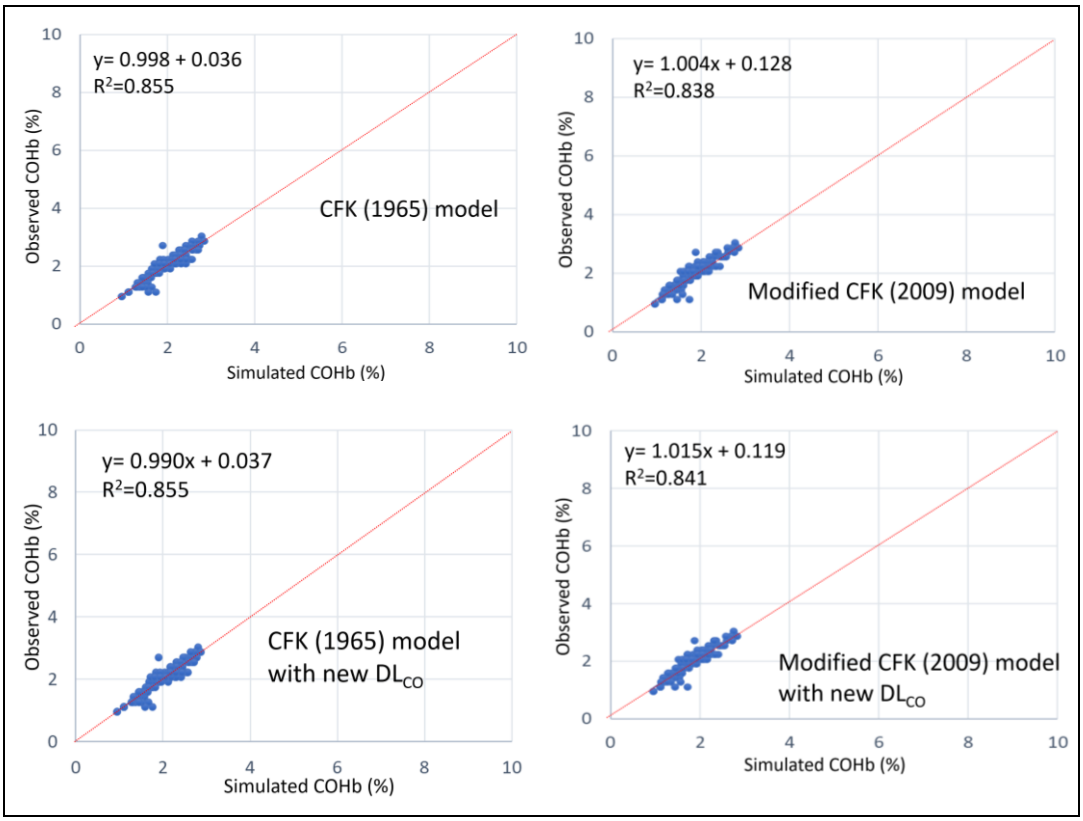

Figure 6-12. The slope between the predicted data from the CFK models and the measured data of Caucasian smokers from the exhaled CO experiment, red reference line, y=x

Table 6-12. Correlation test between the COHb values simulated by the CFK models and the measured data of Caucasian smokers from the exhaled CO experiment

	CFK (1965) model	**CFK (1965) model with estimated DL_{CO}**	**Modified (2009) CFK model**	**Modified CFK (2009) model with estimated DL_{CO}**
Measured data	0.925	0.925	0.915	0.917

Table 6-13. Linear regressions ($y = m \cdot x$) between the COHb values simulated by the CFK models (x) and the experimental data (y) from the exhaled CO experiment of Caucasian smokers

	Estimated slope (m)	**95% CI**	**Adjusted R^2**	**Points**
CFK (1965) model	0.998	(0.914, 1.082)	0.854	96
CFK (1965) model with estimated DL_{CO}	0.990	(0.906, 1.073)	0.853	96
Modified CFK (2009) model	1.004	(0.914, 1.095)	0.836	96
Modified CFK (2009) model with estimated DL_{CO}	1.015	(0.924, 1.105)	0.840	96

6.3.2 Simulation and backcasting of CO exposure in different scenarios

The simulations and backcasting (looking back, which means predicting for a past event; is the opposite of forecasting, which means predicting for a future event) of the CFK (1965) model with the estimated DL_{CO} and the modified CFK (2009) model with the estimated DL_{CO} were used to predict CO uptake and elimination in two different scenarios. The scenarios postulated involved exposure to the same total amount of CO by either a) low CO over a longer period or b) high CO over a short period.

The CO exposure scenarios are based on the real-life examples shown in Table 6-14; the parameters used in the simulation are shown in Table 6-15 and Table 6-16. In Table 6-16, the young and old age were assumed by the definition from WHO (WHO, 1999), which is 20 and 65 years old; the values of height, weight and the concentration of Hb for participants are assumed from the average of the TSGH relatively healthy group; other parameters are calculated from above assumptions.

Table 6-14. The different scenarios of CO exposure

	Time (min)	Level (ppm)	Example
Scenario A	500	100	Hot water boiler with problems (Faruk Tekbaş et al., 2001)
Scenario B	5	10,000	Forklifts at working speed (Fawcett et al., 1992)

Table 6-15. Parameters used in the CFK (1965) model and the modified CFK (2009) model

Parameters and values used in the CFK (1965) model	
P_B (mmHg)	760
Pc,o_2 (mmHg)	100
M	250
$[O_2Hb]_{max}$ (ml/ml)	0.25
Parameters and values used in the modified CFK (2009) model	
R (mmHg (ml_{air})/Kelvin(ml_{CO}))	2.55
M	240
k_{HbS} (min^{-1})	0.002
k_{Sf} (min^{-1})	0.01
T (Kelvin)	310
b_{COHb}^{Max} (ml_{CO}/g_{Hb})	1.68
k_{CO2} (min^{-1})	0.0000333

Table 6-16. The basic characteristics of the four demographic and physiological values of participants

	Young male	Old male	Young female	Old female
Age	20	65	20	65
Height	172.7	172.7	158.5	158.5
Weight	71.6	71.6	56.8	56.8
Hb	15.1	15.1	13.3	13.3
DL_{CO} (ml/min/mmHg)	36.9	25.1	26.6	20.1
CFK (1965) model with estimated DL_{CO}				
V_{AR} (ml/min)	6813	6813	4213	4213
V_{BL} (ml)	5298.4	5298.4	4146.4	4146.4
V_{CO} (ml/min)	0.0087	0.0087	0.0069	0.0069
Initial COHb (%)	0.93	0.93	0.93	0.93
$[O_2Hb]_{max}$ (ml/ml)	0.25	0.25	0.22	0.22
$[COHb]_0$ (ml/ml)	0.0023	0.0023	0.0020	0.0020
Modified CFK (2009) model with estimated DL_{CO}				
V_{BL} (ml)	5656.4	5656.4	4487.2	4487.2
P_{O2} (mmHg)	99.9	89.1	99.9	89.1
V_{ALV} (ml)	582	582	321	321
Q_{ALV} (ml/min)	6982	6982	4496	4496
Endo (ml/min)	0.0087	0.0087	0.0069	0.0069
B_{COHb}^{Max} (ml_{CO})	1434.92	1434.92	1002.62	1002.62
Initial COHb (%)	0.93	0.93	0.93	0.93

	Young male	Old male	Young female	Old female
Initial CO amount (ml)	13.34	13.34	9.32	9.32

Comparison of CO uptake and CO elimination between males and females

Based on the calculation of the parameters for simulation, the simulations of the two scenarios are shown in Figure 6-13 and Figure 6-14. The results show that young males' levels increased more quickly, had a higher peak and decreased faster than those of young females. Also, the difference in peak between males and females increased with the time and concentration of CO they were exposed to. Similar results were obtained when comparing old males and females (see Appendix 9.2.4, Supplementary Figure 9-10 and Supplementary Figure 9-11 for details).

When inhaling the same amount of CO, the longer the period of CO exposure, the longer it took to decrease to a normal value. The CO half-life was longer for all participants in Scenario A, at around 300 minutes, and lower in Scenario B, at around 275 minutes.

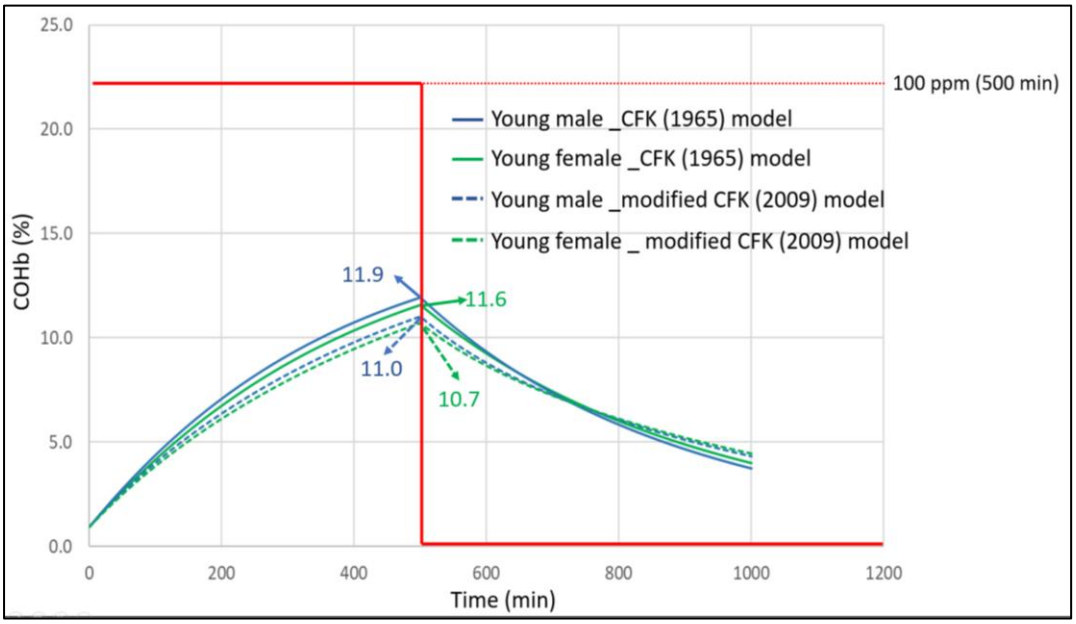

Figure 6-13. Simulation for young males and females in Scenario A (100 ppm for 500 min

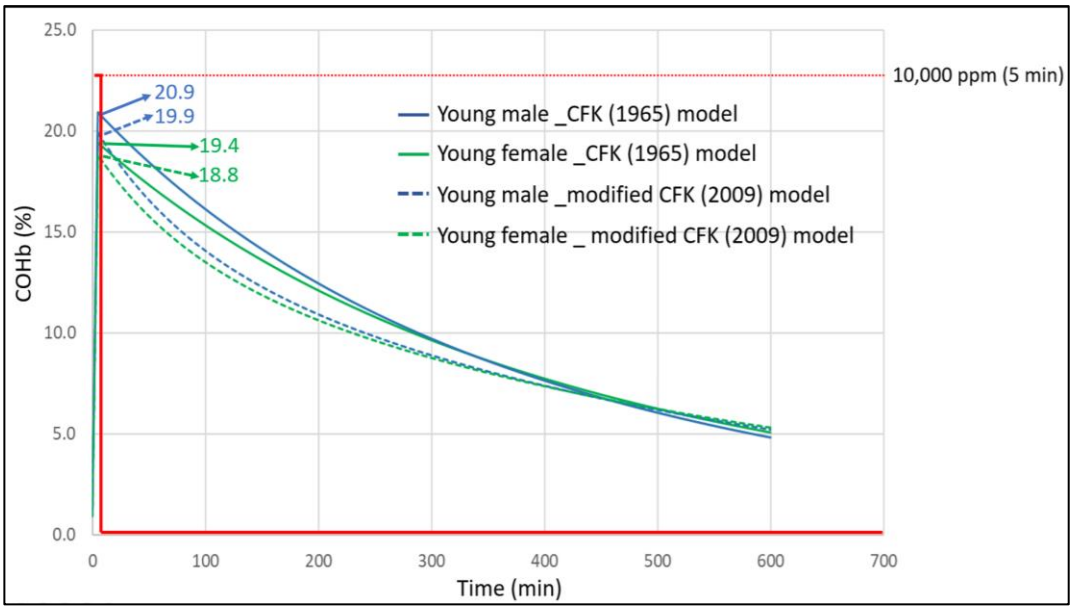

Figure 6-14. Simulation for young males and females in Scenario B (10,000 ppm for 5 min)

Comparison of CO uptake and CO elimination between young and old males

Figure 6-15 and Figure 6-16 show that young males' COHb levels increased more quickly, and they had a higher peak than old males in both exposure scenarios. Also, the difference between young and old males increased with the time and concentration of CO they were exposed to. Similar results were seen when comparing young and old females (see Appendix 9.2.4, Supplementary Figure 9-12 and Supplementary Figure 9-13 for details).

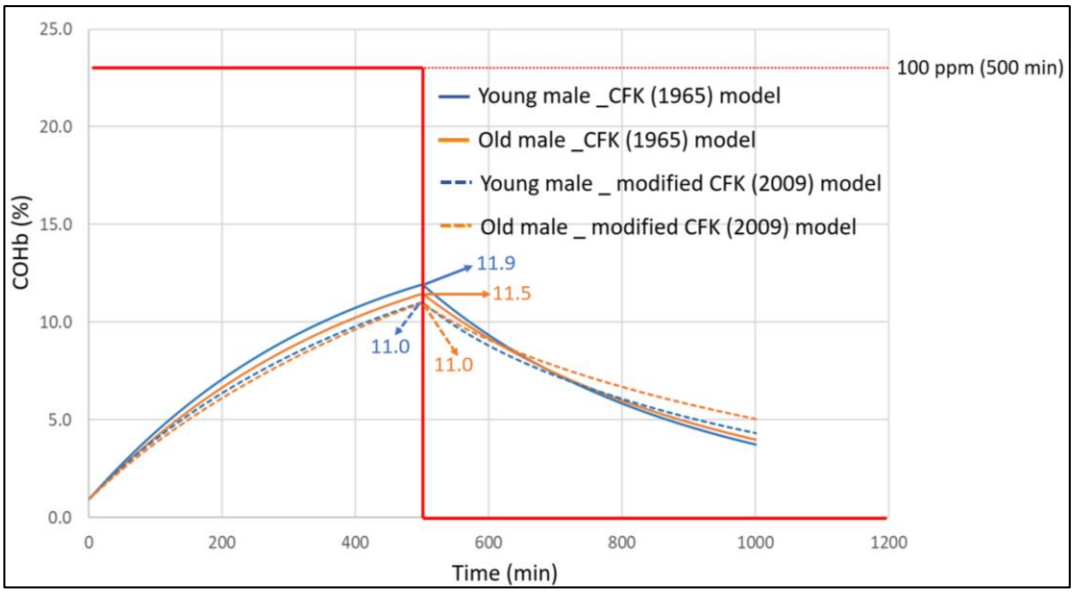

Figure 6-15. Simulation for young males and old males in Scenario A (100 ppm for 500 min

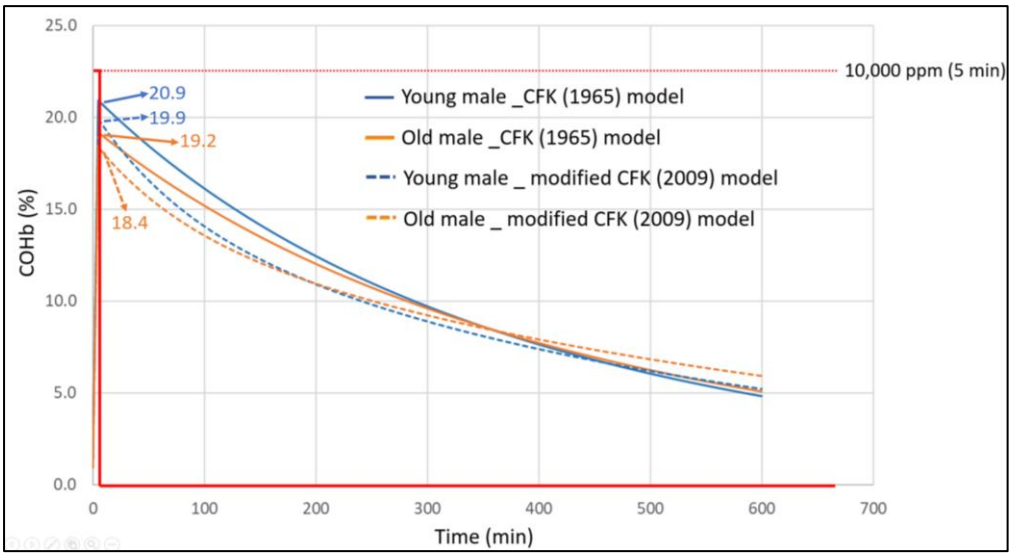

Figure 6-16 Simulation for young males and old males in Scenario B (10,000 ppm for 5 min)

Comparison of CO uptake and CO elimination between tall and short males

When comparing the CO uptake and CO elimination rate between tall and short males, only the DL_{CO} differed (see Table 6-16). The values of height for males were derived from the TSGH relatively healthy group, the value of the percentiles in 25% and 75% for height. The tall young male was assumed as 177 cm with DL_{CO} value as 37.5 ml/min/mmHg, and the short young male was 168 cm with DL_{CO} value as 36.2 ml/min/mmHg. Figure 6-17 shows that tall males had a slightly increased CO uptake and CO elimination rate than short males. Similar results were also seen in Scenario B, Figure 6-18.

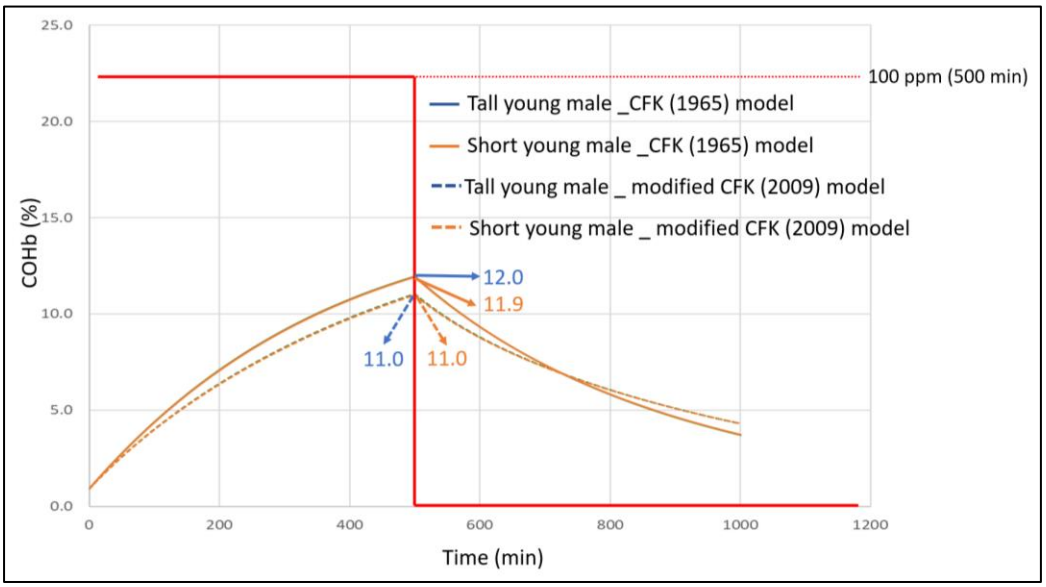

Figure 6-17. Simulation for tall and short young males in Scenario A (100 ppm for 500 mins)

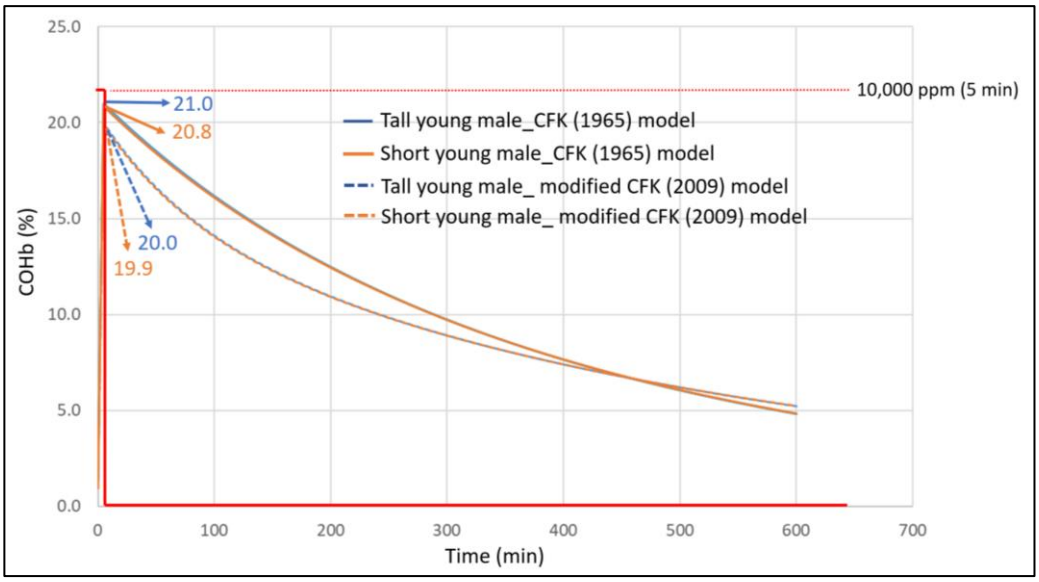

Figure 6-18. Simulation for tall and short males in Scenario B (10,000 ppm for 5 mins)

Comparison of CO uptake and CO elimination between male smokers and male non-smokers

Table 6-16 shows the demographic, physiological and behavioural values used in the simulation and Table 6-17 shows that initial COHb and DL_{CO} differed between smokers and non-smokers and used in the simulation. The simulation focused on males rather than females due to the smaller sample size of female smokers in the predictive model for DL_{CO} from the TSGH relatively healthy group.

The comparison showed that even though smokers had a higher blood COHb value, both CO uptake and CO elimination rate are similar in young male smokers and non-smokers (see Figure 6-19 and Figure 6-20).

Table 6-17. Basic characteristics of the four types of smokers

	Young male	**Young male**	**Old male**	**Old male**
Age	20	20	65	65
Smoking status	Non-smoker	Smoker	Non-smoker	Smoker
DL_{CO} (ml/min/mmHg)	35.8	37.8	26.7	24.5
CFK (1965) model and Modified CFK (2009) model with estimated DL_{CO}				
Initial COHb (%)	0.93	1.73	0.93	1.73
$[COHb]_0$ (ml/ml)	0.0023	0.0043	0.0023	0.0043
Initial CO amount (ml)	13.34	24.82	13.34	24.82

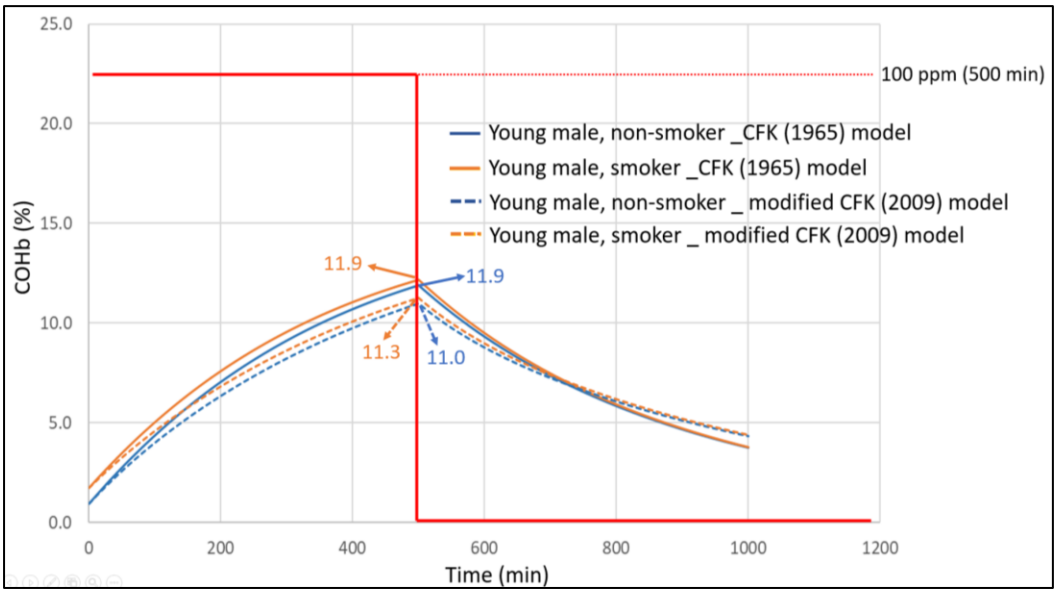

Figure 6-19. Simulation for smokers and non-smokers of young males in Scenario A (100 ppm for 500 min)

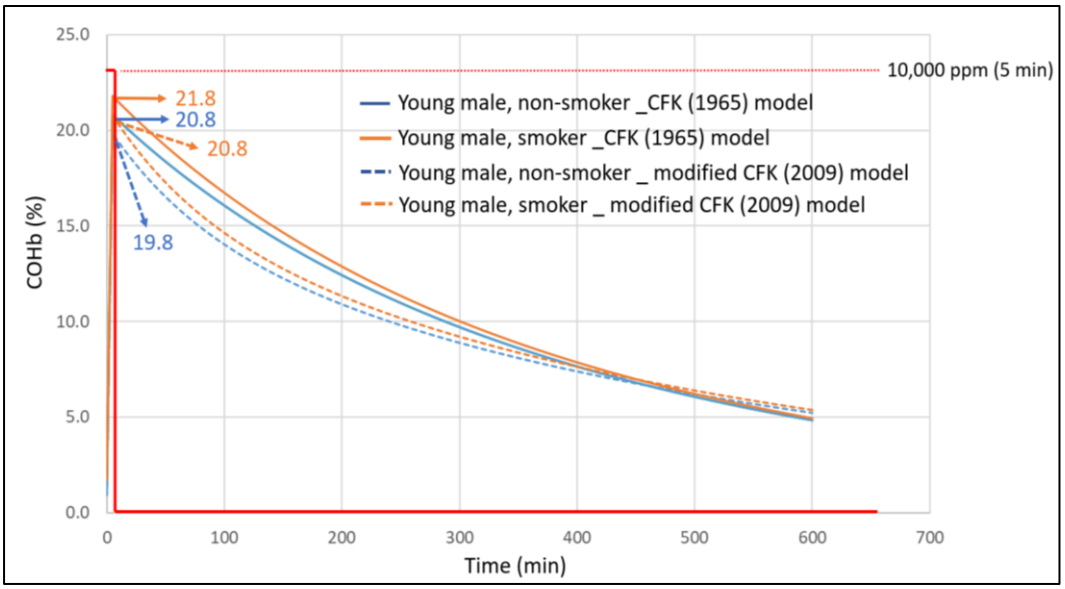

Figure 6-20. Simulation for smokers and non-smokers of young males in Scenario B (10,000 ppm for 5 min)

Even though the differences in the rate of CO uptake and CO elimination between males and females, young and old males, tall and short males, and smokers and non-smokers seem to be small, the differences would increase if all the factors combined or in situations of higher CO concentration or longer duration. Also, it should be noted that factors such as age, sex, height, weight and smoking status would vary from person to person in real life and so their effects on the rate of CO uptake and CO elimination should be taken into account to produce a realistic modelling of CO exposure.

6.4 Discussion and conclusion

After simulating the predicted data from four different models – CFK (1965), modified CFK (2009), and CFK (1965) and modified CFK (2009) with the estimated DL_{CO} – the predicted data was compared to the measured data from the literature, the CO-rebreathing experiment and the exhaled CO experiment. This section offers a discussion and comparison of differences between the original models and the models with the new estimated input of DL_{CO}; the predicted CO uptake and elimination for different individuals in various scenarios are also presented.

6.4.1 Comparison of the original models and the models with estimated DL_{CO}

The difference between the original models and the models with new input was the predictive model for DL_{CO}. The parameter used for the predictive model for DL_{CO} was the body surface area in the CFK (1965) model (Coburn et al., 1965; Peterson and Stewart, 1975), and sex, age and height in the modified CFK (2009) model (Paoletti et al., 1985; Gosselin et al., 2009) (see Section 2.3 and Appendix 9.2.1 for details). In the models with estimated DL_{CO}, the parameters for the predictive model for DL_{CO} were taken from the TSGH relatively healthy group and the RBH group with normal PFT.

The literature shows that DL_{CO} affects the rate of CO uptake and elimination significantly (Filley et al., 1954; Coburn et al., 1965; Bruce and Bruce, 2003; Gosselin et al., 2009). When the value of DL_{CO} increases, the rate of CO uptake and elimination also increases. This relationship was shown in the results of all the simulations.

In the present study, the researcher has compared the predicted data with the measured data from different sources, including the literature (Stewart et al., 1970), our CO-rebreathing experiment and our exhaled CO experiment. When comparing the predicted data with the measured data from Stewart et al.' (1970) study, the predicted data from all the CFK models showed an acceptable prediction, which is close to 1. Generally, in the steady CO exposure scenarios, the CFK models showed a good prediction for the rate of CO uptake and elimination (Coburn et al., 1965; Peterson and Stewart, 1970; Bruce and Bruce, 2003; Gosselin et al., 2009).

When comparing the predicted data with the measured data from our CO-rebreathing experiment (Table 6-9), it showed that modified CFK models seem to have a slightly better prediction than CFK models, which is similar to the previous

study (Gosselin et al., 2009). In the previous studies, they have mentioned that the CFK model might be less accurate when predicting the rapidly varying CO exposure scenarios because the CFK model only contains one compartment (Peterson and Stewart, 1970; Bruce and Bruce, 2003; Gosselin et al., 2009). Moreover, the prediction of CFK models for all subjects are similar, but the prediction of modified CFK models was better in subject C compared to others (Figure 6-9). The possible reason might be the modified CFK model was only validated with male data (Gosselin et al., 2009). Therefore, its prediction of the CO uptake and elimination rate for male subjects would be better than for female subjects.

Moreover, when predicting the time of COHb concentration back to normal for subjects in the CO-rebreathing experiment, it would take around 15 hours for the COHb concentration back to normal in our model, which is similar to the literature. In the literature, the time taken for COHb concentration back to normal would take more than 10 hours depending on the different situation, such as the time and level of CO exposure, age and alveolar ventilation (Coburn et al., 1965; Stewart et al., 1970; Hill et al., 1977; Eichhorn et al., 2018).

When comparing the predicted data with the measured data from our exhaled CO study, the correlation of CFK models for Asians was slightly higher than for Caucasians (Table 6-10 and Table 6-12). The possible reason for the lower correlation for Caucasians might be more females in the Caucasian group. As mentioned in the literature, CFK models were based on healthy, white males (Coburn et al., 1965; Gosselin et al., 2009). Fortunately, in the analysis, there were only 1 female in the Asian group and 3 females in the Caucasian group and they might not have a significant impact on the prediction. Therefore, even though the correlation was slightly higher in Asians all the CFK models showed an acceptable prediction for both Asians and Caucasians.

Overall, when comparing the predicted data with the measured data from the literature, the CO-rebreathing experiment and the exhaled CO experiment, the predicted data from both the CFK models (i.e. whether original DL_{CO} or estimated DL_{CO}) showed a high correlation with an acceptable prediction. Even though the models with estimated DL_{CO} did not show a better prediction than the models with original DL_{CO} in the healthy population, our estimated DL_{CO} opens up a possibility of producing realistic modelling of the rate of CO uptake and elimination among a wide range of individuals, including those in poor health, in future work. The

researcher will build the predictive models for DL_{CO} based on individuals with abnormal PFT from the hospitals' datasets. Therefore, according to each specific lung disease, the DL_{CO} could be predicted and used to simulate a more accurate rate of CO uptake and elimination among these groups.

There are several possible reasons why our estimated DL_{CO} did not show a significantly better prediction than the original DL_{CO} when used in the CFK models. The first was the limited effect of ethnicity for DL_{CO}: although there was a difference in DL_{CO} between ethnicities, the effects of other parameters, such as age and height, might explain more variation in DL_{CO}. Second, our measured data is confined to young and healthy participants, so it is difficult to speculate how the inclusion of older participants would affect the results. Lastly, there are other parameters in the CFK models that also play important roles in predicting the rate of CO uptake and elimination, such as blood volume, alveolar ventilation, Hb concentration, and should be considered together in the future.

6.4.2 Comparison of CO exposure models for different individuals in different scenarios

Effects of different individuals on CO uptake and elimination

In the study, we aimed to see the effects of factors such as age, sex, height, weight, ethnicity and smoking status on the rate of CO uptake and CO elimination. In the PFT section, all these factors exhibited an influence on DL_{CO}, which is an important parameter in the CFK models (Coburn et al., 1965; Gosselin et al., 2009). When we control for the same CO exposure for different individuals, the CO uptake and elimination rates were higher in a younger male compared to an older male, in a taller male compared to a shorter male, and in males compared to females.

Our study revealed that age may play a role in DL_{CO} and may potentially affect CO uptake and elimination according to the CFK models (Figure 6-15 and Figure 6-16). Figure 6-16 showed that young males had a higher rate of CO uptake and elimination than old males. When exposed to 10,000 ppm CO for 5 min, the difference of COHb concentration in the peak would be around 1.5 to 2 % between young males and old males. However, previous studies found age had no significant effect on COHb half-life (Burney et al., 1982; Weaver et al., 2000). In Burney et al.'s (1982) study, despite a wide age range (9-86) of the 146 students and 38 teachers/school employees participating, the average age was only 20: the relative

lack of older participants means that the effects of age may not be significant. Weaver et al. (2000) only separated their participants into two groups: under 40-year-olds and over 40-year-olds: more and narrower age groups may have led to discovery of the effects of age. Similar to previous studies (Filley et al., 1954; Bruce and Bruce, 2003), we found that taller people have a higher value of DL_{CO} (Figure 6-17 and Figure 6-18), and this may increase CO uptake and elimination rate. Also, taller people with higher DL_{CO} would increase the CO uptake and elimination rate when controlling for all other factors.

The effects of sex are complicated. Some studies have found no significant effect of sex in the rate of CO elimination (Burney et al., 1982; Weaver et al., 2000) while others, for example, Pace et al. (1950), stated that females had a shorter COHb half-life than males (however, they did not explain the reasons for this in the study). Zavorsky et al. (2014) conducted an experiment to find any underlying factors that might cause a difference in COHb half-life between males and females. Their results showed that alveolar ventilation and total Hb mass were the main factors that affected the rate of CO elimination between sexes. When alveolar ventilation increased, the CO half-life decreased. However, when they normalised the COHb half-life with total Hb mass, the difference between the sexes disappeared. Therefore, they suggested that the difference in COHb half-life between males and females was mainly affected by total Hb mass, that is, the CO store in the body. It should be noted that they did not, however, consider factors such as DL_{CO} and myoglobin.

In our study, we found that males had an increased CO uptake and elimination compared to females due to the higher value of DL_{CO} (Figure 6-13 and Figure 6-14), which is similar to previous studies (Filley et al., 1954; Bruce and Bruce, 2003). However, we did not consider the effects of alveolar ventilation, total Hb mass or myoglobin which makes it more difficult to ascertain the effects of sex on CO uptake and elimination. Future investigations of the effects of sex on CO uptake and elimination should include a more thorough body assessment, including lung function, DL_{CO}, ventilation, Hb concentration, total Hb mass and myoglobin. When predicting the COHb half-life, the important factor in prolonging COHb half-life is the CO store in the body, including in Hb and myoglobin.

In our study, smoking status did not significantly affect the rate of CO uptake and elimination. However, Cronenberger et al. (2008) stated that the median (range) CO half-life was 30.9 hours (7.13-367) in adult smokers, which is much longer than

the four hours found by (Kao and Nanagas, 2004). We suspect that the reason for this is that the predictions were still based on relatively healthy participants, so the DL_{CO} value was not very different between smokers and non-smokers. Storebø et al. (2016) found that DL_{CO} declined more rapidly with current smoking and more pack years. Additionally, several clinical conditions caused by smoking could also decrease DL_{CO}, including emphysema, COPD and pulmonary fibrosis (Harvey et al., 2015; Nakazawa et al., 2018). Therefore, to understand the effects of smoking on the rate of CO uptake and elimination, the number of cigarettes smoked and any smoking-related diseases should be taken into account in any future studies.

Effects of different scenarios on CO uptake and elimination

The most important factors affecting CO uptake and elimination are CO concentration and CO exposure duration (WHO, 2010). Different CO concentrations and the duration of CO exposure determine the categorisation of different CO exposure scenarios. When exposed to high CO concentration over a short time, the curve of CO uptake and elimination from both the CFK (1965) and modified CFK (2009) models was sharp (Figure 6-14, Figure 6-16, Figure 6-18 and Figure 6-20). When exposed to the same amounts of CO over a longer period, the curve was smoother (Figure 6-13, Figure 6-15, Figure 6-17 and Figure 6-19). The results were similar to those found in most available literature (Forbes et al., 1945; Peterson and Stewart, 1970; Peterson and Stewart, 1975; Bruce and Bruce, 2006).

The possible reason for the difference of CO half-life for the same amount of CO with short-term low and long-term high CO exposure scenarios might be the amount of CO entering the tissue. Generally, if the CO exposure period was longer, more CO would enter into the tissue and increase the CO elimination time (Bruce and Bruce, 2006). From our study, the different CO half-life for the short-term low and long-term high CO exposure scenarios could be calculated. To distinguish the CO exposure scenarios, COHb at two time points should be taken and calculated to CO half-life (Weaver et al., 2000; Ozturan et al., 2019). Then, the calculated CO half-life could be compared with the simulated CO half-life from the study. Therefore, the CO elimination curve could give the information for backcasting the CO exposure scenarios, which means to identify if it was a long-term low or a short-term high exposure of CO.

6.4.3 Limitations

The limitations in the modelling were as follows. First, when simulating and comparing the data with that from Stewart et al.'s (1970) study, it was found that they had only reported the average demographic and physiological factors data of participants. Therefore, the simulation was based on the data from the average of all participants rather than each participant. However, since all their participants were young and healthy, the results showed that the predicted data had a good fit with the reported data.

Second, the actual exposure of CO amount was hard to measure in the exhaled CO experiment. Many factors, such as exercise, cigarette type, puffs and individuals' smoking habit, could make the exposure amount different and are hard to control for the experiment. Therefore, this study used the average CO amounts of exposure of cigarette from the report (WHO, 1999). This may reduce the validity of the predicted data from the simulations.

Third, when predicting the rate of CO uptake and elimination for smokers, the exhaled CO was calculated to COHb by using the empirical relationship formula – a simple linear regression equation from Jarvis et al.'s (1980) study. Although there are some factors may affect the equation, such as being younger and impaired lung function, the regression provided an acceptable prediction for 'average' people ($r = 0.95$, $n=75$) (Jarvis et al., 1980; Jarvis et al., 1986). In our study, we only included healthy adult smokers. Therefore, the equation should work with acceptable prediction.

Fourth, parameters might change over time, such as populations overall get taller, live longer, etc. However, in our study, the predicted data from the simulation in section 6.3.1 was not only compared to the measured data from Stewart et al.' (1970) study but also compared to the data from our CO-rebreathing experiment and our exhaled CO study. In the results, all CFK models showed an acceptable correlation, which was close to 1. Therefore, the parameters change over time, such as height and age, should not have a significant impact on the prediction of models.

Last but not least, lack of any measured COHb data in the literature of the rate of CO uptake and elimination from old(er) people and unhealthy individuals. The study's measured data were all from young healthy participants, and their characteristics may be different from older people, smokers and people with different diseases. Better results would be achieved if we could test the model with these data.

For example, if it was possible, the researcher would like to invite old(er) people and unhealthy individuals to be exposed to CO, such as using the DL_{CO} test or CO-rebreathing experiment. Then, recording their CO uptake and elimination time. After collecting the data of CO uptake and elimination time, the researcher would use these data to simulate the CFK models. If the CFK models still showed a good prediction, the results from the CFK models could be used not only for healthy young adults but also for the population of old(er) people and unhealthy individuals.

6.4.4 Conclusion

In conclusion, both CFK models and modified CFK models showed a good prediction of the CO uptake and elimination rate in stable and long-term CO exposure scenarios. However, in the relatively short-term and high concentration CO exposure scenarios, modified CFK models showed a better prediction than CFK models, especially for males. Moreover, when sex, age, and height are constant, CFK models with estimated DL_{CO} from the present study produces a similar prediction of COHb value to the CFK models with its original DL_{CO} model. However, the model from the present study is capable of producing predictions for individuals of a wide range of ages, heights and weights, either males or females, smokers or non-smokers, and of either Caucasian or Asian ethnic background.

In the simulations from both CFK models and modified CFK models, when inhaling the same amount of CO, the longer the period of CO exposure, the longer the COHb half-life. Moreover, if comparing the different characteristics of people, males were found to have a higher CO uptake and elimination rate than females, as were younger people compared to old people. However, even though smoking status affected the baseline blood COHb, both smokers and non-smokers had a similar rate of CO uptake and CO elimination trends. Although the results show that backcasting of the CO exposure scenarios using only the models may not be highly accurate, it could help discriminate between different exposure scenarios in individuals presenting to health services.

Further studies could validate the CO uptake and elimination rate with not only the relatively healthy population but also females, old people and people with some health issues. Also, the simulation of CO exposure could be more complicated, such as combine the short-term CO exposure and long-term CO exposure.

7 GENERAL DISCUSSION AND CONCLUSIONS

Section 7.1 describes the general discussion of the three constituent parts and lays out the strengths and limitations in the present study. Section 7.2 summarise the main findings, gives a conclusion and provides some recommendations and suggestions for future study.

7.1 General discussion, strengths and limitations

7.1.1 General discussion

Discussion for each section has been described in sections (Section 3 to Section 6). In this section, the general discussion would focus on the concept that links with the whole present study. In the present study, the effects of factors, such as age, gender, height, weight, ethnicity and smoking status, on the rate of CO uptake and elimination were explored. Also, the empirical data in the present study was used to validate the existing human CO exposure models.

The literature showed that the main system related to the rate of CO uptake and elimination are the respiratory system and the blood system (Filley et al., 1954; Coburn et al., 1965; Bruce and Bruce, 2003; Gosselin et al., 2009). In the respiratory system, the main factors related to the rate of CO uptake and elimination were alveolar ventilation (the exchange of gas between the alveoli and the external environment) and DL_{CO} (the efficiency of gas exchange from lungs to blood) (Coburn et al., 1965; Bruce and Bruce, 2003; Gosselin et al., 2009; Strong, 2014c). Section 5 in the present study showed that age, gender, height, weight and ethnicity (Caucasian vs Asian) have impacted the DL_{CO} value, which was similar to other studies (Paoletti et al., 1985; Stanojevic et al., 2008; Stanojevic et al., 2017; Talaminos Barroso et al., 2018). Therefore, if people were younger, male, tailer, heavier or Caucasian, their DL_{CO} value would be higher and then the rate of CO uptake and elimination should be higher as shown in Section 5 and Section 6.

Even though the smoking status did not show a significant effect on the rate of CO uptake and elimination in Section 4 and Section 6, it did show a possible impact on the DL_{CO} among old people in Section 5. The possible reason that smoking status did not show a significant effect on the CO uptake and elimination might be a big variation of the smoking habit among smokers, such as years of smoking, the number of puffs, interval time between puffs and the depth of smoking (Castleden and Cole,

1975; Zhang et al., 2013; Maga et al., 2017; Schimmel et al., 2018). If the smoking habits could be carefully controlled, the effects of smoking status on the rate of CO uptake and elimination might be understood.

In the blood system, the main factors related to the rate of CO uptake and elimination were blood volume and Hb concentration (Coburn et al., 1965; Bruce and Bruce, 2003; Gosselin et al., 2009). Section 3 showed that a person with the lowest Hb concentration, blood volume and total Hb mass, had the shortest CO half-life, which was similar to other studies (Coburn et al., 1965; Woehlck et al., 2001; Bruce and Bruce, 2006; Zavorsky et al., 2014). Blood volume also plays a role: as it increases, CO uptake and elimination rates decrease (Coburn et al., 1965; Bruce and Bruce, 2006). Therefore, if people had a lower value of blood volume, Hb concentration or total Hb mass, their COHb half-life should be shorter as shown in Section 3 and Section 6. However, Section 3 only contains three subjects. It is hard to determine the effects of the blood characteristics on the rate of CO uptake and elimination due to the small sample size. To give a solid conclusion, the sample size should be increased.

Through the different findings from the sections (Section 3 to Section 6) of the present study, the final CO exposure model from the present study is capable of producing predictions for individuals of a wide range of ages, heights and weights, either males or females, smokers or non-smokers, and of either Caucasian or Asian ethnic background. However, as there are many possible causes and ways to have the same values of COHb in different individuals, some studies have measured the CO concentration in tissue, rather than measuring COHb, in their attempts to obtain information about different CO exposure scenarios (Vreman et al., 2006; Oliverio and Varlet, 2020). However, Vreman et al.'s (2006) study was only able to use the concentration of CO in the tissue to distinguish the cause of death rather than for back-casting the CO exposure scenarios.

It was hard to use only current COHb to back-cast the CO exposure scenarios: a doctor who wants to back-cast the CO exposure scenario of a patient who attends an Emergency Department would need to ask further questions of the patient, including exact location relative to CO sources, time-activity, likely source concentration and likely duration of exposure (Hampson et al., 2012; Clarke et al., 2012). However, if the characteristics of the patients could be obtained, the back-

casting of the CO exposure scenarios of the individuals from the present study could give useful indications of varying exposure scenarios for individuals.

7.1.2 Strengths and limitations

Strengths

Several strengths of the present study are described as follows,

First, in the CO-rebreathing experiment (Section 3), even though the sample size was too small to have a solid conclusion, it did indicate that the blood volume, Hb concentration and total Hb mass might have an impact on the rate of CO uptake and elimination. If the blood volume, Hb concentration and total Hb mass increase, the rate of CO uptake and elimination will be slower. Moreover, it is the first study to investigate the measured CO elimination data by measuring total Hb mass not the calculated Hb mass (Zavorsky et al., 2014).

Second, in the exhaled CO study (Section 4), even though no significant factors were found to affect the CO uptake and elimination rate, it is the first study to use a breath CO monitor to calculate exhaled CO half-life and explore factors affecting baseline exhaled CO concentration and exhaled CO half-life. The calculated exhaled CO half-life using a breath CO monitor was relatively similar to the COHb half-life measured in blood, especially in young healthy adults. Therefore, exhaled CO could be used as a marker of CO exposure. Also, this part of study has been published in the *International Journal of Environmental Research and Public Health* in November 2021 (Pan et al., 2021), please see Appendix 9.3.

Third, in the PFT data analysis (Section 5), the PFT data were collected from two hospitals in different countries, Tri-Service General Hospital (Taiwan) and Royal Berkshire Hospital (UK), which needed great effort. Therefore, the effects of not only age, gender, height, weight and smoking status but also ethnicity on the important pulmonary function parameters (DL_{CO}, V_A and K_{CO}) for the rate of CO uptake and elimination could be compared.

Last but not least, in the CO exposure models simulation (Section 6), the CO exposure models were validated by not only the measured data from the literature but also the empirical data from our studies (Section 3, Section 4 and Section 5). The sections in the present study were closely linked with each other to give a solid conclusion. As precision medicine or customised medicine has been emphasised, the

one-size-fits-all approach has been criticised and the approach for treatments should be based on the different characteristics of individuals. From the findings, the CO exposure models could predict the rate of CO uptake and elimination for individuals of a wide range of ages, either males or females, heights and weights, smokers or non-smokers, and of either Caucasian or Asian ethnic background. Therefore, the models could not only give indications of the possible exposure CO scenarios for individuals but also provide useful guides for optimal treatment for individuals with various characteristics.

Limitations

Specific limitations have been presented in each section (Section 3 to Section 6), in this section the overall limitations of the present study are discussed.

As mentioned in the general discussion, CO uptake and elimination occurs in two main systems; the respiratory system (pulmonary function) and the blood system (blood characteristics). In our present study, the CO-rebreathing experiment (Section 3) explores the effects of factors related to blood characteristics on the rate of CO uptake and elimination. The pulmonary function data collection (Section 5) helps understand the effects of factors related to pulmonary function on the rate of CO uptake and elimination. However, there were only 3 subjects collected in the CO-rebreathing experiment. Therefore, the present study was hard to explore the factors related to blood characteristics. Future studies could discuss more the effects of the factors related to blood characteristics on the kinetics on CO uptake and elimination in the human body.

Moreover, in the present study, the researcher tried to validate the CO exposure model with a wide range of different characteristics, such as age, gender, height, weight, ethnicity and smoking status, among the general population. However, each section was based on different settings, including the CO-rebreathing experiment (Southampton General Hospital), the exhaled CO study (UCL), and the pulmonary function data collection (Tri-Service General Hospital and the Royal Berkshire Hospital). Also, there were missing data in the studies. Therefore, the effects of some factors, such as Hb, body fat, menstrual cycle and smoking habits, between each participant can’t easily be controlled for without more subjects with more detailed data.

Also, through the present study, lack of any measured COHb data of the rate of CO uptake and elimination from old people and unhealthy individuals. Therefore, it is hard to validate the CO exposure models among these people. Old people and unhealthy individuals might have different kinetics of CO uptake and elimination from young healthy individuals. In the future, the COHb measured data from these people could be more explored.

7.2 Conclusions and recommendations

CO poisoning is a critical public health issue in the world. CO exposure may cause headache, nausea and even loss of consciousness or death. Moreover, some patients may experience long-lasting neuropsychological sequelae and cognitive and psychological sequelae (DNS). Currently, the accepted approach to treating CO poisoned patients is to flush the CO from the body as quickly as possible, which is supported by the existing CO exposure models. These models predict the kinetics of CO uptake and elimination but are based on a limited dataset: it is necessary to improve these models using a wider population to enable optimal, more personalised treatment.

7.2.1 Conclusions

The literature presents several types of different human CO exposure models but these are often based on a limited number of people that are generally healthy, white and male. This work seeks to expand these CO exposure models to people with wider range of different characteristics, varying in age, sex, height, weight, smoking status and ethnicity. Therefore, different study designs were used to test the relationship between these demographic, physiological and behavioural factors and pulmonary function, the rate of CO uptake and the rate of CO elimination. Results from these three studies indicate how demographic, physiological and behavioural factors affect the results of CO exposure models through changes in pulmonary function. Finally, modified CO exposure models with estimated DL_{CO} were used to simulate the CO uptake and elimination for a wider range of individuals.

The main findings of the three studies are as follows. First, in the exhaled CO experiment, the results indicated that smoking status (light smokers vs heavy smokers) did not affect exhaled CO half-life; this may be due to the fact that the participants in the study were all young healthy smokers and had no history of lung

illness that related to smoking. However, the results may not be representative of the general population due to the small sample size and narrow age band (18-34) of the participants.

Second, analysis of both of the datasets from TSGH relatively healthy group (Asian) and the RBH with normal PFT group (Caucasian) found that demographic, physiological and behavioural factors, such as age, sex, height and weight, played an important role in pulmonary function in terms of the ability of the lungs to transfer gas from inspired air to the bloodstream. It found that age was negatively associated with DL_{CO} and it quantified such association. Similarly, the analysis provided novel quantified evidence of the positive association of height and weight with DL_{CO}. This can be explained by older lungs being less efficient at transferring CO into the body. Height and weight are strongly linked with lung volume and this may be explained by taller and heavier individuals having larger lungs and being able to take in more CO into the blood stream. Also, males had a higher value of DL_{CO} than females which is probably linked to that males have larger lungs than females; and non-smokers had a higher value of DL_{CO} than smokers, due to non-smokers have a higher value of K_{CO} than ever smokers. However, the effect of smoking status on DL_{CO} was reduced in the TSGH 'relatively healthy' group and the RBH with 'normal' PFT group; this may be due to ever-smokers in those two groups may not yet having suffered any lung damage or lung illness related to smoking. For the lung transfer coefficient, K_{CO}, the effect of smoking status was also reduced in the TSGH 'relatively healthy' group. This suggests that if smokers do not yet have any smoking-related lung disease, their DL_{CO} and K_{CO} figures are similar to non-smokers. When we took a closer look at those 'relatively healthy' smokers and 'relatively healthy' non-smokers, the DL_{CO} of smokers decreases more rapidly than non-smokers with age. Even though smoking status did not show a significant effect on DL_{CO} in either the TSGH "relatively healthy" group or the RBH 'normal' PFT group, we would expect a more significant difference in older groups as older smokers have lower DL_{CO} than younger ones: the combined effect of ageing and smoking on DL_{CO} could be an area for further research.

When comparing the resultant predictive models for DL_{CO}, V_A and K_{CO} between Asian (TSGH) and Caucasian (RBH) groups, it indicated that the Asian population might have a slightly lower value of DL_{CO} compared to the Caucasian population even after adjusting for age, sex, height and weight. From the PFT section of the work, we could see the value of lung volume (V_A) was slightly higher in the

Caucasian population, while the value of K_{CO} was similar in both Caucasians and Asians. This explains Asians' lower value of DL_{CO} when compared to the Caucasian population. The value V_A is linked to height in the predictive models, indicating that the difference in DL_{CO} between Asians and Caucasians could be explained by V_A and height differences.

Finally, after obtaining the predictive models for DL_{CO} in the PFT section, we used the estimated DL_{CO} value in the CO exposure models (CFK models) to predict the COHb level for different individuals in various CO exposure scenarios. In the CO exposure models, we focused on DL_{CO}, which is calculated from V_A and K_{CO}. It is the ability of individuals' lungs to transfer gas (CO) from inhaled air to the red blood cells in pulmonary capillaries and is a key parameter in CFK models. Simulations based on the estimated DL_{CO} from the model developed in the present study were shown to produce similar results and predictions as the CFK (1965) and modified CFK (2009) models, especially when the individuals considered had the same age, height and sex as those originally studied. However, the present study can produce simulations for a much wider range of individuals, differing by age, sex, height, ethnic group and smoking status. Therefore, these PFT data have helped to further empirically validate the CFK models. For example, the present CFK models with estimated DL_{CO}, predicted that CO uptake and elimination rate would be higher in males compared to females, in a taller male compared to a shorter one, and in a younger male compared to an older one. However, it did not show a specific difference between smoking and non-smoking young males.

The CFK models with estimated DL_{CO} from the predictive models developed during research could be used to predict the rate of CO uptake and elimination more accurately for different sub-sets of a population with different health conditions in future work. They provide more reliable and realistic modelling of predicted COHb values in different CO uptake and elimination scenarios for different individuals, which could provide useful and practical guidance in clinical science and practice.

Overall, the present study found the important factors affecting the rate of CO uptake and elimination, such as age, gender, height, weight, ethnicity and pulmonary function. Even though the smoking status did not show a significant impact on the rate of CO uptake and elimination, its effect should not be excluded in the older population. Moreover, the study helped provide empirical validation of existing human CO exposure models to better predict the rate of CO uptake and elimination

for a wider population by revealing the underlying pulmonary function. If these characteristics could be collected in the hospital, the possible exposure scenarios could be predicted. This could help medical staff distinguish the susceptible patients (who might have a longer CO elimination time) and provide more efficient treatment strategies to the different scenarios for CO poisoned patients.

Building on the current research, there are several future research perspectives. First, future work could further explore the effects of CO exposure in old people and people with different health statuses. Although it would be unethical to expose susceptible people (such as old people and people in poor health) to CO in an experiment, it should be possible to follow their CO elimination rate after a DL_{CO} test or CO-rebreathing experiment under clinical and medical staff control. Second, future studies could consider the effects of other factors, especially on blood characteristics and smoking habits; on the rate of CO uptake and elimination. Furthermore, future work could provide a comprehensive overview of demographic, physiological and behavioural factors' effects on not only pulmonary function but also blood characteristics in order to gain a more thorough understanding of factors affecting CO poisoning and personal CO exposure.

7.2.2 Recommendations

The results of the present study could help prevent, and provide treatment for, CO poisoning. Recommendations for different perspectives are given below.

From the public health perspective, it is important to set a customized standard in different settings or buildings according to the characteristics of the residents. For example, in places such as nursing homes or hospices, the residents are more susceptible (older and may have lung disease) than, for example, people working in an office; the standards for indoor CO exposure for those settings should be stricter. Also, if the exhaled CO concentration for a non-smoker was equal to or above 5 ppm, they might have exogenous CO exposure and would trigger a home investigation. For smokers, it is hard to determine if smokers have CO exogenous CO exposure. However, according to the present study, if the exhaled CO concentration is above 10 ppm for smokers whose last smoked is more the four hours ago, the possibility of exogenous CO exposure and an investigation should be considered.

From the CO exposure modelling perspective, under stable CO exposure, the CFK model and modified CFK model both showed good predictions for the rate of

CO uptake and elimination. However, if people are exposed to a high concentration of CO for a relatively short time, the modified CFK model would be recommended. Moreover, some important factors affecting the rate of CO uptake and elimination have been found in the models in the present study. Therefore, for modellers, if they considered predicting the CO exposure of the population, different characteristics should be considered, such as; age, gender, height, weight, ethnicity and pulmonary function. For example, when doing the empirical modelling, different target groups with different characteristics could be set (ie. Female group, male group, young group, older group, etc.); when doing the microenvironmental exposure modelling, individuals who have a longer CO elimination time could be simulated in different groups, such as people with older age and those with lower DL_{CO} value.

From the health care perspective, the present study could help medical staff to identify more susceptible patients and design more accurate individual treatment strategies. To identify the susceptible patients for CO poisoning, the factors affecting the rate of CO uptake and elimination from the present study should be collected, such as age, gender, height, weight and ethnicity. Even though the smoking status did not show a significant impact on healthy young individuals, the factor should be also collected in the hospital, especially for old patients. Moreover, after these factors are collected, two possible CO exposure scenarios (long-term CO exposure and short-term CO exposure) could be simulated by CFK models. If the concentrations of COHb at 2 different time points (ie. at scene, at ED, before HBO treatment, etc.) for a patient are collected, the COHb half-life will be calculated and compared with the simulated data from CFK models. Then, the possible CO exposure scenarios of the patient could be predicted. Therefore, for susceptible patients who may have a longer CO half-life, the initial rapid treatment should be supplemented by follow-up procedures to check the amount of CO remaining in the body. This would also guide any decisions regarding testing for DNS for these patients.

From the built environment perspective, the study informs architects, engineers, and designers about populations who are particularly susceptible to CO poisoning. When designing buildings for different settings, they should pay attention to the characteristics of the residents in the settings, such as old people, children, or people suffering chronic diseases; and the environment around the building, such as heavy road traffic or fossil-fuel power stations. Sufficient ventilation design for kitchens,

heating and hot water boiler or places with CO sources should be kept in mind, especially in nursing homes or hospices.

CO poisoning consequences are influenced by CO uptake and elimination which are, in turn, affected by various factors. Several CO exposure models have been built to predict the COHb in the human body. However, these models are based on a limited dataset and a clear and thorough picture of the factors and underlying mechanism for CO uptake and elimination is yet to be achieved. Much work on CO exposure and the potential factors affecting CO uptake and elimination for people with different characteristics still needs to be done. Our present study has empirically validated the CO exposure models with PFT datasets to enable them to cover a wider population and has given a better understanding of CO exposure and CO poisoning in the general population. Additionally, it opened up the possibility to produce simulations tailored to a wide range of specific physiological and clinical conditions. This research has laid a foundation for future researchers to build on, exploring the effects of different CO exposure scenarios on differing individuals in order to guide medical staff in designing a more customised treatment for CO poisoned patients.

8 REFERENCES

Agostoni, P., Bussotti, M., Cattadori, G., et al. (2006) 'Gas diffusion and alveolar–capillary unit in chronic heart failure', *Eur Heart J*, 27(21), pp. 2538-2543.

Ahlgrim, C., Birkner, P., Seiler, F., et al. (2018) 'Applying the optimized CO rebreathing method for measuring blood volumes and hemoglobin mass in heart failure patients', *Front physiol*, 9, 1603.

Allen, A. M., Scheuermann, T. S., Nollen, N., et al. (2016) 'Gender differences in smoking behavior and dependence motives among daily and nondaily smokers', *Nicotine Tob Res*, 18(6), pp. 1408-1413.

Alm, S., Reponen, A., Mukala, K., et al. (1994) 'Personal exposures of preschool children to carbon monoxide: Roles of ambient air quality and gas stoves', *Atmos Environ*, 28(22), pp. 3577-3580.

Anderson, T. W. and Shephard, R. J. (1969) 'Normal values for single-breath diffusing capacity – the influence of age, body size and smoking habits', *Respiration*, 26(1), pp. 1-7.

Antczak, A., Ciebiada, M., Kharitonov, S. A., et al. (2012) 'Inflammatory markers: exhaled nitric oxide and carbon monoxide during the ovarian cycle', *Inflammation*, 35(2), pp. 554-559.

Ashcroft, J., Fraser, E., Krishnamoorthy, S. and Westwood-Ruttledge, S. (2019) 'Carbon monoxide poisoning', *BMJ*, 365, pp. l2299.

Ashok, V., Gupta, T., Dubey, S. and Jat, R. (2014) 'Personal exposure measurement of students to various microenvironments inside and outside the college campus', *Environ Monit Assess*, 186(2), pp. 735-750.

Asuero, A. G., Sayago, A. and González, A. G. (2006) 'The correlation coefficient: an overview', *Crit Rev Anal Chem*, 36(1), pp. 41-59.

Babb, T. G., Wyrick, B. L., DeLorey, et al. (2008) 'Fat distribution and end-expiratory lung volume in lean and obese men and women', *Chest*, 134(4), pp. 704-711.

Badireddy, M. and Baradhi, K. (2019) *Chronic Anemia*. Treasure Island (FL): StatPearls. Available at: https://www.ncbi.nlm.nih.gov/books/NBK534803/ (Accessed: 14 Mar 2020).

Bal, U., Sönmez, B. M., Inan, S., et al. (2019) 'The efficiency of continuous positive airway pressure therapy in carbon monoxide poisoining in the emergency department', *Eur J Emerg Med*, 27(3), 217-222.

Barn, P., Giles, L., Héroux, M.-E. and Kosatsky, T. (2018) 'A review of the experimental evidence on the toxicokinetics of carbon monoxide: the potential role of pathophysiology among susceptible groups', *Environ Health*, 17(1), pp. 13.

Barreiro, T. J. and Perillo, I. (2004) 'An approach to interpreting spirometry', *Am Fam Physician*, 69(5), pp. 1107-1114.

Batubara, T. L., Yunus, F., Ratnawati, Y. and Nurwidya, F. (2017) 'Pulmonary diffusion capacity for carbon monoxide (DLCO) in Indonesian patients with end-stage renal disease', *Maedica*, 12(4), pp. 235-241.

Bellin, P. and Spengler, J. D. (1980) 'Indoor and outdoor carbon monoxide measurements at an airport', *J Air Pollut Control Assoc*, 30(4), pp. 392-394.

Benignus, V. A., Hazucha, M. J., Smith, M. V. and Bromberg, P. A. (1994) 'Prediction of carboxyhemoglobin formation due to transient exposure to carbon monoxide', *J Appl Physiol*, 76(4), pp. 1739-1745.

Biener, L. and Albers, A. B. (2004) 'Young adults: vulnerable new targets of tobacco marketing', *Am J Public Health*, 94(2), pp. 326-330.

Biglan, A., Magis, K., Dirocco, A. and Silverblatt, A. (1986) 'First versus second portion of expired air and duration of breath holding in the sampling of expired air carbon monoxide', *Br J Addict*, 81(2), pp. 283-286.

Bisby, J. A., Ouw, K. H., Humphries, M. and Shandar, A. G. (1977) 'Absorption of lead and carbon monoxide in Sydney traffic policement', *Med J Aust*, 1(13), pp. 437-439.

Bjartveit, K. and Tverdal, A. (2005) 'Health consequences of smoking 1–4 cigarettes per day', *Tob Control*, 14(5), pp. 315-320.

Blakemore, W. S., Forster, R. E., Morton, J. W. and Ogilvie, C. M. (1957) 'A standardized breath holding technique for the clinical measurement of the diffusing capacity of the lung for carbon monoxide', *J Clin Invest*, 36(1 Part 1), pp. 1-17.

Blumenthal, I. (2001) 'Carbon monoxide poisoning', *J R Soc Med*, 94(6), pp. 270-272.

Boulbou, M. S., Gourgoulianis, K. I., Klisiaris, V. K., et al. (2003) 'Diabetes mellitus and lung function', *Med Princ Pract*, 12(2), pp. 87-91.

Bowdish, D. M. E. (2019) 'The aging lung: is lung health good health for older adults?', *Chest*, 155(2), pp. 391-400.
Boyer, L., Chouaïd, C., Bastuji-Garin, S., et al. (2015) ' Aging-Related Systemic Manifestations in COPD Patients and Cigarette Smokers', *PLoS One*, 10(3), pp. e0121539.
Braubach, M., Algoet, A., Beaton, M., et al. (2013) 'Mortality associated with exposure to carbon monoxide in WHO European Member States', *Indoor Air*, 23(2), pp. 115-125.
British Lung Foundation. (2020) Available at: https://www.blf.org.uk/support-for-you/breathing-tests/exhaled-carbon-monoxide-test (Accessed: 11 December 2021).
Brown, R. P., Delp, M. D., Lindstedt, S. L., et al. (1997) 'Physiological parameter values for physiologically based pharmacokinetic models', *Toxicol Ind Health*, 13(4), pp. 407-484.
Bruce, E. N. and Bruce, M. C. (2003) 'A multicompartment model of carboxyhemoglobin and carboxymyoglobin responses to inhalation of carbon monoxide', *J Appl Physiol*, 95(3), pp. 1235-1247.
Bruce, E. N., Bruce, M. C. and Erupaka, K. (2008) 'Prediction of the rate of uptake of carbon monoxide from blood by extravascular tissues', *Respir Physiol Neurobiol*, 161(2), pp. 142-159.
Bruce, M. C. and Bruce, E. N. (2006) 'Analysis of factors that influence rates of carbon monoxide uptake, distribution, and washout from blood and extravascular tissues using a multicompartment model', *J Appl Physiol*, 100(4), pp. 1171-1180.
Bruinen de Bruin, Y., Carrer, P., Jantunen, M., et al. (2004) 'Personal carbon monoxide exposure levels: contribution of local sources to exposures and microenvironment concentrations in Milan', *J Expo Anal Environ Epidemiol*, 14(4)**,** pp 312-322
Buckley, N. A., Juurlink, D. N., Isbister, G., et al. (2011) 'Hyperbaric oxygen for carbon monoxide poisoning', *Cochrane Database Syst Rev*, (4), pp. CD002041.
Burney, R. E., Wu, S.-C. and Nemiroff, M. J. (1982) 'Mass carbon monoxide poisoning: Clinical effects and results of treatment in 184 victims', *Ann Emerg Med*, 11(8), pp. 394-399.
Burrows, B., Kasik, J. E., Niden, A. H. and Barclay, W. R. (1961) 'Clinical usefulness of the single-breath pulmonary diffusing capacity test', *Am Rev Respir Dis*, 84(6), pp. 789-806.
Bush, A. and Gabriel, R. (1991) 'Pulmonary function in chronic renal failure: effects of dialysis and transplantation', *Thorax*, 46(6), pp. 424-428.
Caglar, B., Serin, S., Yilmaz, G., et al. (2019) 'The impact of treatment with continuous positive airway pressure on acute carbon monoxide poisoning', *Prehosp Disaster Med*, 34(6), pp. 588-591.
Carlsten, A., Holmgren, A. L. F., Linroth, K., et al. (1954) 'Relationship between low values of alveolar carbon monoxide concentration and carboxyhemoglobin percentage in human blood', *Acta Physiol Scand*, 31(1), pp. 62-74.
Castleden, C. M. and Cole, P. V. (1975) 'Carboxyhaemoglobin levels of smokers and non-smokers working in the City of London', *Br J Ind Med*, 32(2), pp. 115-118.
Cattaneo, A., Taronna, M., Consonni, D., et al. (2010) 'Personal exposure of traffic police officers to particulate matter, carbon monoxide, and benzene in the city of Milan, Italy', *J Occup Environ Hyg*, 7(6), pp. 342-351.
CDC, US. (2005) *Key Outcome Indicators for Evaluating Comprehensive Tobacco Control Programs*. Available at: https://stacks.cdc.gov/view/cdc/5472 (Accessed: 04 June 2020).
Chaloulakou, A., Mavroidis, I. and Duci, A. (2003) 'Indoor and outdoor carbon monoxide concentration relationships at different microenvironments in the Athens area', *Chemosphere*, 52(6), pp. 1007-1019.
Chang, Y.-H., Hsu, C.-Y., Cheng, Q., et al. (2019) 'The evolution of the characteristics of charcoal-burning suicide in Hong Kong, 2002–2013', *J Affect Disord*, 257, pp. 390-395.
Chen, C.-C., Sung, G.-N., Chen, W.-C., et al. (2016) 'A wireless and batteryless intelligent carbon monoxide sensor', *Sensors*, 16(10), pp. 1568.
Chen, Y.-Y., Sung, F.-C., Chen, M.-L., et al. (2016) 'Indoor Air Quality in the Metro System in North Taiwan', *Int J Environ Res Public Health*, 13(12), pp. 1200.
Cheung, H. J. and Cheung, L. (2015) 'Coaching patients during pulmonary function testing: a practical guide', *Can J Respir Ther*, 51(3), pp. 65-68.
Chhabra, S. K., Kumar, R. and Gupta, U. A. (2016) 'Prediction equations for diffusing capacity (transfer factor) of lung for North Indians', *Lung India*, 33(5), pp. 479-486.
Chiang, S. T. and Wang, B. C. (1970) 'Acute effects of cigarette smoking on pulmonary function', *Am Rev Respir Dis*, 101(6), pp. 860-868.
Chinwong, D., Mookmanee, N., Chongpornchai, J. and Chinwong, S. (2018) 'A comparison of gender differences in smoking behaviors, intention to quit, and nicotine dependence among Thai university students', *J Addict*, 2018, pp. 8081670-8081670.

Clarke, S., Keshishian, C., Murray, V., et al. (2012) 'Screening for carbon monoxide exposure in selected patient groups attending rural and urban emergency departments in England: a prospective observational study', *BMJ Open*, 2(6), pp. e000877.

Clarys, P., Deliens, T., Huybrechts, I., et al. (2014) 'Comparison of nutritional quality of the vegan, vegetarian, semi-vegetarian, pesco-vegetarian and omnivorous diet', *Nutrients*, 6(3), pp. 1318-1332.

Cobb, N. and Etzel, R. A. (1991) 'Unintentional carbon monoxide—related deaths in the United States, 1979 through 1988', *JAMA*, 266(5), pp. 659-663.

Coburn, R. F., Blakemore, W. S. and Forster, R. E. (1963) 'Endogenous carbon monoxide production in man', *J Clin Invest*, 42(7), pp. 1172-1178.

Coburn, R. F., Forster, R. E. and Kane, P. B. (1965) 'Considerations of the physiological variables that determine the blood carboxyhemoglobin concentration in man', *J Clin Invest*, 44(11), pp. 1899-1910.

Cohen, S., Perkins, N., Ury, H. and Goldsmith, J. R. (1971) 'Carbon monoxide uptake in cigarette smoking', *Arch Environ Health*, 22(1), pp. 55-60.

Commodore, A. A., Hartinger, S. M., Lanata, C. F., et al. (2013) 'Carbon monoxide exposures and kitchen concentrations from cookstove-related woodsmoke in San Marcos, Peru', *Int J Occup Environ Health*, 19(1), pp. 43-54.

Corder, G. W. (2014) *Nonparametric statistics : a step-by-step approach.* 2ed edn.: Hoboken, New Jersey: Wiley.

Cotes, J. E. (1975) *Lung function, Assessment and application in medicine.* 3rd edn. Osney Mead, Oxford: Blackwell Scientific Publications.

Cotes, J. E., Chinn, D. J. and Miller, M. R. M. (2006) *Lung Function.* 6th edn. Osney Mead, Oxford: Blackwell Scientific Publications.

Crapo, R. O., Hankinson, J. L., Irvin, C., et al. (1995) 'American thoracic society. single-breath carbon monoxide diffusing capacity (transfer factor). recommendations for a standard technique--1995 update', *Am J Respir Crit Care Med*, 152(6), pp. 2185-2198.

Cronenberger, C., Mould, D. R., Roethig, H.-J. and Sarkar, M. (2008) 'Population pharmacokinetic analysis of carboxyhaemoglobin concentrations in adult cigarette smokers', *Br J Clin Pharmacol*, 65(1), pp. 30-39.

Cross government group on gas safety and CO awareness (2019). *Annual Report 2017/18.* Available at: https://www.hse.gov.uk/gas/domestic/cross-government-group-1718.pdf (Accessed: 07 December 2021).

Crowley, T. J., Andrews, A. E., Cheney, J., et al. (1989) 'Carbon monoxide assessment of smoking in chronic obstructive pulmonary disease', *Addict Behav*, 14(5), pp. 493-502.

Croxford, B., Leonardi, G. and Kreis, I. (2008) 'Self-reported neurological symptoms in relation to CO emissions due to problem gas appliance installations in London: a cross-sectional survey', *Environ Health*, 7, pp. 34.

Defra's UKAir (2021) *Annual and Exceedance Statistics.* Available at: https://uk-air.defra.gov.uk/data/exceedance (Accessed: 10 December 2021).

Delivoria-Papadopoulos, M., Coburn, R. and Forster, R. (1974) 'Cyclic variation of rate of carbon monoxide production in normal women', *J Appl Physiol*, 36(1), pp. 49-51.

Department of Civil Affairs, Taipei City Government (2019). *Annual population in Taipei city*. Available at: https://ca.gov.taipei/News_Content.aspx?n=8693DC9620A1AABF&sms=D19E9582624D83CB&s=78DC4B104D9D374E (Accessed: June 04 2020).

Deveci, S. E., Deveci, F., Açik, Y. and Ozan, A. T. (2004) 'The measurement of exhaled carbon monoxide in healthy smokers and non-smokers', *Respir Med*, 98(6), pp. 551-556.

Dimitroulopoulou, C., Ashmore, M. R., Hill, M. T. R., et al. (2006) 'INDAIR: a probabilistic model of indoor air pollution in UK homes', *Atmos Environ*, 40(33), pp. 6362-6379.

Dixon, A. E. and Peters, U. (2018) 'The effect of obesity on lung function', *Expert Rev Respir Med*, 12(9), pp. 755-767.

Dolan, M. C. (1985) 'Carbon monoxide poisoning', *CMAJ*, 133(5), pp. 392-399.

Dukić, L., Kopčinović, L. M., Dorotić, A. and Baršić, I. (2016) 'Blood gas testing and related measurements: national recommendations on behalf of the Croatian society of medical biochemistry and laboratory medicine', *Biochem Med*, 26(3), pp. 318-336.

Dunn, K. H., Shulman, S., Stock, A. L. and Naeher, L. P. (2013) 'Personal carbon monoxide exposures among firefighters at prescribed forest burns in the southeastern united states', *Arch Environ Occup Health*, 68(1), pp. 55-59.

Durussel, J., Ross, R., Kodi, P. R., et al. (2013) 'Precision of the optimized carbon monoxide rebreathing method to determine total haemoglobin mass and blood volume', *Eur J Sport Sci*, 13(1), pp. 68-77.

Elbehairy, A. F., Hockmann, K., Ciavaglia, C. E., et al. (2017) 'Unraveling the cause of severe exertional dyspnea in a heavy smoker', *Ann Am Thorac Soc*, 14(12), pp. 1849-1855.

Ely, W. E., Moorehead, B. and Haponik, E. F. (1995) 'Warehouse workers' headache: emergency evaluation and management of 30 patients with carbon monoxide poisoning', *Am J Med*, 98(2), pp. 145-155.

Enache, I., Oswald-Mammosser, M., Scarfone, S., et al. (2011) 'Impact of altered alveolar volume on the diffusing capacity of the lung for carbon monoxide in obesity', *Respiration*, 81(3), pp. 217-222.

Environmental Protection Agency, R.O.C. (2012) *Air Quality Standards in R.O.C.* Available at: https://taqm.epa.gov.tw/taqm/tw/b0206.aspx (Accessed: 14 Mar 2020).

Environmental Protection Agency, US. (1970) *Clean Air Act*. Available at: https://www.epa.gov/laws-regulations/summary-clean-air-act (Accessed: Mar 14 2020).

Environmental Protection Agency, US. (1991) *Air quality criteria for carbon monoxide*, (EPA-600/B-90/045F).

Enzi, G., Bevilacqua, M. and Crepaldi, G. (1976) 'Disturbances in pulmonary gaseous exchange in primary hyperlipoproteinemias', *Bull Eur Physiopathol Respir*, 12(3), pp. 433-42.

Ernst, A. and Zibrak, J. D. (1998) 'Carbon monoxide poisoning', *N Engl J Med.*, 339(22), pp. 1603-8.

Erupaka, K., Bruce, E. N. and Bruce, M. C. (2010) 'Prediction of extravascular burden of carbon monoxide (CO) in the human heart', *Ann Biomed Eng*, 38(2), pp. 403-438.

Farha, S., Asosingh, K., Laskowski, D., et al. (2007) 'Pulmonary gas transfer related to markers of angiogenesis during the menstrual cycle', *J Appl Physiol*, 103(5), pp. 1789-1795.

Faruk Tekbaş, Ö., Vaizoglu, S. A., Didem Evci, E., et al. (2001) 'Carbon monoxide levels in bathrooms using hot water boilers', *Indoor Built Environ*, 10(3-4), pp. 167-171.

Fatemi, S. H. and Clayton P. J. (2008) *The Medical Basis of Psychiatry*. 3rd edn. Totowa, New Jersey: Humana Press.

Fawcett, T. A., Moon, R. E., Fracica, P. J., et al. (1992) 'Warehouse workers' headache. Carbon monoxide poisoning from propane-fueled forklifts', *J Occup Med*, 34(1), pp. 12-15.

Fernandez-Bremauntz, A. A. and Ashmore, M. R. (1995) 'Exposure of commuters to carbon monoxide in Mexico city—I. Measurement of in-vehicle concentrations', *Atmos Environ*, 29(4), pp. 525-532.

Filley, G. F., MacIntosh, D. J. and Wright, G. W. (1954) 'Carbon monoxide uptake and pulmonary diffusing capacity in normal subjects at rest and during exercise', *J Clin Invest*, 33(4), pp. 530-539.

Fisher, D. S., Bowskill, S., Saliba, L. and Flanagan, R. J. (2013) 'Unintentional domestic non-fire related carbon monoxide poisoning: data from media reports, UK/Republic of Ireland 1986–2011', *Clin Toxicol*, 51(5), pp. 409-416.

Fisher, D. S., Leonardi, G. and Flanagan, R. J. (2014) 'Fatal unintentional non-fire-related carbon monoxide poisoning: England and Wales, 1979–2012', *Clin Toxico*, 52(3), pp. 166-170.

Fisher, J. A., Iscoe, S., Fedorko, L. and Duffin, J. (2011) 'Rapid elimination of CO through the lungs: coming full circle 100 years on', *Exp Physiol*, 96(12), pp. 1262-1269.

Flachsbart, P. G., Howes, J. E., Mack, G. A. and Rodes, C. E. (1987) 'Carbon monoxide exposures of Washington commuters', *JAPCA*, 37(2), pp. 135-142.

Forbes, W. H., Sargent, F. and Roughton, F. J. W. (1945) 'The rate of carbon monoxide uptake by normal men', *Am J Physiol*, 143(4), pp. 594-608.

Forrest, J. B. (1970) 'The effect of changes in lung volume on the size and shape of alveoli', *J Physiol*, 210(3), pp. 533-547.

Foster, R. (1964) *Handbook of Physiology*. Washinton, D.C.: American Physiological Society.

Frans, A., Nemery, B., Veriter, C., et al. (1997) 'Effect of alveolar volume on the interpretation of single breath DLCO', *Respir Med*, 91(5), pp. 263-273.

Frans, A., Stănescu, D. C., Veriter, C., et al. (1975) 'Smoking and pulmonary diffusing capacity', *Scand J Respir Dis*, 56(3), pp. 165-183.

Friedman, P., Guo, X. M., Stiller, R. J. and Laifer, S. A. (2015) 'Carbon monoxide exposure during pregnancy', *Obstet Gynecol Surv*, 70(11), 705-712.

Fröhlich, E., Mercuri, A., Wu, S. and Salar-Behzadi, S. (2016) 'Measurements of deposition, lung surface area and lung fluid for simulation of inhaled compounds', *Front Pharmacol*, 7, pp. 181.

Gagnon, P., Guenette, J. A., Langer, D., et al. (2014) 'Pathogenesis of hyperinflation in chronic obstructive pulmonary disease', *Int J Chron Obstruct Pulmon Dis*, 9, pp. 187-201.
Georgoulis, L. B., Hänninen, O., Samoli, E., et al. (2002) 'Personal carbon monoxide exposure in five European cities and its determinants', *Atmos Environ*, 36(6), pp. 963-974.
Ghosh, R. E., Close, R., McCann, L. J., et al. (2016) 'Analysis of hospital admissions due to accidental non-fire-related carbon monoxide poisoning in England, between 2001 and 2010', *J Public Health*, 38(1), pp. 76-83.
Gluschko, A., Befurt, R., Brock, T. H., et al. (2019) 'Method for the determination of carbon monoxide in workplace air using a Fourier-transform infrared spectrometer (FTIR spectrometer) [Air Monitoring Methods, 2019]', *MAK collect occup health saf*, 4(2), pp. 980-989.
Gosselin, N. H., Brunet, R. C. and Carrier, G. (2009) 'Determination of carboxyhaemoglobin in humans following low-level exposures to carbon monoxide', *Inhal Toxicol*, 21(13), pp. 1077-1091.
Graham, B. L., Brusasco, V., Burgos, F., et al. (2017) '2017 ERS/ATS standards for single-breath carbon monoxide uptake in the lung', *Eur Respir J*, 49(1), pp. 1600016.
Graham, B. L., Mink, J. T. and Cotton, D. J. (2002) 'Effects of increasing carboxyhemoglobin on the single breath carbon monoxide diffusing capacity', *Am J Respir Crit Care Med*, 165(11), pp. 1504-1510.
Global Adult Tobacco Survey Collaborative Group. (2011) *Tobacco Questions for Surveys: A Subset of Key Questions from the Global Adult Tobacco Survey (GATS)* , 2ed edn. Atlanta, Georgia: Centers For Disease Control and Prevention.
Guvener, N., Tutuncu, N. B., Akcay, S., et al. (2003) 'Alveolar gas exchange in patients with type 2 diabetes mellitus', *Endocr J*, 50(6), pp. 663-667.
Hackshaw, A. and Kirkwood, A. (2011) 'Interpreting and reporting clinical trials with results of borderline significance', *BMJ*, 343, pp. d3340.
Hagler, G. S. W., Thoma, E. D. and Baldauf, R. W. (2010) 'High-resolution mobile monitoring of carbon monoxide and ultrafine particle concentrations in a near-road environment', *J Air Waste Manag Assoc*, 60(3), pp. 328-336.
Hair Jr, J. F., Black, W. C., Babin, B. J. and Anderson, R. E. (2014) *Multivariate Data Analysis.* 7th edn. London, UK: Pearson Education Limited.
Haldane, J. and Smith, J. L. (1897) 'The absorption of oxygen by the lungs', *J Physiol*, 22(3), pp. 231-258.
Hampson, N. B. (2016) 'U.S. mortality due to carbon monoxide poisoning, 1999–2014. Accidental and intentional deaths', *nn Am Thorac Soc*, 13(10), pp. 1768-1774.
Hampson, N. B., Piantadosi, C. A., Thom, S. R., and Weaver, L. K. (2012) 'Practice recommendations in the diagnosis, management, and prevention of carbon monoxide poisoning', *Am J Respir Crit Care Med.*, 186(11), pp. 1095-101.
Hampson, N. B. and Weaver, L. K. (2007) 'Carbon monoxide poisoning: a new incidence for an old disease', *Undersea Hyperb Med*, 34(3), pp. 163-168.
Harrison, R. M., Thornton, C. A., Lawrence, R. G., et al. (2002) 'Personal exposure monitoring of particulate matter, nitrogen dioxide, and carbon monoxide, including susceptible groups', *Occup Environ Med*, 59(10), pp. 671-679.
Harvey, B.-G., Strulovici-Barel, Y., Kaner, R. J., et al. (2015) 'Risk of COPD with obstruction in active smokers with normal spirometry and reduced diffusion capacity', *Eur Respir J*, 46(6), pp. 1589-1597.
Hawkins, L. H., Cole, P. V. and Harris, J. R. W. (1976) 'Smoking habits and blood carbon monoxide levels', *Environ Res*, 11(3), pp. 310-318.
Health Canada. (2010) *Residential Indoor Air Quality Guideline: Carbon Monoxide (Canada)*. Available at: https://www.canada.ca/en/health-canada/services/publications/healthy-living/residential-indoor-air-quality-guideline-carbon-monoxide.html (Accessed: 04 June 2020).
Health Promotion Administration, ROC. (2019) *Smoking rate among people over 18 years old*. Available at: https://www.gender.ey.gov.tw/gecdb/Stat_Statistics_DetailData.aspx?sn=ORVNSDIGVFTYgkN%2B7bdIyA%3D%3D (Accessed: 04 June 2020).
Hedengren, J. D. (2018) *Process Dynamics and Control in Python*. Available at: http://apmonitor.com/pdc/index.php (Accessed: 20 Dec 2018).
Heinicke, K., Wolfarth, B., Winchenbach, P., et al. (2001) 'Blood volume and hemoglobin mass in elite athletes of different disciplines', *Int J Sports Med*, 22(7), pp. 504-512.

Henn, S. A., Bell, J. L., Sussell, A. L. and Konda, S. (2013) 'Occupational carbon monoxide fatalities in the US from unintentional non-fire related exposures, 1992-2008', *Am J Ind Med,* 56(11), pp. 1280-1289.

Hepper, N. G. G., Fowler, W. S. and Helmholz, F. (1960) 'Relationship of height to lung volume in healthy men', *Diseases of the Chest,* 37(3), pp. 314-320.

Herbert, S. J., Weill, H., Stuckey, W. J., et al. (1965) 'Pulmonary diffusing capacity in polycythemic states before and after phlebotomy', *Dis Chest,* 48(4), pp. 408-415.

Hill, E. P., Hill, J. R., Power, G. G. and Longo, L. D. (1977) 'Carbon monoxide exchanges between the human fetus and mother: a mathematical model', *Am J Physiol,* 232(3), pp. H311-H323.

HM Government. (2006) *Health Act*. Available at: https://www.legislation.gov.uk/ukpga/2006/28/contents (Accessed: 04 June 2020).

HM Government. (2019) *Approved Document F – Ventilation*. Available at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/835549/AD_F_vol_1.pdf (Accessed: 11 December 2021).

HM Government. (2021) *Approved Document F – Ventilation*. Available at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1045918/ADF1.pdf (Accessed: 04 February 2022).

HM Government. (2015) *The Smoke and Carbon Monoxide Alarm (England) Regulations.* Available at: https://www.legislation.gov.uk/ukdsi/2015/9780111133439/contents (Accessed: 04 June 2020).

HM Government. (2021) *The Smoke and Carbon Monoxide Alarm (England) Regulations.* Available at: https://www.propertymark.co.uk/resource/smoke-and-carbon-monoxide-alarm-rules-extended.html (Accessed: 10 December 2021).

Horvath, S. M. and Bedi, J. F. (1989) 'Alteration in carboxyhemoglobin concentrations during exposure to 9 ppm carbon monoxide for 8 hours at sea level and 2134 m altitude in a hypobaric chamber', *JAPCA,* 39(10), pp. 1323-7.

Hsu, C.-L., Wang, H.-T., Tsai, M.-J., et al. (2011) 'Survey on nicotine, tar and carbon monoxide in mainstream smoke of domestic and imported tobacco products', *Ann Rep Food Drug Res,* 2, pp. 427-438.

Htun, C., Pope, A., Lahzami, S., et al. (2018) 'Contribution of peripheral airway function to changes in FEV1/FVC and RV/TLC with aging', *J Appl Physiol,* 125(5), pp. 1378-1383.

Hughes, J. M. B. (2008) 'Pulmonary function: the basics', *Medicine,* 36(3), pp. 142-150.

Hughes, J. M. B. and Pride, N. B. (2012) 'Examination of the carbon monoxide diffusing capacity (DLCO) in relation to its Kco and Va components', *Am J Respir Crit Care Med,* 186(2), pp. 132-139.

ICRP (1994) 'Human respiratory tract model for radiological protection. A report of a task group of the International Commission on Radiological Protection', *Ann ICRP,* 24(1-3), pp. 1-482.

Ilano, A. L. and Raffin, T. A. (1990) 'Management of carbon monoxide poisoning', *Chest,* 97(1), pp. 165-9.

Ip, M. S. M., Lam, W.-K., Lai, A. Y. K., et al. (2007) 'Reference values of diffusing capacity of non-smoking Chinese in Hong Kong', *Respirology,* 12(4), pp. 599-606.

Jarvis, M. J., Belcher, M., Vesey, C. and Hutchison, D. C. (1986) 'Low cost carbon monoxide monitors in smoking assessment', *Thorax,* 41(11), pp. 886-887.

Jarvis, M. J., Russell, M. A. and Saloojee, Y. (1980) 'Expired air carbon monoxide: a simple breath test of tobacco smoke intake', *BMJ,* 281(6238), pp. 484-485.

Jimenez, L. (2019) *Reach Full Capacity of Pulmonary Function Test Coding*. Utah, US: American Academy of Professional Coders.

Jo, W.-K. and Oh, J.-W. (2003) 'Evaluation of CO exposure in active smokers while smoking using breath analysis technique', *Chemosphere,* 53(3), pp. 207-216.

Johnson, J. D. and Theurer, W. M. (2014) 'A stepwise approach to the interpretation of pulmonary function tests', *Am Fam Physician,* 89(5), pp. 359-66.

Kaminsky, D. A., Daud, A. and Chapman, D. G. (2014) 'Relationship between the baseline alveolar volume-to-total lung capacity ratio and airway responsiveness', *Respirology,* 19(7), pp. 1046-1051.

Kao, L. W. and Nanagas, K. A. (2004) 'Carbon monoxide poisoning', *Emerg Med Clin North Am,* 22(4), pp. 985-1018.

Kawai, S., Yokota, T., Onozawa, Y., et al. (2017) 'Risk factors for aspiration pneumonia after definitive chemoradiotherapy or bio-radiotherapy for locally advanced head and neck cancer: a monocentric case control study', *BMC Cancer,* 17(1), pp. 59.

Kendrick, A. (2015) 'FRC%TLC and RV%TLC to assess effects of lung disease: comparison to FEV1%FVC', *Eur Respir J*, 46(suppl 59), pp. PA1576.
Kendrick, A. H. and Laszlo, G. (1990) 'CO transfer factor on exercise: age and sex differences', *Eur Respir J*, 3(3), pp. 323.
Kiefer, E. M., Hankinson, J. L. and Barr, R. G. (2011) 'Similar relation of age and height to lung function among Whites, African Americans, and Hispanics', *Am J Epidemiol*, 173(4), pp. 376-387.
Kim, Y.-M., Shin, H.-J., Choi, D.-W., et al. (2019) 'Comparison of high-flow nasal cannula oxygen therapy and conventional reserve-bag oxygen therapy in carbon monoxide intoxication: A pilot study', *Am J Emerg Med*, 38(8), pp. 1621-1626..
Kinoshita, H., Tanaka, N., Takakura, A., et al. (2017) *Application of CO-oximeter for forensic samples, post mortem examination and autopsy - current issues from death to laboratory analysis*: IntechOpen. Available at: https://www.intechopen.com/chapters/57175 (Accessed: 04 June 2020).
Kinoshita, H., Türkan, H., Vucinic, S., et al. (2020) 'Carbon monoxide poisoning', *Toxicol Rep*, 7, pp. 169-173.
Kirk, P. W. W., Hunter, M., Baek, S.-O., et al. (1988) 'British indoor air quality: regional and locational variations in the concentration of three environmental tobacco smoke components', *Environ Technol Lett*, 9(5), pp. 437-448.
Kirkham, T. L., Koehoorn, M. W., Davies, H. and Demers, P. A. (2011) 'Characterization of noise and carbon monoxide exposures among professional firefighters in British Columbia', *The Ann Occup Hyg*, 55(7), pp. 764-774.
Kirkwood, B. R. and Sterne, J. A. C. (2003) *Essential Medical Statistics.* Hoboken, UK: John Wiley & Sons, Incorporated.
Klasner, A. E., Smith S. R., Thompson, M. W. and Scalzo, A. J. (1998) 'Carbon monoxide mass exposure in a pediatric population', *Acad Emerg Med.*, 5(10), pp. 992-6.
Knudson, R. J., Kaltenborn, W. T., Knudson, D. E. and Burrows, B. (1987) 'The single-breath carbon monoxide diffusing capacity', *Am Rev Respir Dis*, 135(4), pp. 805-811.
Korotzer, B., Ong, S. and Hansen, J. E. (2000) 'Ethnic differences in pulmonary function in healthy nonsmoking Asian-Americans and European-Americans', *Am J Respir Crit Care Med*, 161(4), pp. 1101-1108.
Kreck, T., Shade, E., Lamm, W., et al. (2001) 'Isocapnic Hyperventilation Increases Carbon MonoxideElimination and Oxygen Delivery', *Am J Respir Crit Care Med*, 163(2):458-62.
Kuo, Y. W., Harris, R. S., Hess, D. R., et al. (2020) ' Endogenous Carbon Monoxide Production and Diffusing Capacity of the Lung for Carbon Monoxide in Sepsis-Induced Acute Respiratory Distress Syndrome', *Crit Care Explor*, 2(12), pp. e0286.
Kutlar Joss, M., Eeftens, M., Gintowt, E., et al. (2017) 'Time to harmonize national ambient air quality standards', *Int J Public Health*, 62(4), pp. 453-462.
Lalley, P. M. (2013) 'The aging respiratory system—pulmonary structure, function and neural control', *Respir Physiol Neurobiol*, 187(3), pp. 199-210.
Laugesen, M., Epton, M., Frampton, C. M. A., et al. (2009) 'Hand-rolled cigarette smoking patterns compared with factory-made cigarette smoking in New Zealand men', *BMC public health*, 9, pp. 194-194.
Leader, D. (2019) *An Overview of Obstructive vs. Restrictive Lung Diseases*. NY 10036, US: verywellhealth. Available at: https://www.verywellhealth.com/obstructive-and-restrictive-lung-diseases-914741 (Accessed: 24 Mar 2020).
Li, F., Chan, H. C., Liu, S., et al. (2015) 'Carbon monoxide poisoning as a cause of death in Wuhan, China: a retrospective six-year epidemiological study (2009–2014)', *Forensic Sci Int*, 253, pp. 112-118.
Lin, S-Y., Yang, Y-C., Lin, C-C., et al. (2021) 'Increased Incidence of Dysmenorrhea in Women Exposed to Higher Concentrations of NO, NO 2, NO x, CO, and PM 2.5: A Nationwide Population-Based Study', *Front Public Health*, 17(9), pp. 682341.
Lioy, P. and Weisel, C. (2014) *Chapter 5 - Exposure science research design*, Oxford, UK: Academic Press.
Littleton, S. W. (2012) 'Impact of obesity on respiratory function', *Respirology*, 17(1), pp. 43-49.
Liu, K. Y., Beautrais, A., Caine, E., et al. (2007) 'Charcoal burning suicides in Hong Kong and urban Taiwan: an illustration of the impact of a novel suicide method on overall regional rates', *J Epidemiol Community Health*, 61(3), pp. 248-53.
Liu, Q., Zhou, L., Zheng, N., et al. (2009) 'Poisoning deaths in China: type and prevalence detected at the Tongji Forensic Medical Center in Hubei', *Forensic Sci Int*, 193(1), pp. 88-94.

Luomanmäki, K. and Coburn, R. F. (1969) 'Effects of metabolism and distribution of carbon monoxide on blood and body stores', *Am J Physiol*, 217(2), pp. 354-363.
Longo, L.D. (1970) ' Carbon monoxide in the pregnant mother and fetus and its exchange across the placenta', *Ann NY Acad Sc*, 174, pp. 313-341.
MacDonald, R. D., Thomas, L., Rusk, F. C., et al. (2010) 'Occupational health and safety assessment of exposure to jet fuel combustion products in air medical transport', *Prehosp Emerg Care*, 14(2), pp. 202-208.
Maga, M., Janik, M. K., Wachsmann, A., et al. (2017) 'Influence of air pollution on exhaled carbon monoxide levels in smokers and non-smokers. A prospective cross-sectional study', *Environ Res*, 152, pp. 496-502.
Magnussen, H., Canepa, M., Zambito, P. E., et al. (2017) 'What can we learn from pulmonary function testing in heart failure?', *Eur J Heart Fail*, 19(10), pp. 1222-1229.
Malczewska-Lenczowska, J., Sitkowski, D., Orysiak, J., et al. (2013) ' Total haemoglobin mass, blood volume and morphological indices among athletes from different sport disciplines, *Arch Med Sci*, 9(5), pp. 780-787.
Manel, J., Gabrion, I., Raspiller, M. F., et al. (1999) 'Epidemiology and prevention of accidental poisoning by carbon monoxide in the east of France', *Indoor Built Environ*, 8(3), pp. 179-183.
Mannino, D. M. and Buist, A. S. (2007) 'Global burden of COPD: risk factors, prevalence, and future trends', *Lancet*, 370(9589), pp. 765-773.
Mannino, D. M., Sonia Buist, A. and Vollmer, W. M. (2007) 'Chronic obstructive pulmonary disease in the older adult: what defines abnormal lung function?', *Thorax*, 62(3), pp. 237-241.
Marrades, R. M., Diaz, O., Roca, J., et al. (1997) 'Adjustment of DLCO for hemoglobin concentration', *Am J Respir Crit Care Med*, 155(1), pp. 236-241.
Mattiuzzi, C. and Lippi, G. (2019) 'Worldwide epidemiology of carbon monoxide poisoning', *Hum Exp Toxicol*, 39(4), pp. 0960327119891214.
McCann, L. J., Close, R., Staines, L., et al. (2013) 'Indoor carbon monoxide: a case study in England for detection and interventions to reduce population exposure', *J Environ Public Health*, 2013, pp. 735952.
McNeill, A., West, R., Jarvis, M. and Russell, M. A. (1986) 'Do older children take in more smoke from their cigarettes? Evidence from carbon monoxide levels', *J Behav Med*, 9(6), pp. 559-65.
Mellors, A. (1976) 'The haldane relationship enzymes & equilibrium', *Biochem Educ*, 4(4), pp. 71-71.
Meredith, T. and Vale, A. (1988) 'Carbon Monoxide Poisoning', *BMJ*, 296(6615), pp. 77-79.
Miller, A. and Enright, P. L. (2012) 'PFT interpretive strategies: American thoracic society/ European respiratory society 2005 guideline gaps', *Respir Care*, 57(1), pp. 127-133.
Miller, A., Thornton, J. C., Warshaw, R., et al. (1983) 'Single breath diffusing capacity in a representative sample of the population of Michigan, a large industrial state', *Am Rev Respir Dis*, 127(3), pp. 270-277.
Ministry of Health and Welfare, Health Promotion Administration. (2009) *Tobacco Hazards Prevention Act (Taiwan)*. Available at: https://law.moj.gov.tw/ENG/LawClass/LawAll.aspx?pcode=L0070021 (Accessed: 04 June 2020).
Mo, X., Jian, W., Su, Z., et al. (2020) 'Abnormal pulmonary function in COVID-19 patients at time of hospital discharge', *Eur Respir J*, 55(6), pp. 2001217.
Mohsenifar, Z., Brown, H., Schnitzer, B., et al. (1982) 'The effect of abnormal levels of hematocrit on the single breath diffusing capacity', *Lung*, 160(6), pp. 325-330.
Moscato, U., Poscia, A., Gargaruti, R., et al. (2014) 'Normal values of exhaled carbon monoxide in healthy subjects: comparison between two methods of assessment', *BMC Pulm Med*, 14, pp. 204-204.
Mosteller, R. D. (1987) 'Simplified calculation of body-surface area', *N Engl J Med*, 317(17), pp. 1098-1098.
Mott, J. A., Wolfe, M. I., Alverson, C. J., et al. (2002) 'National vehicle emissions policies and practices and declining US carbon monoxide–related mortality', *JAMA*, 288(8), pp. 988-995.
Muhammad-Kah, R., Liang, Q., Frost-Pineda, K., et al. (2011) 'Factors affecting exposure to nicotine and carbon monoxide in adult cigarette smokers', *Regul Toxicol Pharmacol*, 61(1), pp. 129-136.
Mutluoglu, M., Metin, S., Ibrahim, A., et al. (2016) 'The use of hyperbaric oxygen therapy for carbon monoxide poisoning in Europe', *Undersea Hyperb Med*, 43(1), pp. 49-56.

Nadler, S. B., Hidalgo, J. H. and Bloch, T. (1962) 'Prediction of blood volume in normal human adults', *Surgery*, 51(2), pp. 224-232.
Najeeb, H. M. (2010) 'Lung diffusing capacity for carbon monoxide (DLco-SB): the iInfluence of cigarette smoking', *Iraqi Postgrad Med J*, 9(3), pp. 328-334.
Nakazawa, S., Shimizu, K., Mogi, A. and Kuwano, H. (2018) 'Low diffusing capacity, emphysema, or pulmonary fibrosis: who is truly pulling the lung cancer strings?', *J Thorac Dis*, 10(2), pp. 600-602.
Nash, D. G. and Leith, D. (2010) 'Use of passive diffusion tubes to monitor air pollutants', *J Air Waste Manag Assoc*, 60(2), pp. 204-209.
Nordenberg, D., Yip, R. and Binkin, N. J. (1990) 'The effect of cigarette smoking on hemoglobin levels and anemia screening', *JAMA*, 264(12), pp. 1556-1559.
O'Donnell, C. R., Bankier, A. A., Stiebellehner, L., et al. (2010) 'Comparison of plethysmographic and helium dilution lung volumes: which is best for COPD?', *Chest*, 137(5), pp. 1108-1115.
Office for National Statistics (UK). (2019) *Male and female populations (UK)*. Available at: https://www.ethnicity-facts-figures.service.gov.uk/uk-population-by-ethnicity/demographics/male-and-female-populations/latest#full-page-history (Accessed: 04 June 2020).
Office for National Statistics and Public Health England (2019) *Adult smoking habits in the UK*. Available at: https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/healthandlifeexpectancies/bulletins/adultsmokinghabitsingreatbritain/2015 (Accessed: 04 June 2020).
Oliverio, S. (2020) *Carbon monoxide poisonings: exploring new approaches for quantification and evaluating measurement errors from an analytical and epidemiological point of view*. Doctor of Philosophy (PhD), Brunel University, London, UK.
Oliverio, S. and Varlet, V. (2018) 'Total blood carbon monoxide: alternative to carboxyhemoglobin as biological marker for carbon monoxide poisoning determination', *J Anal Toxicol*, 43(2), pp. 79-87.
Oliverio, S. and Varlet, V. (2020) 'New strategy for carbon monoxide poisoning diagnosis: carboxyhemoglobin (COHb) vs total blood carbon monoxide (TBCO)', *Forensic Sci Int*, 306, pp. 110063.
Olson, K. and Smollin, C. (2008) 'Carbon monoxide poisoning (acute)', *BMJ clin Evid*, 2008, pp. 2103.
Ott, W., Switzer, P. and Willits, N. (1994) 'Carbon monoxide exposures inside an automobile traveling on an urban arterial highway', *Air Waste*, 44(8), pp. 1010-8.
Otto, J. M., Plumb, J. O. M., Clissold, E., et al. (2017) ' Hemoglobin concentration, total hemoglobin mass and plasma volume in patients: implications for anemia', *Haematologica*, 102(9), pp. 1477-1485.
Owens, E. O. (2010) 'Endogenous carbon monoxide production in disease', *Clin Biochem*, 43(15), pp. 1183-1188.
Ozturan, I. U., Yaka, E., Suner, S., et al. (2019) 'Determination of carboxyhemoglobin half-life in patients with carbon monoxide toxicity treated with high flow nasal cannula oxygen therapy', *Clin Toxicol*, 57(7), pp. 1-7.
Pace, N., Consolazio, W. V., White, W. A. and Behnke, A. R. (1946) 'Formulation of the principal factors affecting the rate of uptake of carbon monoxide by men', *Am J Physiol*, 147(2), pp. 352-359.
Pace, N., Strajman, E. and Walker, E. L. (1950) 'Acceleration of carbon monoxide elimination in man by high pressure oxygen', *Science*, 111(2894), pp. 652-654.
Pan, K.-T. (2013) *Related factors contributing to prognosis of carbon monoxide poisoning*. Master of Science (MSc), National Defense Medical Center, Taipei, Taiwan.
Pan, K.-T., Shen, C.-H., Lin, F.-G., et al. (2019) 'Prognostic factors of carbon monoxide poisoning in Taiwan: a retrospective observational study', *BMJ Open*, 9(11), pp. e031135.
Pan, K.-T., Leonardi, G. S. and Croxford, B. (2020) 'Factors contributing to CO uptake and elimination in the body: a critical review', *Int J Environ Res Public Health*, 17(2), pii: E528.
Paoletti, P., Viegi, G., Pistelli, G., et al. (1985) 'Reference equations for the single-breath diffusing capacity. A cross-sectional analysis and effect of body size and age', *Am Rev Respir Dis*, 132(4), pp. 806-13.
Park, J. O., Choi, I. S. and Park, K. O. (1986) 'Normal predicted values of single-breath diffusing capacity of the lung in healthy nonsmoking adults', *Korean J Intern Med*, 1(2), pp. 178-184.
Parkinson, C. M., Hammond, D., Fong, G. T., et al. (2009) 'Smoking beliefs and behavior among youth in Malaysia and Thailand', *Am J Health Behav*, 33(4), pp. 366-375.

Partridge, M. R., Hughes, J. M. and Thompson, G. R. (1979) 'Effect of hyperlipidaemia on pulmonary diffusing capacity for carbon monoxide', *Thorax,* 34(2), pp. 265.
Peces-Barba, G., Rodríguez-Nieto, M. J., Verbanck, S., et al. (2004) 'Lower pulmonary diffusing capacity in the prone vs. supine posture', *J Appl Physiol,* 96(5), pp. 1937-1942.
Pellegrino, R., Viegi, G., Brusasco, V., et al. (2005) 'Interpretative strategies for lung function tests', *Eur Respir J,* 26(5), pp. 948.
Penney, D. G. (1996) *Carbon Monoxide.* 1st edn., Boca Raton, Florida: CRC Press.
Penney, D. G. (2007) *Carbon Monoxide Poisoning.* 1st edn. Boca Raton, Florida: CRC Press.
Pepe, G., Castelli, M., Nazerian, P., et al. (2011) 'Delayed neuropsychological sequelae after carbon monoxide poisoning: predictive risk factors in the Emergency Department. A retrospective study', *Scand J Trauma Resusc Emerg Med,* 19, pp. 16.
Peterson, J. E. and Stewart, R. D. (1975) 'Predicting the carboxyhemoglobin levels resulting from carbon monoxide exposures', *J Appl Physiol,* 39(4), pp. 633.
Peterson, J. F. and Stewart, R. D. (1970) 'Absorption and elimination of carbon monoxide by inactive young men', *Arch Environ Health,* 21(2), pp. 165-71.
Plumb, J. O. M., Otto, J. M., Kumar, S. B., et al. (2020) 'Application of the optimized carbon monoxide rebreathing method for the measurement of total haemoglobin mass in chronic liver disease', *Physiol Rep,* 8(6), pp. e14402.
Popović-Grle, S., Pavicić, F. and Bicanić, V. (1992) '[The effect of cigarette smoking on pulmonary diffusing capacity in asymptomatic smokers]', *Lijec Vjesn,* 114(5-8), pp. 118-21.
Potchter, O., Oz, M., Brenner, S., et al. (2014) 'Exposure of motorcycle, car and bus commuters to carbon monoxide on a main road in the Tel Aviv metropolitan area, Israel', *Environ Monit Assess,* 186(12), pp. 8413-8424
Poulton, T. J. (1987) 'Medical helicopters: carbon monoxide risk?', *Aviat Space Environ Med,* 58(2), pp. 166-168.
Public Health England (PHE). (2014) Antenatal checks: Carbon Monoxide (CO). Available at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/485608/CO_antenatal_checks_algorithm_2015.pdf (Accessed: 11 December 2021).
Schroeder, T. and Irish, C. (2005) 'Unintentional non-fire-related carbon monoxide exposures--United States, 2001-2003', *MMWR Morb Mortal Wkly Rep,* 54(2), pp. 36-9.
Prockop, L. D. and Chichkova, R. I. (2007) 'Carbon monoxide intoxication: an updated review', *J Neurol Sci,* 262(1-2), pp. 122-30.
Quanjer, P. H., Capderou, A., Mazicioglu, M. M., et al. (2014) 'All-age relationship between arm span and height in different ethnic groups', *Eur Respir J,* 44(4), pp. 905-912.
Rankin, J., McNeill, R. S. and Forster, R. E. (1961) 'The effect of anemia on the alveolar-capillary exchange of carbon monoxide in man', *J Clin Invest,* 40(7), pp. 1323-1330.
Ranu, H., Wilde, M. and Madden, B. (2011) 'Pulmonary function tests', *Ulster Med J,* 80(2), pp. 84-90.
Raphael, J.-C., Elkharrat, D., Jars-Guincestre, et al. (1989) 'Trial of normobaric and hyperbaric oxygen for acute carbon monoxide intoxication', *Lancet,* 334(8660), pp. 414-419.
Raub, J. A., Mathieu-Nolf, M., Hampson, N. B. and Thom, S. R. (2000) 'Carbon monoxide poisoning — a public health perspective', *Toxicology,* 145(1), pp. 1-14.
Ren, W., Li, L., Zhao, R.-y. and Zhu, L. (2012) 'Age-associated changes in pulmonary function: a comparison of pulmonary function parameters in healthy young adults and the elderly living in Shanghai', *Chin Med J,* 125 17, pp. 3064-3068.
Reynolds, S. J., Black, D. W., Borin, S. S., et al. (2001) 'Indoor environmental quality in six commercial office buildings in the midwest United States', *Appl Occup Environ Hyg,* 16(11), pp. 1065-1077.
Rickert, W. S., Robinson, J. C., Young, J. C., et al. (1983) 'A comparison of the yields of tar, nicotine, and carbon monoxide of 36 brands of Canadian cigarettes tested under three conditions', *Prev Med,* 12(5), pp. 682-694.
Robinson, J. C. and Forbes, W. F. (1975) 'The role of carbon monoxide in cigarette smoking', *Arch Environ Health,* 30(9), pp. 425-434.
Roca-Barceló, A., Crabbe, H., Ghosh, R., et al. (2020) 'Temporal trends and demographic risk factors for hospital admissions due to carbon monoxide poisoning in England', *Pre Med,* 136, pp. 106104.
Roca, J., Rodriguez-Roisin, R., Cobo, E., et al. (1990) 'Single-breath carbon monoxide diffusing capacity prediction equations from a Mediterranean Population', *Am Rev Respir Dis,* 141(4 pt 1), pp. 1026-1032.

Roderique, L. J. D., Josef, C. S., Newcomb, A. H., et al. (2015) 'Preclinical evaluation of injectable reduced yydroxocobalamin as an antidote to acute carbon monoxide poisoning', *J Trauma Acute Care Surg*, 79(4 s2), pp. S116-S120.
Ronald, F. and Coburn, M. D. (1970) 'The carbon monoxide body store', *Ann N Y Acad Sci*, 174(1), pp. 11-22.
Rose, J. J., Wang, L., Xu, Q., et al. (2017) 'Carbon monoxide poisoning: pathogenesis, management, and future directions of therapy', *Am J Respir Crit Care Med*, 195(5), pp. 596-606.
Rubinstein, I., Zamel, N., DuBarry, L. and Hoffstein, V. (1990) 'Airflow limitation in morbidly obese, nonsmoking men', *Ann Intern Med*, 112(11), pp. 828-832.
Sakornsakolpat, P., Bakke, P., Gulsvik, A., et al. (2018) 'Genetic determinants of diffusing capacity of carbon monoxide: genome-wide association analyses and meta-analysis in four cohorts', *ATS 2018 conference*..
Salome, C. M., King, G. G. and Berend, N. (2009) 'Physiology of obesity and effects on lung function', *J Appl Physiol*, 108(1), pp. 206-211.
Sansores, R. H., Abboud, R. T., Kennell, C. and Haynes, N. (1995) 'The effect of menstruation on the pulmonary carbon monoxide diffusing capacity', *Am J Respir Crit Care Med*, 152(1), pp. 381-384.
Sansores, R. H., Pare, P. D. and Abboud, R. T. (1992) 'Acute effect of cigarette smoking on the carbon monoxide diffusing capacity of the lung', *Am Rev Respir Dis*, 146(4), pp. 951-958.
Sargent, J. D. and Dalton, M. (2001) 'Does parental disapproval of smoking prevent adolescents from becoming established smokers?', *Pediatrics*, 108(6), pp. 1256-1262.
Saydain, G., Beck, K. C., Decker, P. A., et al. (2004) 'Clinical significance of elevated diffusing capacity', *Chest*, 125(2), pp. 446-452.
Scarlata, S., Paladini, L., Cesari, M. and Incalzi, R. A. (2010) 'Lung volumes in COPD - not only the total lung capacity', *Chest*, 138(1), pp. 233.
Schaefer, T. (2019) 'Age-related changes of pulmonary diffusion capacity components in healthy adolescents', *Eur Respir J*, 54(suppl 63), pp. PA4987.
Scherer, G. (2006) 'Carboxyhemoglobin and thiocyanate as biomarkers of exposure to carbon monoxide and hydrogen cyanide in tobacco smoke', *Exp Toxicol Pathol*, 58(2), pp. 101-124.
Schimmel, J., George, N., Schwarz, J., Yousif, S., et al. (2018) 'Carboxyhemoglobin levels induced by cigarette smoking outdoors in smokers', *J Med Toxicol*, 14(1), pp. 68-73.
Schmidt, W. and Prommer, N. (2005) 'The optimised CO-rebreathing method: a new tool to determine total haemoglobin mass routinely', *Eur J Appl Physiol*, 95(5), pp. 486-495.
Sein Anand, J., Schetz, D., Waldman, W. and Wiśniewski, M. (2017) 'Hyperventilation with maintenance of isocapnia. An "old new" method in carbon monoxide intoxication', *PloS one*, 12(1), pp. e0170621.
Selvakumar, S., Sharan, M. and Singh, M. P. (1993) 'A mathematical model for the elimination of carbon monoxide in humans', *J Theor Biol*, 162(3), pp. 321-336
Sharp, J. T., Henry, J. P., Sweany, S. K., et al. (1964) 'The total work of breathing in normal and obese men', *J Clin Invest*, 43(4), pp. 728-739.
Shie, H-G and Li, C-Y. (2007) 'Population-based case-control study of risk factors for unintentional mortality from carbon monoxide poisoning in Taiwan', *Inhal Toxicol*, 19(10), PP. 905-912.
Shin, T. R., Oh, Y.-M., Park, J. H., et al. (2015) 'The prognostic value of residual volume/total lung capacity in patients with chronic obstructive pulmonary disease', *J Korean Med Sci*, 30(10), pp. 1459-1465.
Shinkawa, T., Hayashida, N., Mori, K., et al. (2009) 'Poor chewing ability is associated with lower mucosal moisture in elderly individuals', *Tohoku J Exp Med*, 219(4), pp. 263-267.
Shrubsole, C., Symonds, P. and Taylor, J. (2017) *Determinants of CO Exposure in the English Housing Stock: Modelling Current and Possible Future Risks*, London: University College London (UCL).
Siebenmann, C., Keiser, S., Robach, P. and Lundby, C. (2017) 'CORP: The assessment of total hemoglobin mass by carbon monoxide rebreathing', *J Appl Physiol*, 123(3), pp. 645.
Sill, J. (2016) 'The effects of smoking on pulmonary function testing', *Chest*, 149(4), pp. A591.
Smith, M. V., Hazucha, M. J., Benignus, V. A. and Bromberg, P. A. (1994) 'Effect of regional circulation patterns on observed HbCO levels', *J Appl Physiol*, 77(4), pp. 1659-1665.
Son, J.-Y., Bell, M. L. and Lee, J.-T. (2010) 'Individual exposure to air pollution and lung function in Korea: spatial analysis using multiple exposure approaches', *Environ Res*, 110(8), pp. 739-749.
Staniforth, A. (1997) 'Regional modeling: a theoretical discussion', *Meteorol Atmos Phys*, 63(1), pp. 15-29.

Stanojevic, S. (2018) 'Standardisation of lung function test interpretation: global lung function initiative', *Lancet Respir Med*, 6(1), pp. 10-12.
Stanojevic, S., Graham, B. L., Cooper, B. G., Thompson, Bruce R., Carter, K. W., Francis, R. W. and Hall, Graham L. (2017) 'Official ERS technical standards: global lung function initiative reference values for the carbon monoxide transfer factor for Caucasians', *Eur Respir J*, 50(3), pp. 1700010.
Stanojevic, S., Wade, A. and Stocks, J. (2010) 'Reference values for lung function: past, present and future', *Eur Respir J*, 36(1), pp. 12.
Stanojevic, S., Wade, A., Stocks, J., et al. (2008) 'Reference ranges for spirometry across all ages: a new approach', *Am J Respir Crit Care Med*, 177(3), pp. 253.
Stevenson, D. K., Wong, R. J., Ostrander, C. R., et al. (2019) 'Increased carbon monoxide washout rates in newborn infants', *Neonatology*, 117(1), pp.118-122.
Stewart, R. D., Peterson, J. E., Baretta, E. D., et al. (1970) 'Experimental human exposure to carbon monoxide', *Arch Environ Health*, 21(2), pp. 154-164.
Stewart, R. I. (1988) 'Carbon monoxide diffusing capacity in asthmatic patients with mild airflow limitation', *Chest*, 94(2), pp. 332-336.
Storebø, M. L., Eagan, T. M. L., Eide, G. E., et al. (2016) 'Change in pulmonary diffusion capacity in a general population sample over 9 years', *Eur Clin Respir J*, 3, pp. 31265.
Strasser, A. A., Lerman, C., Sanborn, P. M., et al. (2007) 'New lower nicotine cigarettes can produce compensatory smoking and increased carbon monoxide exposure', *Drug Alcohol Depend*, 86(2), pp. 294-300.
Strong, E. (2014a) *Pulmonary Function Tests (PFT): Lesson 1 - An Introduction*. YouTube: Strong Medicine. Available at: https://www.youtube.com/watch?v=6TApeMJ-rkc (Accessed: 25 Mar 2020).
Strong, E. (2014b) *Pulmonary Function Tests (PFT): Lesson 3 - Lung Volumes*. YouTube: Strong Medicine. Available at: https://www.youtube.com/watch?v=4nQApZ8Q0XM&t=414s (Accessed: 25 Mar 2020).
Strong, E. (2014c) *Pulmonary Function Tests (PFT): Lesson 4 - DLCO*. YouTube: Strong Medicine. Available at: https://www.youtube.com/watch?v=j_oRQS2dnck (Accessed: 25 Mar 2020).
Strong, E. (2014d) *Pulmonary Function Tests (PFT): Lesson 5 - Summary and Practice Cases*. YouTube: Strong Medicine. Available at: https://www.youtube.com/watch?v=6mZmpHycSuQ (Accessed: 25 Mar 2020).
Takeuchi, A., Vesely, A., Rucker, J., et al. (2000) 'A simple "new" method to accelerate clearance of carbon monoxide', *Am J Respir Crit Care Med*, 161(6), pp. 1816-1819.
Taiwan Air Quality Monitoring Database (TAQMD) (2021) Available at: https://airtw.epa.gov.tw/cht/EnvMonitoring/Central/CentralMonitoring.aspx (Accessed: 10 December 2021).
Talaminos Barroso, A., Marquez Martin, E., Roa Romero, L. M. and Ortega Ruiz, F. (2018) 'Factors affecting lung function: a review of the literature', *Arch Bronconeumol*, 54(6), pp. 327-332.
Tantisuwat, A. and Thaveeratitham, P. (2014) 'Effects of smoking on chest expansion, lung function, and respiratory muscle strength of youths', *J Phys Ther Sci*, 26(2), pp. 167-170.
Thomas, E. T., Guppy, M., Straus, S. E., et al. (2019) 'Rate of normal lung function decline in ageing adults: a systematic review of prospective cohort studies', *BMJ Open*, 9(6), pp. e028150.
Thompson, B. R., Johns, D. P., Bailey, M., et al. (2008) 'Prediction equations for single breath diffusing capacity (TLco) in a middle aged caucasian population', *Thorax*, 63(10), pp. 889-893.
Tikuisis, P., Kane, D. M., McLellan, T. M., et al. (1992) 'Rate of formation of carboxyhemoglobin in exercising humans exposed to carbon monoxide', *J Appl Physiol*, 72(4), pp. 1311-1319.
Townsend, C. L. and Maynard, R. L. (2002) 'Effects on health of prolonged exposure to low concentrations of carbon monoxide', *Occup Environ Med*, 59(10), pp. 708.
Townsend, E. A., Miller, V. M. and Prakash, Y. S. (2012) 'Sex differences and sex steroids in lung health and disease', *Endocr Rev*, 33(1), pp. 1-47.
Tseng, H.-J., Henry, T. S., Veeraraghavan, S., et al. (2017) 'Pulmonary function tests for the radiologist', *Radiographics*, 37(4), pp. 1037-1058.
Valent, F., McGwin, G., Jr., Bovenzi, M. and Barbone, F. (2002) 'Fatal work-related inhalation of harmful substances in the United States', *Chest*, 121(3), pp. 969-975.
van der Lee, I., Zanen, P., van den Bosch, J. M. M. and Lammers, J. W. J. (2006) 'Pattern of diffusion disturbance related to clinical diagnosis: the KCO has no diagnostic value next to the DLCO', *Respir Med*, 100(1), pp. 101-109.

van Ganse, W. F., Ferris, B. G. and Cotes, J. E. (1972) 'Cigarette smoking and pulmonary diffusing capacity (Transfer Factor)', *Am Rev Respir Dis*, 105(1), pp. 30-41
Vandevoorde, J., Verbanck, S., Schuermans, D., et al. (2006) 'Obstructive and restrictive spirometric patterns: fixed cut-offs for FEV1/FEV6 and FEV6', *Eur Respir J*, 27(2), pp. 378-383.
Varon, J., Marik, P. E., Fromm, R. E., et al. (1999) 'Carbon monoxide poisoning: a review for clinicians', *J Emerg Med*, 17(1), pp. 87-93.
Vázquez-García, J. C., Pérez-Padilla, R., Casas, A., et al. (2016) 'Reference values for the diffusing capacity determined by the single-breath technique at different altitudes: the Latin American single-breath diffusing capacity reference project', *Respir Care*, 61(9), pp. 1217-1223.
Verbanck, S., Van Muylem, A., Schuermans, D., et al. (2016) 'Transfer factor, lung volumes, resistance and ventilation distribution in healthy adults', *Eur Respir J*, 47(1), pp. 166-176.
Vogt, T. M., Selvin, S. and Hulley, S. B. (1979) 'Comparison of biochemical and questionnaire estimates of tobacco exposure', *Prev Med*, 8(1), pp. 23-33.
Volans, G., Armstrong, B., Croxford, B., et al. (2007) *Neuropsychological effects of chronic exposure to carbon monoxide in the indoor air*, London: University College London, Guys and St Thomas NHS Foundation Trust.
Vreman, H. J., Wong, R. J., Stevenson, D. K., et al. (2006) 'Concentration of carbon monoxide (CO) in postmortem human tissues: effect of environmental CO exposure', *J Forensic Sci*, 51(5), pp. 1182-1190.
Wallace, L. A. (1983) 'Carbon monoxide in air and breath of employees in an underground office', *J Air Pollut Control Assoc*, 33(7), pp. 678-682.
Wang, X., Yano, E., Nonaka, K., et al. (1997) 'Respiratory impairments due to dust exposure: a comparative study among workers exposed to silica, asbestos, and coalmine dust', *Am J Ind Med.*, 31(5), pp. 495-502.
Wanger, J., Clausen, J. L., Coates, A., et al. (2005) 'Standardisation of the measurement of lung volumes', *Eur Respir J*, 26(3), pp. 511
Warner, D. O. (2005) 'Preoperative smoking cessation: the role of the primary care provider', *Mayo Clin Proc*, 80(2), pp. 252-258.
Warner, R. M. (2008) *Applied statistics : from bivariate through multivariate techniques.* 2ed edn. Los Angeles, California: SAGE Publications, Incorporated,.
Weaver, L. K. (2009) 'Carbon monoxide poisoning', *N Engl J Med*, 360(12), pp. 1217-1225.
Weaver, L. K., Hopkins, R. O., Chan, K. J., et al. (2002) 'Hyperbaric oxygen for acute carbon monoxide poisoning', *N Engl J Med*, 347(14), pp. 1057-1067.
Weaver, L. K., Howe, S., Hopkins, R. and Chan, K. J. (2000) 'Carboxyhemoglobin half-life in carbon monoxide-poisoned patients treated with 100% oxygen at atmospheric pressure', *Chest*, 117(3), pp. 801-808.
Weibel, E. R. and Gomez, D. M. (1962) 'Architecture of the human lung', *Science*, 137(3530), pp. 577.
Weinhold, L. L. and Stitzer, M. L. (1989) 'Effects of puff number and puff spacing on carbon monoxide exposure from commercial brand cigarettes', *Pharmacol Biochem Behav*, 33(4), pp. 853-858.
Weitzman, R. H. and Wilson, A. F. (1974) 'Diffusing capacity and over-all ventilation: Perfusion in asthma', *Am J Med*, 57(5), pp. 767-774.
Wémeau-Stervinou, L., Perez, T., Murphy, C., et al. (2012) 'Lung capillary blood volume and membrane diffusion in idiopathic interstitial pneumonia', *Respir Med*, 106(4), pp. 564-570.
West, J. B. (1995) *Pulmonary Pathophysiology: the Essentials*. 5th edn. Baltimore, Maryland: Williams & Wilkins.
West, R. J. (1984) 'The effect of duration of breath-holding on expired air carbon monoxide concentration in cigarette smokers', *Addict Behav*, 9(3), pp. 307-309.
WHO (1958) *Air pollution: fifth report of the Expert Committee on Environmental Sanitation.* Available at: http://apps.who.int/iris/handle/10665/40416 (Accessed: 15 December 2021).
WHO (1964) *Atmospheric pollutants.* Available at: http://apps.who.int/iris/handle/10665/40578 (Accessed: 15 December 2021).
WHO (1972) *Air quality criteria and guides for urban air pollutants.* Available at: http://apps.who.int/iris/handle/10665/40989 (Accessed: 15 December 2021).
WHO (1987) *Air quality guidelines for Europe.* Available at: https://extranet.who.int/iris/restricted/handle/10665/107364 (Accessed: 15 December 2021).
WHO (1999) *Environmental health criteria 213: carbon monoxide*. Available at: https://www.who.int/ipcs/publications/ehc/ehc_213_part_1.pdf?ua=1 (Accessed: 09 April 2020)

WHO (1999) *Men Ageing And Health- Achieving health across the life span.* Available at: http://apps.who.int/iris/bitstream/handle/10665/66941/WHO_NMH_NPH_01.2.pdf;jsessionid=23FE834A2539F10935396BE8F52A0F3F?sequence=1 (Accessed: 28 December 2021).

WHO (2000) *Air quality guidelines for europe.* Available at: https://apps.who.int/iris/bitstream/handle/10665/107335/9789289013581-eng.pdf?sequence=1&isAllowed=y (Accessed: 09 April 2020).

WHO (2010) *WHO guidelines for indoor air quality: selected pollutants.* Available at: https://www.euro.who.int/__data/assets/pdf_file/0009/128169/e94535.pdf (Accessed: 09 April 2020).

WHO (2014) *WHO indoor air quality guidelines: household fuel combustion*. Available at: https://www.who.int/publications/i/item/9789241548885 (Accessed: 09 April 2020).

WHO (2017). *Cardiovascular diseases (CVDs)* Available at: https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds) (Accessed: 09 April 2020).

WHO (2020) *Obesity and overweight.* Available at: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight (Accessed: 06 April 2020).

Whitrow, M. J. and Harding, S. (2008) 'Ethnic differences in adolescent lung function: anthropometric, socioeconomic, and psychosocial factors', *Am J Respir Crit Care Med,* 177(11), pp. 1262-1267.

Widmaier, E. P., Raff, H. and Strang K. T. (2008) *Vander's human physiology: the mechanisms of body funstion.* 10 edn. New York, New York: McGraw-Hill Education,.

Woehlck, H. J., Mei, D., Dunning, M. B. and Ruiz, F. (2001) 'Mathematical modeling of carbon monoxide exposures from anesthetic breakdown Effect of subject size, hematocrit, fraction of inspired oxygen, and quantity of carbon monoxide', *Anesthesiology,* 94(3), pp. 457-460.

Wu, L. and Wang, R. (2005) 'Carbon monoxide: endogenous production, physiological functions, and pharmacological applications', *Pharmaco Rev,* 57(4), pp. 585-630.

Wyka, K., Mathews, P. and Rutkowski, J. (2011) *Foundations of Respiratory Care.* 2 edn. New York, New York: CENGAGE Delmar Learning.

Yang, S. (1992) 'Single-breath carbon monoxide diffusing capacity: effect of body size and age in healthy nonsmoking Chinese', *J Formos Med Assoc,* 91(2), pp. 131-40.

Yang, S. C. (1993) '[Pulmonary diffusing capacity in normal smoking Chinese]', *J Formos Med Assoc,* 92 Suppl 2, pp. S61-8.

Yoshioka, E., Hanley, S. J. B., Kawanishi, Y. and Saijo, Y. (2014) 'Epidemic of charcoal burning suicide in Japan', *Br J Psychiatry,* 204(4), pp. 274-282.

Yoshioka, E., Hanley, S. J. B. and Saijo, Y. (2019) 'Time trends in suicide rates by domestic gas or car exhaust gas inhalation in Japan, 1968–1994', *Epidemiol Psychiatr Sci,* 28(6), pp. 644-654.

Yuan, W., He, X., Xu, Q.-F., Wang, et al. (2014) 'Increased difference between slow and forced vital capacity is associated with reduced exercise tolerance in COPD patients', *BMC pulm Med,* 14, pp. 16-16.

Zahir, M., Patolia, S., Eneh, K., et al. (2010) 'Effect of sex and smoking on DLCO - What about female smoker?', *ATS 2010 conference*.

Zaouter, C. and Zavorsky, G. S. (2012) 'The measurement of carboxyhemoglobin and methemoglobin using a non-invasive pulse CO-oximeter', *Respir Physiol Neurobiol,* 182(2), pp. 88-92.

Zavorsky, G. S., Kim, D. J., Sylvestre, J.-L. and Christou, N. V. (2008) 'Alveolar-membrane diffusing capacity improves in the morbidly obese after bariatric surgery', *Obes Surg,* 18(3), pp. 256-263.

Zavorsky, G. S., Smoliga, J. M., Longo, L. D., et al. (2012) 'Increased carbon monoxide clearance during exercise in humans', *Med. Sci. Sports Exerc,* 44(11), pp. 2118-2124.

Zavorsky, G. S., Tesler, J., Rucker, J., et al. (2014) 'Rates of carbon monoxide elimination in males and females', *Physio Rep,* 2(12), pp. e12237.

Zhang, Q., Li, L., Smith, M., et al. (2013) 'Exhaled carbon monoxide and its associations with smoking, indoor household air pollution and chronic respiratory diseases among 512,000 Chinese adults', *Int J Epidemiol,* 42(5), pp. 1464-1475.

Zou, B., Wilson, J. G., Zhan, F. B. and Zeng, Y. (2009) 'Air pollution exposure assessment methods utilized in epidemiological studies', *J Environ Monit,* 11(3), pp. 475-490.

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9 APPENDIX

9.1Ethical approval confirmation and documents for projects

9.1.1 Ethical document from Southampton Hospital

NIHR Southampton Respiratory Biomedical Research Unit

UNIVERSITY OF Southampton

Southampton NHS University Hospitals NHS Trust

Dr James O. M. Plumb

University Hospital Southampton
Tremona Road, Southampton SO16 6YD
Tel: 02381 205308
E-mail: j.plumb@soton.ac.uk

CONSENT FORM Version 1.2 **07/01/20Long**
title: Cardiopulmonary exercise testing before and after intravenous iron: a prospective clinical study
Name of Principal Investigator: Dr James O.M. Plumb
Thank you for reading the ***Patient Information Sheet***. If you would like to take part, please read and sign this form.
Study number:_____

PLEASE INITIAL THE BOXES IF YOU AGREE WITH EACH SECTION:

1.	I have read the Patient Information Sheet for this study and have been given a copy to keep. I have had the opportunity to consider the information and to ask questions, and have had any questions answered satisfactorily.	
2.	I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.	
3.	I understand that as part of this study I will have 3 and possibly 4 additional visits to hospital (depending on the dose of intravenous iron that I require), twice for a blood tests and finally to repeat a CPET and total haemoglobin mass test. This is in addition to the visit I would have anyway as part of my routine care.	
4.	I understand that as part of this study I will have blood taken 4 times in addition to my standard care.	
5.	I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from regulatory authorities or from the NHS Trust, where relevant to the research. I give permission for these individuals to have access to my records. I understand that the information will be kept confidential.	
6.	I understand that I will not benefit financially if this research leads to the development of a new treatment or test.	
7.	I know how to contact research team if I need to.	
8.	I agree to participate in this study.	
9.	I agree to the study team informing my GP of my participation in this study.	
10.	(Optional) I agree that in the future, my anonymised data may be shared with researchers outside the UK and outside the European Union.	Yes☐ No ☐
11.	(Optional) I agree to gift my blood samples to the research team for analysis in this study and for use in future research studies.	Yes☐ No ☐

Patient name	Date	Signature
Researcher taking consent	Date	Signature

Original for Investigator Site File, 1 copy for participant, 1 copy for medical record/hospital note

The Southampton Respiratory Biomedical Research Unit is funded by the National Institute for Health Research (NIHR V1.2 07/01/2018)

Supplementary Figure 9-1. Consent form from Southampton Hospital for CO-rebreathing project

9.1.2 Ethical approval from Tri-Service General Hospital

TEL: 886-2-87923311 ext 10552
FAX:886-2-2793-6049 / 2792-9201
E-mail: tsghirb@ndmctsgh.edu.tw

國防醫學院三軍總醫院
人體試驗審議會

11490 台北市內湖區成功路二段
325 號 醫療大樓五樓 5113 室
No. 325, Sec.2, Cheng-Kung Rd.
Neihu 11490, Taipei, Taiwan, R.O.C

人體試驗計畫同意函

本審議會核准編號：1-108-05-066
計畫名稱：肺功能中一氧化碳瀰散量與其相關因子之研究
執行機構：三軍總醫院
計畫主持人：高壓氧中心黃坤崙醫師
計畫書版本日期：108.05.02. v3.0
本會審核通過之其他文件版本及日期：
中文摘要：108.04.12. v6.0；個案報告表：108.04.30. v2.0

業經本院 2019 年 5 月 7 日人體試驗審議會第一審議會審查通過，該計畫案經評估屬低度風險，(持續審查頻率為每年一次)，有效期限至 2020 年 5 月 6 日，特此證明。
本審議會的運作，遵循藥品優良臨床試驗準則及政府相關法律規章。計畫主持人應於同意函有效期屆滿前二個月，提出展延申請，本案須經本院人體試驗審議會通過後，方可繼續執行。

Letter of Approval
Institutional Review Board, Tri-Service General Hospital

TSGHIRB No.: 1-108-05-066
Protocol title：The related factors to DLco and CO uptake and elimination
Research institution：Tri-Service General Hospital
Principle investigator：Dr. Kun-Lun Huang
Protocol version：108.05.02. v3.0
Other documents：
Chinese Abstract：108.04.12. v6.0；Case Report Form：108.04.30. v2.0

On 05/07/2019, the Institutional Review Board I of the Tri-Service General Hospital approved the above-named application. Assessed as Low Risk, the protocol is subject to follow-up review annually. The Board is organized and operated in compliance with International Conference on Harmonization (ICH) / WHO Good Clinical Practice (GCP) and applicable laws and regulations.
This approval is valid for 1 year till 05/06/2020. The principle investigator is required to submit the application for extension 2 months before the expiration date.

軍總醫院 TSGHIRB 人體試驗審議會

Institutional Review Board
余慕賢
Chairman_____.

The Committee is Organized and operates in accordance with ICH6 GCP regulations and guideline.
本審議會組織與運作皆遵守ICH6 GCP規定

Supplementary Figure 9-2. Ethical approval from Tri-Service General Hospital for PFT project

9.1.3 Ethical approval from Royal Berkshire Hospital (HRA)

Ymchwil Iechyd a Gofal Cymru
Health and Care Research Wales

NHS
Health Research Authority

Dr Ben Croxford
4.02 Central House
14 Upper Woburn Place
London
WC1H 0NN

Email: approvals@hra.nhs.uk
HCRW.approvals@wales.nhs.uk

02 April 2020

Dear Dr Croxford

HRA and Health and Care Research Wales (HCRW) Approval Letter

Study title:	**Improved modelling of factors affecting carbon monoxide (CO) uptake and elimination by humans**
IRAS project ID:	**253541**
Protocol number:	**127205**
REC reference:	**20/NW/0133**
Sponsor	**University College London**

I am pleased to confirm that **HRA and Health and Care Research Wales (HCRW) Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

Please now work with participating NHS organisations to confirm capacity and capability, in line with the instructions provided in the "Information to support study set up" section towards the end of this letter.

How should I work with participating NHS/HSC organisations in Northern Ireland and Scotland?
HRA and HCRW Approval does not apply to NHS/HSC organisations within Northern Ireland and Scotland.

If you indicated in your IRAS form that you do have participating organisations in either of these devolved administrations, the final document set and the study wide governance report (including this letter) have been sent to the coordinating centre of each participating nation. The relevant national coordinating function/s will contact you as appropriate.

Please see IRAS Help for information on working with NHS/HSC organisations in Northern Ireland and Scotland.

How should I work with participating non-NHS organisations?
HRA and HCRW Approval does not apply to non-NHS organisations. You should work with your non-NHS organisations to obtain local agreement in accordance with their procedures.

What are my notification responsibilities during the study?

The standard conditions document "*After Ethical Review – guidance for sponsors and investigators*", issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

Who should I contact for further information?
Please do not hesitate to contact me for assistance with this application. My contact details are below.

Your IRAS project ID is **253541**. Please quote this on all correspondence.

Yours sincerely,

Kevin Ahmed
Approvals Manager

Email: approvals@hra.nhs.uk

Copy to: *Miss Sabrina Kassim*

Supplementary Figure 9-3. Ethical approval from HRA for PFT project

NHS
Royal Berkshire
NHS Foundation Trust

Research & Development
Level 2, North Block
Royal Berkshire NHS Foundation Trust
London Road
Reading RG1 5AN

Tel: 0118 322 8223 / 8140
Fax: 0118 322 8425
jo.jones@royalberkshire.nhs.uk

Mr. Mark Unstead
Chief Respiratory Physiologist
Respiratory Department
Royal Berkshire NHS Foundation Trust
London Road
Reading
RG1 5AN

30 September 2020

Dear Mr Unstead,

RE: Improved modelling of factors affecting carbon monoxide (CO) uptake and elimination by humans / Respiratory Department
REC Ref: NRES Committee North West – Greater Manchester West REC, 20/NW/0133
EudraCT number: n/a
ISRCTN No: n/a
UKCRN: n/a
HRA: 253541
PID number: 15232
Protocol Version: 1.2, 05/03/2020

Capacity and capability to conduct this study at the Royal Berkshire NHS Foundation Trust is confirmed. A list of the letters containing documents reviewed is listed below.

Please also note that recruitment into the study is expected to start within 38 days of the date of this letter or by 07/11/2020. If you foresee any issues with achieving this please do let R&D know.

As Principal Investigator, you have agreed to obtain data from 200-500 patient records by the date of 30/11/2020 (recruitment end date).

As Principal Investigator, please may we remind you that R&D should be informed of any amendments to the Protocol, closure of the study or any changes made during the course of the study.

Recruitment figures should also be sent to R&D every month from the start of the study until completion.

Should any problems arise with recruitment or any other aspects of the study, please do not hesitate to contact R&D on the above numbers.

We would also like to take this opportunity to remind you of your responsibilities under the UK Policy Framework for Health and Social Care Research. An extract from this framework outlining these responsibilities are included with this letter for your reference.

We wish you all the best with the study.

Yours sincerely,

[redacted]

Jo Jones
Clinical Research Facilitator

Cc – Leslie Mokogwu – R&D Manager, RBFT
Student – Ke-Ting Pan, University College London
Sponsor contact – Sabrina Kassim – UCL/ UCLH Joint Research Office
Chief Investigator / Supervisor– Dr Ben Croxford - University College London
Studyline RBFT Database - royalberkshire@studyline.uk.com

Enclosure: Extract From UK Policy Framework for Health and Social Care Research (Version 3.3, 07/11/2017) Responsibilities of Researcher, PI/CI, Funder and Sponsor
Organisation Information Document (OID)

Regulatory Approval letters listing approved documents:

- Letter of HRA approval dated 02/04/2020
- North West - Greater Manchester West REC Proportionate Review Favourable Opinion Letter dated 02/04/2020

Supplementary Figure 9-4. Ethical approval from RBH for PFT project

9.1.4 Ethical and related documents from UCL

THE BARTLETT SCHOOL OF ENVIRONMENT, ENERGY AND RESOURCES

BSEER Research Ethics – Low Risk Application – Review (v1.11)

Applicant UCL email address: ke-ting.pan.16@ucl.ac.uk
Energy Institute / IEDE / ISH / ISR (Cross out as applicable)
Student / Staff (Cross out as applicable)
(If Student) **Course: PhD**
(If Student) **Supervisor: Ben Croxford**
(If Staff) **Principal Investigator:**
Title of Study: The related factors to DLco and CO uptake and elimination
Date of Application: 26/06/2019

	Unsatisfactory	Satisfactory	N/A
STUDY DETAILS			
Sufficient study details provided to evaluate ethical implications		**x**	
Study does not seem to include sensitive topics (see High Risk checklist)		x	
Sufficient sampling details provided to evaluate ethical implications		**x**	
Sample does not seem to include vulnerable individuals (see High Risk checklist) & active steps to exclude <18yo		x	
CONSENT			
Information for participants covers necessary issues adequately (Researcher & says if student, Institution, funder, study title & purpose, how participant selected, excludes <18yo, what happens to participant, how long it will take, benefits, potential risks/harms, anonymity/confidentiality, voluntariness, right to withdraw, contact details)		**x**	
Information for participants is sufficiently concise			x
Information for participants is written in an appropriate style (Study title and content appropriately phrased for participants, level of detail appropriate for participants)			x
(Where participants known to researcher) appropriate procedures to ensure participants feel free to not participate & withdraw from the study			x
EVALUATION & MITIGATION OF HARM			
Risk of harm to participants seems to be minimal (see High Risk checklist)		x	
Recognises & addresses potential risks/harms to participants		x	
(Where risks to researcher beyond those experienced in daily life) has appropriate risk assessment been completed?			x
DATA PROTECTION & PRIVACY			
Correctly identifies whether/not personal data are being collected / used / processed (Definition of personal data is embedded in the low risk form Q42…check whole application to ensure applicant answered this Q correctly)		x	
Correctly identifies whether/not *special category* or *criminal records* personal data are being collected / used / processed (Definitions embedded in the low risk form Q43…check whole application to ensure applicant answered this Q correctly)		x	
(If personal data are being collected / used / processed) has registered study with UCL Data Protection Officer		x	
(Where participants are known to researcher) appropriate procedures to protect participants' privacy (EG data collected &/or collection method)			x

Review (delete as applicable):

x Study is low risk and may commence.

☐ Study is low risk and may commence AFTER you obtain a UCL Data Protection number from UCL Legal – you are collecting personal data

☐ Study is low risk and may commence AFTER you meet the following conditions and demonstrate that to the evaluators:

☐ Study requires revised submission to BSEER Research Ethics Team. Data collection/processing cannot start until the research is evaluated as low risk.

☐ Study requires approval from UCL Research Ethics Committee prior to data collection/processing.

Name(s) of BSEER reviewer(s): Jonathon Taylor

Date: 26/06/2019

Supplementary Figure 9-5. Ethical approval from UCL for TSGH PFT project

UCL RESEARCH ETHICS COMMITTEE
OFFICE FOR THE VICE PROVOST RESEARCH

25th January 2019

Dr Ben Croxford
Behavioural Science and Health
UCL

Dear Dr Croxford,

Notification of Ethics Approval with Provisos
Project ID/Title: 14201/001: The effect of smoking on carbon monoxide in the human body

Further to your satisfactory responses to my comments, I am pleased to confirm in my capacity as Joint Chair of the UCL Research Ethics Committee (REC) that I have ethically approved your research study until **1st December 2019.**

Ethical approval is subject to the following conditions:

Notification of Amendments to the Research
You must seek Chair's approval for proposed amendments (to include extensions to the duration of the project) to the research for which this approval has been given. Each research project is reviewed separately and if there are significant changes to the research protocol you should seek confirmation of continued ethical approval by completing an 'Amendment Approval Request Form'
http://ethics.grad.ucl.ac.uk/responsibilities.php

Adverse Event Reporting – Serious and Non-Serious
It is your responsibility to report to the Committee any unanticipated problems or adverse events involving risks to participants or others. The Ethics Committee should be notified of all serious adverse events via the Ethics Committee Administrator (ethics@ucl.ac.uk) immediately the incident occurs. Where the adverse incident is unexpected and serious, the Joint Chairs will decide whether the study should be terminated pending the opinion of an independent expert. For non-serious adverse events the Joint Chairs of the Ethics Committee should again be notified via the Ethics Committee Administrator within ten days of the incident occurring and provide a full written report that should include any amendments to the participant information sheet and study protocol. The Joint Chairs will confirm that the incident is non-serious and report to the Committee at the next meeting. The final view of the Committee will be communicated to you.

Final Report
At the end of the data collection element of your research we ask that you submit a very brief report (1-2 paragraphs will suffice) which includes in particular issues relating to the ethical implications of the research i.e. issues obtaining consent, participants withdrawing from the research, confidentiality, protection of participants from physical and mental harm etc.
In addition, please:

Office of the Vice Provost Research, 2 Taviton Street
University College London
Tel: +44 (0)20 7679 8717
Email: ethics@ucl.ac.uk
http://ethics.grad.ucl.ac.uk/

Supplementary Figure 9-6. Ethical approval from UCL for exhaled CO project

20190117 Email confirm Z6364106 2019 01 77

Crouch, Spenser on behalf of Finance.Data Protection

Thu 17/01/2019 11:11

To:Pan, Ke-Ting <ke-ting.pan.16@ucl.ac.uk>;

Cc:Croxford, Ben <b.croxford@ucl.ac.uk>;

1 attachments (3 MB)

data protection application_KE-TING PAN (002).pdf;

Hi,

Thank you for your application to register with the Data Protection Office. I am pleased to confirm that this project is now registered under, reference No Z6364106/2019/01/77 social research in line with UCL's Data Protection Policy.

You may quote this reference on your Ethics Application Form, or any other related forms.

When all essential documents are ready to archive, contact the UCL Records Office by email records.office@ucl.ac.uk to arrange ongoing secure storage of your research records unless you have made specific alternative arrangements with your department, or funder. Please note the UCL Records Office does not store student research data.

For data protection enquiries, please contact the data protection team at data-protection@ucl.ac.uk

For ethics enquiries, please contact the ethics team at ethics@ucl.ac.uk.

Regards,

Spenser Crouch
Data Protection & Freedom of Information Administrator & Chief Web Editor
Legal Services, UCL | Gower Street | London | WC1E 6BT
Internal Address: 6th floor | Bidborough House | 38-50 Bidborough Street | Kings Cross | London | WC1H 9BT
Email: s.crouch@ucl.ac.uk Data Protection: data-protection@ucl.ac.uk FOI: foi@ucl.ac.uk.
Telephone: 0203 108 8764 (internal 58764)

Office: Tuesday & Thursday 7.30am – 3.30pm; Home: Monday, Wednesday & Friday

Please protect the Environment. Print only if necessary.

UCL

Supplementary Figure 9-7. Data protection confirmation from UCL for exhaled CO project

Research Questionnaire

Thank you for participating in the study.
All the data will be confidential and only accessed by researcher. Ask us if there is anything that is not clear or if you would like more information.

Please click the best description of you.

Demographic Questions:

1. What is your gender? ☐ Male (skip Question 10) ☐ Female ☐ other _____
2. How old are you? _____
3. Height: _____ cm Weight: _____ kg
4. What is your race/ethnicity?

   ☐ Asian ☐ Black/Africa American ☐ Hispanic/Latino ☐ White/Caucasian

   ☐ other_____
5. Diet?

   ☐ Vegetarian ☐ Vegan ☐ gluten free ☐ Pescatarian (no meat, but eat fish and/or shellfish)

   ☐ None of the above ☐ other_____
6. Do you currently smoke tobacco?

   ☐ Yes, on a regular basis. ☐ Not anymore, I quit. ☐ No, I have never used tobacco.
7. If you smoke tobacco, what type of cigarettes do you smoke?

   ☐ tobacco cigarettes ☐ roll-up cigarettes ☐ others _____
8. If you smoke tobacco,

   Daily? How many cigarettes do you smoke per day? _____

   Weekly? How many cigarettes do you smoke per week? _____
9. When did you smoke last time? _____

   ☐ within 1 hour ☐ 1 hour ago ☐ 2 hours ago ☐ 3 hours ago ☐ 4 hours ago or above

---

10. Question for women only

The question is related to the menstrual cycle. Because the relationship between menstrual cycle and CO concentration in the body has been described in literature. The exhaled CO concentration is lower in the period time than not in the period time (Foster et al. 1974; Antczak et al. 2012). It will be very appreciated if you could answer the question. Also, it is okay to leave blank if you don't feel comfortable to answer.

Are you happy to answer the question? ☐ Yes ☐ No

Menstrual cycle –

Date of the first day of your last period _____ (DDMMYY)

UCL Institute for Environmental Design & Engineering
University College London
Gower Street London WC1E 6BT

Open questions:

1. What exposure have you had to CO in the last few hours? For example, kitchen busy road or gas fire…
   □ Yes □ No
   □ 1. Kitchen □ 2. busy road (car exhaust) □ 3. gas fire □ 4. other _____

2. Have you done any activities that have raised your heart rate in the last 2 hours, for example, running and climbing stairs?
   □ Yes □ No
   □ 1. Light _____ □ 2. Medium_____ □ 3. High_____

Record precise smoking method, e.g. how many puffs taken per cigarette, time taken to smoke cigarette?

Time start smoking: _____

Time finish smoking: _____

Puffs: _____

The last length of cigarette: _____

Supplementary Figure 9-8. Questionnaire for exhaled CO experiment at UCL

Research Recruitment!! UCL

Hello,
We are seeking participants for a study on exhaled CO concentration. The experiment will last between 15-30 minutes for non-smokers and up to 2.5 hours for smokers. You will need to be able to hold your breath for up to 35 seconds, at varying intervals we will measure your exhaled CO.

Payment:
Non-smokers: £5 per person.
Smokers: £30 per person.

Criteria to participate:
Over 18 years old to 34 years old
Healthy with no history of lung function illness
Not pregnant
Fluent English speaker

If you are interested in participating in the study, please contact:
KE-TING PAN (Katy)
Tel: 07874944050
E-mail: ke-ting.pan.16@ucl.ac.uk

Supplementary Figure 9-9. The poster and flyer for exhaled CO experiment at UCL

9.2Supplemental information for literature review and supplemental data for results

This section contains the supplemental information for literature review and supplemental data for Section 5.

9.2.1 Supplemental information for literature review and methods

Equations for parameters in CFK (1965) model

Below are the assumptions and equations for the parameters of the CFK (1965) model. Haldane's coefficient, which quantifies the relative affinity of CO and O_2 for Hb, was assumed to be 250 in the CFK (1965) model (Haldane and Smith, 1897; Coburn et al., 1965). For $[COHb]_0$ and $[HbO_2]$, the values were derived from Peterson and Stewart's (1975) study. The maximum Hb capacity for CO (δ) is 1.389 ml/g based on STPD conditions and 1.68 ml/g based on BTPS conditions, for which the conversion factor β is 1.21 (West, 1995; WHO, 1999; Gosselin et al., 2009). The $[O_2Hb]_{max}$ used the data from Peterson and Stewart (1975) which reported that the value of $[O_2Hb]_{max}$ was around 0.25 ml/ml. The equations are as follows:

$$[HbO2]_{max} = \delta \times [Hb] \div 100$$

$$[COHb]_0(\%) = [COHb]_0(ml/ml) \times 100 \div [HbO2]_{max}$$

The value of V_{CO} (rate of endogenous CO production) in the CFK model was calculated based on the first equation below and was then converted into the value of BTPS conditions by using the second equation from Gosselin et al.'s study, where β is 1.21 (Coburn et al., 1963; West, 1995; Gosselin et al., 2009).

$$V_{CO}(STPD) = 0.007 \times \frac{Weight(kg)}{69.59(kg)}$$

$$V_{CO}(BTPS) = \beta \times V_{CO}(STPD)$$

The equations used to generate V_{AR} (alveolar ventilation rate), f (respiration rate), V_{BL} (blood volume), V_T (tidal volume) and DL_{CO} were from Peterson and Stewart' (1975) study, where BSA means body surface area (m^2).

$$V_{AR} = 0.933 \times f(min^{-1}) \times V_T - 132f$$

$$V_{BL}\,(Male) = 74\,(ml/kg) \times Weight$$

$$V_{BL}\,(Female) = 73\,(ml/g) \times Weight$$

$$DL_{CO}(BTPS) = 1/(-0.0287 + 0.1188/BSA))$$

BSA (body surface area) was presented as around 0.25 to 2.25 m^2. The most common value was around 2 m^2 (Foster, 1964). The most widely used equation to calculate BSA was published in *The New England Journal of Medicine* by Mosteller (1987).

$$BSA\ (m^2) = \sqrt{\frac{Height\ (cm) \times Weight(kg)}{3600}}$$

Equations for parameters in the modified CFK (2009) model

The following shows the equations for the parameters in the CFK model in BTPS condition (Coburn et al., 1965; Peterson and Stewart, 1975; Gosselin et al., 2009). The equations for V_{ALV}, Q_{ALV}, Endo, P_{O2}, V_{BL} and B_{COHb}^{Max} were defined as follows (Cotes, 1975; Brown et al., 1997),

$$V_{ALV} = V_T(tidal\ volume, ml_{air}) - V_D(physiological\ dead\ space, ml_{air})$$

$$Q_{ALV} = f_R(respiratory\ frequency, min^{-1}) \times (V_T - V_D)$$

$$Endo = 1.21 \times 0.007(ml_{CO}) \times \frac{Weight(kg)}{69.5(kg)}$$

$$P_{O2} = -0.24 \times Age(year) + 104.7$$

$$B_{COHb}^{Max} = b_{COHb}^{Max} \times C_{Hb} \times V_{BL}$$

$$V_{BL}(Blood\ Volume) = 7.9\% \times Weight$$

Gosselin et al. (2009) calculated DL_{CO} using the equations from Paoletti et al.' (1985) study, as shown below (see also details of the equation shown in Section 2.5.3 .

$$DL_{CO}(Male) = -31.3822 - 0.1936 \times Age(yr) + 0.4410 \times Height(cm)$$

$$DL_{CO}(Female) = 5.0767 - 0.0677 \times Age(yr) + 0.1569 \times Height(cm)$$

The DL_{CO} was shown in STPD condition. Gosselin et al. (2009) converted it into BTPD condition through multiplied the β, which was equal to 1.21 in model simulation (West, 1995).

$$DL_{CO}\ (BTPS) = \beta \times DL_{CO}\ (STPD)$$

Equations for CO-rebreathing experiment

Total Hb mass calculation (oCOR method)

$$\text{tHb} - \text{mass(g)} = (\text{K} \times \text{MCO} \times 100) \div (\Delta\text{COHb}(\%) \times 1.39)$$

a. K was calculated from '$current\ barometric\ pressure \times 760^{-1} \times [1 + (0.003661 \times current\ temperature)]$'
b. MCO was calculated from '$CO_{adm} - (CO_{system+lung\ (after\ disconnection)} + CO_{exhaled(after\ disconnection)})$'
c. CO_{adm} means the CO volume administered to the system

d. $CO_{system+lung\ (after\ disconnection)}$ was calculated from '$CO\ concentration\ in\ spirometer \times (spirometer\ volume + lung\ residual\ volume)$'
e. $CO_{exhaled\ (after\ disconnection)}$ was calculated from '$end - tidal\ CO\ cencentration \times alveolar\ ventilation \times time$'
f. $\Delta COHb(\%)$ means the difference between basal HbCO and HbCO in the blood samples after CO administration
g. 1.39 (ml_{CO}/g Hb) = Hüfner number (the amount of CO that can bind with one gram of Hb when fully saturated)

Details for method of equations for the CFK (1965) model

Supplementary Table 9-1. Parameters in the CFK (1965) model and their associated units

Parameter	Unit
COHb, a ratio of COHb to Hb, expressed as a percentage	%
DLco, diffusing capacity of the lung for CO,	(ml/min)/mmHg
M, the ratio of the affinity of blood for CO to that for O_2	No units
O_2Hb, a ratio of OHb to Hb expressed as a percentage	%
PB pressure,	mmHg
Pco_2, the average partial pressure of O2 in lung capillaries	mmHg
PH_2O, the saturated water vapour pressure at body temperature	mmHg
Pico, partial pressure of CO in inhaled air minus water vapour	mmHg
t, time	min
V_{AR}, alveolar ventilation rate	ml/min
V_{BL}, blood volume	ml
V_{CO}, rate of endogenous CO production	ml/min
$[COHb]_0$, the measure of CO in the blood at time t=0, a ratio of COHb to Hb expressed as a percentage	-

Supplementary Table 9-2. Measured COHb following exposure to 50 ppm of CO (Stewart et al., 1970, page 157)

	Mean	Range	No. of Subjects
Time during exposure			
Preexposure	0.7	0.4-1.5	11
30 min	1.3	1.3	3
1 hour	2.1	1.9-2.7	11
3 hours	3.8	3.6-4.2	10
6 hours	5.1	4.9-5.5	5
8 hours	5.9	5.4-6.2	5
12 hours	7.0	6.5-7.9	3
15.5 hours	7.6	7.2-8.2	3
22 hours	8.5	8.1-8.7	3
24 hours	7.9	7.6-8.2	3
Time after 1 hour of exposure			
30 min	1.8	1.8	3
1 hour	1.7	1.6-1.8	3
2 hours	1.5	1.4-1.5	3
5 hours	1.1	1.0-1.1	2
Time after 3 hours of exposure			
30 min	3.7	3.4-3.9	3
1 hour	3.3	2.7-3.8	3
2 hours	2.7	2.3-3.0	3
Time after 8 hours of exposure			
30 min	5.6	5.1-5.9	3
1 hour	5.1	4.8-5.4	3
1 hour 45 min	4.0	-	-

11 hours	1.5	1.4-1.7	
Time after 24 hours of exposure			
30 min	7.5	7.2-7.8	3
1 hour	6.7	6.4-7.1	3
2 hours	5.8	5.6-6.2	3

Calculations for the parameters used in the CFK (1965) model

Haldane's coefficient, which quantifies the relative affinity of CO and O_2 for Hb, was assumed to be 250 in the CFK (1965) model (Haldane and Smith, 1897; Coburn et al., 1965). The person was assumed male with height of 174.4 cm, weight of 70 kg and Hb concentration of 15 g/dL.

$$[HbO2]_{max} = \delta \times [Hb] \div 100 = 1.68 \times 15 \div 100 = 0.25$$

$$[COHb]_0 \left(\frac{ml}{ml}\right) = [COHb]_0(\%) \times [HbO2]_{max} \div 100 = 0.7 \times 0.25 \div 100 = 0.0018$$

$$V_{CO}(STPD) = 0.007 \times \frac{Weight(kg)}{69.59(kg)} = 0.007 \times \frac{70}{69.59} = 0.007$$

$$V_{CO}(BTPS) = \beta \times V_{CO}(STPD) = 1.21 \times 0.007 = 0.0085$$

The equations used to generate V_A and V_{BL} are from Peterson and Stewart's (1975) study. The researcher then used the data from Gosselin et al.'s (2009) study to calculate the results, where respiration rate (f) is assumed to be 12 min^{-1} and tidal volume (V_T) is assumed to be 750 ml.

$$V_{AR} = 0.933 \times f \times V_T - 132f = 0.933 \times 12 \times 750 - 132 \times 12 = 6813$$

$$V_{BL}(\text{male}) = 74 \times weight(kg) = 74 \times 70 = 5180$$

$$BSA\ (m^2) = \sqrt{\frac{Height\ (cm) \times Weight(k)}{3600}} = \sqrt{\frac{174.4 \times 70}{3600}} = 26.81$$

$$DL_{CO} = 1/(-0.0287 + 0.1188/A) = 1/(-0.0287 + 0.1188/1.8) = 26.81$$

Supplementary Table 9-3. The values of the parameters used for the CFK (1965) model simulation

Parameter	Description	Value
DL_{CO}	Diffusion capacity of CO	26.8 (ml/min)/mmHg
M	The ratio of the affinity of blood for CO to that for O2	250
P_B	Barometric pressure	750 mmHg
PCo_2 (Pa_{O2})	The average partial pressure of oxygen in lung capillaries	100 mmHg
V_{AR}	Alveolar ventilation rate	6000 ml/min
V_{BL}	Blood volume	5500 ml

V_{CO}	Rate of endogenous CO production	0.07 ml/min
$[COHb]_0$	Percentage of ml of CO per ml of blood at the beginning of the exposure	0.7 %

Details for method of equations for the modified CFK (2009) model

Supplementary Table 9-4. Parameters in the modified CFK (2009) model and their associated units

Parameter	unit
$A_{CO}(t)$, Amount of CO in alveoli as a function of time	ml_{CO}
$B_{CO}(t)$, Total amount of CO in the blood as a function of time	ml_{CO}
B_{COHb}^{Max}, The maximum amount of CO bound to Hb	ml_{CO}
$C_{EXT}(t)$, The concentration of CO in ambient air as a function of time	ml_{CO}/mlair
DL_{CO}, Diffusing capacity of lungs for CO	(ml_{CO}/min)/mmHg
$Endo$, rate of endogenous production of CO	ml_{CO}/min
M, Haldane's coefficient which quantifies the relative affinity of CO and O2 for Hb	- No units
P_{O2}, Partial pressure of oxygen in lung capillaries	mmHg
Q_{ALV}, The alveolar ventilation rate of inhaled air	ml_{air}/min
R, Avogadro's constant	mmHg (ml_{air})/Kelvin(ml_{CO})
V_{ALV}, Alveolar volume	ml
$S_{CO}(t)$, Amount of CO bound to haem proteins in the extravascular spaces as a function of time	ml_{CO}
T, Temperature BTPS	Kelvin
b_{COHb}^{Max}, The maximum amount of CO bound to 1g of Hb	ml_{CO}/g_{Hb}
k_{HS}, Capture rate of CO from blood to haem proteins in extravascular spaces	1/min
k_{Sf}, The release rate of CO from haem proteins in extravascular spaces to blood	1/min
k_{CO2}, Oxidising rate of CO	1/min
t, time	min

The simulations used the reference values for respiratory parameters, including tidal volume (V_T), respiratory frequency (f_R), physiological dead space (V_D) and alveolar ventilation rate (Q_{ALV}) in BTPS condition (body temperature of 37 °C, ambient barometrical pressure conditions and breathing gas saturated with water vapour) in Supplementary Table 9-5 and reference values for DL_{CO} are shown in Table 2-10 in Section 2.5.3 (Gosselin et al., 2009).

Supplementary Table 9-5. Reference values for the respiratory parameters of the general population (in BTPS condition)

Age group	People at rest			
	Tidal volume (ml)	Respiratory frequency (min^{-1})	Physiological dead space (ml)	Alveolar ventilation rate (ml/min)
1 year old	102	36	23	2843
5 years old	213	25	53	4000
10 years old				
Boys	333	19	90	4620
Girls	333	19	90	4620
15 years old				
Boys	533	15	150	5749
Girls	417	16	131	4571
Adults				

Men	750	12	168	6982
Women	464	14	143	4496

Because the individual value for participants in Stewart et al.'s (1970) study was not reported, simulations used average physiological parameters for men at rest. The final data used in the simulation of the modified CFK model was shown in Supplementary Table 9-6.

Supplementary Table 9-6. The values of parameters used for modified CFK (2009) model simulation

Parameter	Value	Unit	Calculation
Age	30	years-old	
Sex	Male		
Height	174.4	cm	
Weight	70	kg	
B_{COHb}^{Max}	1393.56	ml_{CO}	1.68 × 150 × 0.079 × 70
CHb	150	g_{Hb}/L_{blood}	
DL_{CO}	48.1	$(ml_{CO}/min)/mmHg$	(-31.3822-0.1936×30+0.4410×174.4) × 1.21
$Endo$	0.0085	ml_{CO}/min	1.21 × 0.007 × (70/69.5)
M	240		
P_{O2}	97.5	mmHg	-0.24 × 30 + 104.7
Q_{ALV}	6982	ml_{air}/min	(750-168) × 12
R	2.55	mmHg (ml_{air})/Kelvin(ml_{CO})	
T	310	Kelvin	
V_D	168	ml_{air}	
V_{BL}	5.53	L_{blood}	7.9% × 70
V_T	750	ml_{air}	
V_{ALV}	582	ml	750-168
b_{COHb}^{Max}	1.68	ml_{CO}/g_{Hb}	
f_R	12 min^{-1}		
k_{HbS}	0.002	1/min	
k_{Sf}	0.01	1/min	
k_{CO2}	0.0000333	1/min	

9.2.2 Supplemental tables for exhaled CO study

Supplementary Table 9-7. Basic demographic and behavioural factors overview for smokers and comparison of characteristics between light smokers and heavy smokers by chi-square test in the exhaled CO experiment dataset

Characteristics	Total (n=48) n (%)	Light Smokers (n=28) n (%)	Heavy smokers (n=20) n (%)	p-value
Sex				-
Male	39(81.3)	20(71.4)	19(95.0)	
Female	9(18.7)	8(28.6)	1(5.0)	
Ethnicity				0.497
Asian	27(56.3)	14(58.3)	13(68.4)	
White/Caucasian	16(33.3)	10(41.7)	6(31.6)	
Type of cigarette				0.883
Tobacco cigarettes	33(68.8)	19(67.9)	14(70.0)	
Roll-ups	11(22.9)	7(25.0)	4(20.0)	
Both	4(8.3)	2(7.1)	2(10.0)	
Exposure to CO before study				0.301

None	32(66.7)	17(60.7)	15(75.0)	
Yes	16(33.3)	11(39.3)	5(25.0)	
Exercise before study				0.762
None	30(62.5)	18(64.3)	12(60.0)	
Yes	18(37.5)	10(35.7)	8(40.0)	

*Note: *Where there was a significant difference found between light smokers and heavy smokers these values are shown in bold.*

Supplementary Table 9-8. Exhaled CO value for smokers and comparison of the data from light smokers and heavy smokers by t-test in the exhaled CO experiment dataset

Exhaled CO (ppm)	Total (n=48) mean ±SD	Light Smokers (n=28) mean ±SD	Heavy smokers (n=20) mean ±SD	p-value
Baseline	6.9 ±4.9	4.8 ±2.6	10.0 ±5.8	<0.001
Right after smoking	11.7 ±5.9	9.4 ±4.2	14.9 ±6.5	<0.001
30 mins after smoking	10.4 ±4.8	8.4 ±3.2	13.1 ±5.4	<0.001
60 mins after smoking	9.5 ±4.4	7.8 ±3.1	12.0 ±4.7	<0.001
90 mins after smoking	8.8 ±4.0	7.3 ±2.9	10.9 ±4.4	0.001
120 mins after smoking	8.2 ±3.7	6.7 ±2.6	10.4 ±4.1	<0.001

Supplementary Table 9-9. Variation of baseline exhaled CO with demographic and behavioural factors for non-smokers analysed with t-test in the exhaled CO experiment dataset

Variable (n=26)	Baseline exhaled CO (ppm) mean ±SD	p-value
Sex		0.392
Male (n=14)	2.0 ±0.6	
Female (n=12)	1.8 ±0.4	
Ethnicity		0.575
Asian (n=18)	2.0 ±0.3	
Hispanic/Latino (n=2)	1.5 ±0.7	
White/Caucasian (n=5)	2.0 ±0.7	
Exposure to CO before the study		0.724
None (n=21)	1.9 ±0.4	
Yes (n=5)	2.0 ±0.7	
Exercise before the study		0.772
None (n=22)	1.9 ±0.4	
Yes (n=4)	2.0 ±0.8	

Supplementary Table 9-10. Univariable linear regression of the relationship between baseline exhaled CO and each demographic and physiological factors for non-smokers in the exhaled CO experiment dataset

Variable (n=26)	Baseline exhaled CO (ppm)			
	β [a]	Beta [b]	p-value	R^2
Age (year)	-0.028	-0.159	0.437	0.025
Height (cm)	0.011	0.237	0.245	0.056
Weight (kg)	0.0003	0.009	0.967	0.0001
BMI (kg/m2)	0.002	0.009	0.965	0.0001

Note: [a] β (un-standardised coefficient), [b] Beta (standardised coefficient).

Supplementary Table 9-11. Variation of CO increase after smoking with demographic and smoking-related factors for smokers analysed with t-test in the exhaled CO experiment dataset

Variable (n=48)	CO increased after smoking (ppm) mean ±SD	p-value
Sex		**0.037***
Male (n=39)	**4.4 ±2.2**	
Female (n=9)	**6.3 ±3.0**	

Ethnicity		0.802
Asian (n=27)	4.4 ±2.6	
White/Caucasian (n=16)	4.5 ±1.7	
Smoking group		0.688
Light smokers (n=28)	4.6 ±2.8	
Heavy smokers (n=20)	4.9 ±1.8	
Exercise before study		0.180
None (n=30)	5.1 ±2.2	
Yes (n=18)	4.1 ±2.8	

*Note: *Where a significant difference was found in CO uptake amounts, these values are shown in bold.*

Supplementary Table 9-12. Univariable linear regression of the relationship between CO increase after smoking and each demographic, physiological, behavioural factors and smoking-related factor for smokers in the exhaled CO experiment dataset

Variable (n=48)	CO increased after smoking (ppm)			
	β [a]	Beta [b]	p-value	R^2
Age (year)	-0.087	-0.158	0.282	0.025
Height (cm)	**-0.104**	**-0.341**	**0.018***	**0.116**
Weight (kg)	**-0.056**	**-0.316**	**0.029***	**0.100**
BMI (kg/m2)	-0.142	-0.208	0.157	0.043
Years of smoking (year)	0.026	0.051	0.732	0.003
Time since last cigarette (hr ago) (n=47)	0.003	0.054	0.714	0.003
Cigarettes smoked (daily)	0.008	0.018	0.905	0.0003
Cigarettes smoked (weekly)	0.004	0.059	0.693	0.003
Puffs taken per cigarette	0.102	0.170	0.249	0.029
Smoking duration (min)	0.076	0.033	0.826	0.001

*Note: *Significant variables affect CO uptake amounts are shown in bold, [a] β (un-standardised coefficient), [b] Beta (standardised coefficient).*

Supplementary Table 9-13. Variation of CO decrease after smoking with demographic and smoking-related factors for smokers analysed by t-test in the exhaled CO experiment dataset

Variable (n=47)	CO decreased after smoking (ppm) mean ±SD	p-value
Sex		0.826
Male (n=38)	3.6 ±2.6	
Female (n=9)	3.4 ±1.7	
Ethnicity		0.834
Asian (n=27)	3.7 ±2.9	
White/Caucasian (n=15)	3.2 ±1.7	
Smoking status		**0.013***
Light smokers (n=27)	**2.8 ±1.8**	
Heavy smokers (n=20)	**5.6 ±2.8**	

*Note: *Where a significant difference was found in CO elimination, these values are shown in bold.*

Supplementary Table 9-14. Univariable linear regression of the relationship between CO decrease after smoking and each demographic, physiological, behavioural and smoking related factor for smokers in the exhaled CO experiment dataset

Variable (n=47)	CO decreased after smoking (ppm)			
	β [a]	Beta [b]	p-value	R^2
Age (year)	0.016	0.030	0.838	0.001
Height (cm)	-0.045	-0.148	0.319	0.022
Weight (kg)	-0.006	-0.037	0.805	0.001
BMI (kg/m2)	0.023	0.035	0.816	0.001
Years of smoking (year)	0.056	0.107	0.466	0.009
Time since last cigarette (hrs ago)	-0.008	-0.171	0.257	0.029
Cigarettes smoked (daily)	0.118	0.271	0.065	0.074
Cigarettes smoked (weekly)	**0.020**	**0.334**	**0.022***	**0.111**

Puffs taken per cigarette	-0.060	-0.103	0.491	0.011
Smoking duration (min)	-0.128	-0.056	0.707	0.003

Note: Significant variables affect CO elimination are shown in bold, [a] β (un-standardised coefficient), [b] Beta (standardised coefficient).

9.2.3 Supplemental tables for PFT groups

TSGH group with any PFT

Supplementary Table 9-15. Collinearity test *of variables by VIF in the TSGH group with any PFT*

Variable (n=1,943)	VIF (variance inflation factor)
Age (yr)	1.30
Sex (male)	2.32
Height (cm)	34.71
Weight (kg)	125.78
BMI (kg/m2)	91.60
Ever smoker	1.24
Obesity	1.76
Obstructive lung disease	1.12
Restrictive lung disease	1.11
Mixed obstructive and restrictive lung disease	1.09

Supplementary Table 9-16. Correlation test of all possible factors affecting DL_{CO} in the TSGH group with any PFT

	Height	***Weight***	***BMI***
Height	1.000		
Weight	0.532	1.000	
BMI	0.036	**0.860**	1.000

TSGH group with normal PFT

Supplementary Table 9-17. Multicollinearity test of variables by VIF in the TSGH group with normal PFT

Variable (n=169)	VIF (variance inflation factor)
Age (yr)	1.47
Sex (male)	3.24
Height (cm)	62.83
Weight (kg)	199.48
BMI (kg/m2)	118.41
Hb (g/dL)	2.59
Period of smoking (yr)	5.55
Ever smoker	5.33
Obesity	1.48
Anaemia	2.37
Hypertension	1.39
Hyperlipidaemia	1.24
DM	1.37
Cardiovascular diseases	1.37
Kidney disease	1.19

Supplementary Table 9-18. Correlation test of all possible factors affecting DL_{CO} in the TSGH group with normal PFT

	Height	***Weight***	***BMI***
Height	1.000		

Weight	0.603	1.000	
BMI	0.081	**0.839**	1.000

TSGH relatively healthy group

Supplementary Table 9-19. Multicollinearity test of variables by VIF in the TSGH relatively healthy group

Variable (n=40)	VIF (variance inflation factor)
Age (yr)	1.65
Sex (male)	7.16
Height (cm)	112.61
Weight (kg)	416.48
BMI (kg/m2)	268.17
Hb (g/dL)	4.30
Period of smoking (yr)	10.46
Ever smoker	11.64
Obesity	2.37

Supplementary Table 9-20. Correlation test of all possible factors affecting DL_{CO} in the TSGH relatively healthy group

	Sex (male)	***Height***	***Weight***	***BMI***	***Period of smoking***	***Ever smoker***
***Sex** (male)*	1.000					
Height	**0.755**	1.000				
Weight	0.578	0.674	1.000			
BMI	0.211	0.155	**0.830**	1.000		
Period of smoking	0.173	0.133	0.094	0.022	1.000	
Ever smoker	0.288	0.326	0.182	-0.006	**0.889**	1.000

RBH group with any PFT

Supplementary Table 9-21. Multicollinearity test of variables by VIF in the RBH group with any PFT

Variable (n=686)	VIF (variance inflation factor)
Age (yr)	1.13
Sex (male)	2.08
Height (cm)	26.95
Weight (kg)	106.23
BMI (kg/m2)	91.56
Ever smoker	1.09
Obesity	2.63
Obstructive lung disease	1.23
Restrictive lung disease	1.13
Mixed obstructive and restrictive lung disease	1.13

Supplementary Table 9-22. Correlation test of all possible factors affecting DL_{CO} in the RBH group with any PFT

	Height	***Weight***	***BMI***
Height	1.000		
Weight	0.358	1.000	
BMI	-0.134	**0.873**	1.000

RBH group with normal PFT

Supplementary Table 9-23. Multicollinearity test of variables by VIF in the RBH group with normal PFT

Variable (n=106)	VIF (variance inflation factor)
Age (yr)	1.15
Sex (male)	2.04
Height (cm)	41.10
Weight (kg)	137.69
BMI (kg/m2)	97.36
Ever smoker	1.09
Obesity	2.56

Supplementary Table 9-24. Correlation test of all possible factors affecting DL_{CO} in the RBH group with normal PFT

	Height	***Weight***	***BMI***
Height	1.000		
Weight	0.512	1.000	
BMI	-0.034	**0.837**	1.000

Predictive models for DL_{CO}, V_A and K_{CO} in the TSGH groups

Supplementary Table 9-25. Predictive models for DL_{CO} of non-smokers in the TSGH relatively healthy group

	Regression A	**Regression B**	**Regression C**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**
Sex (male)	2.671† (-0.421, 5.763)	**2.760*** (1.414, 4.106)**	**4.154*** (3.025, 5.283)**
Age	**-0.159*** (-0.218, -0.100)**	**-0.168*** (-0.198, -0.139)**	**-0.182*** (-0.211, -0.152)**
Height	**0.200* (0.064, 0.335)**	**0.149* (0.063, 0.236)**	excluded
Weight	0.054 (-0.023, 0.131)	**0.099*** (0.051, 0.146)**	**0.134*** (0.089, 0.179)**
Hb	-0.043 (-1.204, 1.117)	excluded	excluded
Intercept	-7.417 (-34.158, 19.324)	-2.612 (-15.838, 10.614)	19.685*** (16.599, 22.770)
N	33	113	113
SEE	1.948	2.370	2.485
R^2	0.880	0.795	0.773
Adjusted R^2	0.857	0.787	0.766

*Note: *Where a significant difference was found in DL_{CO} value, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

Supplementary Table 9-26. Predictive models for DL_{CO} of those with a history of smoking in the TSGH relatively healthy group

	Regression A	**Regression B**	**Regression C**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**
Sex (male)	4.786 (-1.165, 10.737)	**2.849* (0.445, 5.254)**	**3.499* (1.688, 5.329)**
Age	**-0.264* (-0.390, -0.138)**	**-0.233*** (-0.289, -0.177)**	**-0.246*** (-0.293, -0.198)**
Height	0.001 (-0.260, 0.261)	0.060 (-0.083, 0.202)	excluded
Weight	0.117† (-0.016, 0.250)	**0.135** (0.057, 0.214)**	**0.155** (0.091, 0.218)**

Hb	-0.097 (-2.015, 1.821)	excluded	excluded
Intercept	24.884 (-24.218, 73.986)	12.688 (-9.091, 34.467)	21.626 (17.548, 25.704)
N	20	64	64
SEE	2.284	2.613	2.607
R^2	0.836	0.740	0.737
Adjusted R^2	0.778	0.723	0.724

*Note: *Where a significant difference was found in DL_{CO} value, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

Supplementary Table 9-27. Predictive models for DL_{CO} for females in the TSGH relatively healthy group

	Regression A	**Regression B**	**Regression C**	**Regression D**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
Age	**-0.147*** **(-0.210, -0.084)**	**-0.142*** **(-0.204, -0.080)**	**-0.121*** **(-0.159, -0.082)**	**-0.121*** **(-0.159, -0.083)**
Height	**0.191*** **(0.035, 0.347)**	**0.193*** **(0.037, 0.349)**	**0.131*** **(0.032, 0.230)**	**0.131*** **(0.033, 0.230)**
Weight	0.062 (-0.015, 0.140)	0.070† (-0.007, 0.146)	**0.120*** **(0.067, 0.173)**	**0.119*** **(0.067, 0.172)**
Hb	0.148 (-0.977, 1.273)	0.059 (-1.051, 1.170)	excluded	excluded
Smoking status	-0.973 (-2.920, 0.974)	excluded	0.259 (-0.924, 1.444)	excluded
Intercept	-9.666 (-38.327, 18.994)	-9.659 (-38.294, 18.975)	-3.098 (-18.287, 12.092)	-3.089 (-18.188, 12.009)
N	28	28	75	75
SEE	1.840	1.843	2.054	2.042
R^2	0.711	0.697	0.649	0.648
Adjusted R^2	0.645	0.644	0.629	0.633

*Note: *Where a significant difference was found in DL_{CO} value, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

Supplementary Table 9-28. Predictive models for DL_{CO} for males in the TSGH relatively healthy group

	Regression A	**Regression B**	**Regression C**	**Regression D**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
Age	**-0.230*** **(-0.322, -0.139)**	**-0.238*** **(-0.300, -0.176)**	**-0.213*** **(-0.247, -0.179)**	**-0.216*** **(-0.249, -0.182)**
Height	0.101 (-0.093, 0.925)	0.090 (-0.076, 0.256)	**0.136**** **(0.037, 0.236)**	**0.119*** **(0.024, 0.215)**
Weight	0.077 (-0.042, 0.196)	0.080 (-0.033, 0.193)	**0.104*** **(0.048, 0.160)**	**0.107*** **(0.051, 0.163)**
Hb	-0.231 (-1.877, 1.416)	-0.271 (-1.839, 1.297)	excluded	excluded
Smoking status	-0.350 (-3.255, 2.555)	excluded	-0.639 (-1.716, 0.438)	excluded
Intercept	15.726 (-23.720, 55.172)	18.196 (-14.609, 51.000)	4.120 (-12.119, 20.359)	6.607 (-9.109, 22.324)
N	25	25	102	102
SEE	2.349	2.294	2.622	2.628
R^2	0.835	0.834	0.705	0.701
Adjusted R^2	0.791	0.801	0.693	0.692

*Note: *Where a significant difference was found in DL_{CO} value, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

Supplementary Table 9-29. Predictive models for the V_A of all participants in the TSGH relatively healthy group

	Regression A	**Regression B**	**Regression C**	**Regression D**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
Sex (male)	0.488† (-0.022, 0.998)	0.482† (-0.023, 0.987)	0.462† (0.355, 0.823)	**0.576*** (0.343, 0.808)**
Age	**-0.014* (-0.024, -0.005)**	**-0.015** (-0.025, -0.005)**	**-0.016** (-0.026, -0.007)**	**-0.010*** (-0.015, -0.005)**
Height	**0.051*** (0.026, 0.075)**	**0.049*** (0.026, 0.071)**	**0.047*** (0.025, 0.069)**	**0.055*** (0.042, 0.068)**
Weight	-0.003 (-0.017, 0.011)	excluded	excluded	excluded
Hb	0.099 (-0.100, 0.299)	0.090 (-0.103, 0.284)	0.080 (-0.111, 0.272)	excluded
Smoking status	-0.152 (-0.468, 0.165)	-0.142 (-0.453, 0.488)	excluded	excluded
Intercept	-4.114 (-8.646, 0.418)	-3.864 (-8.218, 0.488)	-3.410 (-7.640, 0.819)	-3.917*** (-5.998, -1.936)
N	53	53	53	177
SEE	0.460	0.456	0.455	0.494
R^2	0.779	0.778	0.774	0.720
Adjusted R^2	0.750	0.754	0.755	0.715

*Note: *Where a significant difference was found in DL_{CO} value, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

Supplementary Table 9-30. Predictive models for V_A of non-smokers in the TSGH relatively healthy group

	Regression A	**Regression B**	**Regression C**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**
Sex (male)	0.534 (-0.207, 1.273)	**0.649*** (0.376, 0.921)**	**0.642*** (0.374, 0.910)**
Age	-0.008 (-0.023, 0.006)	**-0.008** (-0.014, -0.003)**	**-0.009** (-0.014, -0.003)**
Height	**0.044** (0.011, 0.076)**	**0.054*** (0.036, 0.071)**	**0.052*** (0.037, 0.068)**
Weight	0.005 (-0.013, 0.024)	-0.002 (-0.011, 0.008)	excluded
Hb	0.127 (-0.150, 0.405)	excluded	excluded
Intercept	-4.156 (-10.552, 2.239)	-3.668** (-6.348, -0.998)	-3.564** (-6.153, -0.974)
N	33	113	113
SEE	0.466	0.480	0.478
R^2	0.776	0.720	0.720
Adjusted R^2	0.735	0.710	0.712

*Note: *Where a significant difference was found in V_A value, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

Supplementary Table 9-31. Predictive models for V_A of those with a history of smoking in the TSGH relatively healthy group

	Regression A	**Regression B**	**Regression C**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**
Sex (male)	0.987 (-1.139, 2.113)	0.434† (-0.050, 0.919)	0.449† (-0.038, 0.936)
Age	-0.024	-0.008	**-0.012***

	(-0.048, -0.001)	(-0.020, 0.003)	**(-0.022, -0.001)**
Height	**0.061* (0.011, 0.110)**	**0.072*** **(0.043, 0.101)**	**0.061*** **(0.036, 0.084)**
Weight	-0.014 (-0.039, 0.011)	-0.010 (-0.026, 0.005)	excluded
Hb	-0.143 (-0.506, 0.220)	excluded	excluded
Intercept	-1.518 (-10.810, 7.774)	-6.095* (-10.484, -1.706)	-4.769* (-8.695, -0.842)
N	20	64	64
SEE	0.432	0.527	0.530
R^2	0.838	0.686	0.677
Adjusted R^2	0.780	0.665	0.661

*Note: *Where a significant difference was found in V_A value, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

Supplementary Table 9-32. Predictive models for V_A for females in the TSGH relatively healthy group

	Regression A	**Regression B**	**Regression C**	**Regression D**	**Regression E**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
Age	-0.009 (-0.025, 0.007)	-0.009 (-0.024, 0.007)	-0.008 (-0.024, 0.007)	-0.005 (-0.012, 0.003)	excluded
Height	**0.055**** **(0.015, 0.095)**	**0.055*** **(0.016, 0.094)**	**0.053**** **(0.017, 0.089)**	**0.052***** **(0.034, 0.069)**	**0.056***** **(0.040, 0.072)**
Weight	-0.004 (-0.024, 0.016)	-0.003 (-0.022, 0.016)	excluded	excluded	excluded
Hb	0.177 (-0.111, 0.464)	0.167 (-0.111, 0.446)	0.151 (-0.105, 0.408)	excluded	excluded
Smoking status	-0.102 (-0.600, 0.395)	excluded	excluded	excluded	excluded
Intercept	-6.059 (-13.385, 1.267)	-6.058† (-13.235, 1.118)	-5.698† (-12.414, 1.019)	-3.648* (-6.545, -0.752)	-4.477*** (-7.035, -1.919)
N	28	28	28	75	75
SEE	0.470	0.462	0.453	0.413	0.414
R^2	0.402	0.397	0.394	0.405	0.393
Adjusted R^2	0.266	0.292	0.318	0.389	0.385

*Note: *Where a significant difference was found in V_A value, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

Supplementary Table 9-33. Predictive models for V_A for males in the TSGH relatively healthy group

	Regression A	**Regression B**	**Regression C**	**Regression D**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
Age	**-0.021* (-0.040, -0.002)**	**-0.022**** **(-0.034, -0.009)**	**-0.022**** **(-0.034, -0.009)**	**-0.012**** **(-0.019, -0.005)**
Height	**0.045* (0.005, 0.085)**	**0.044* (0.010, 0.078)**	**0.043**** **(0.013, 0.074)**	**0.058***** **(0.040, 0.076)**
Weight	-0.002 (-0.026, 0.023)	-0.001 (-0.025, 0.022)	excluded	excluded
Hb	-0.032 (-0.371, 0.307)	-0.034 (-0.357, 0.288)	-0.034 (-0.348, 0.280)	excluded
Smoking status	-0.022 (-0.620, 0.576)	excluded	excluded	excluded
Intercept	-0.541	-0.385	-0.341	-3.776*

	(-8.663, 7.581)	(-7.129, 6.359)	(-6.864, 6.182)	(-6.938, -0.613)
N	25	25	25	102
SEE	0.484	0.472	0.460	0.544
R^2	0.659	0.659	0.659	0.421
Adjusted R^2	0.569	0.591	0.610	0.409

*Note: *Where a significant difference was found in V_A value, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

Supplementary Table 9-34. Predictive models for K_{CO} of all participants in the TSGH relatively healthy group

	Regression A	**Regression B**	**Regression C**	**Regression D**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
Sex (male)	-0.013 (-0.630, 0.605)	excluded	excluded	excluded
Age	**-0.025*** (-0.037, -0.013)**	**-0.025*** (-0.037, -0.014)**	**-0.026*** (-0.037, -0.016)**	**-0.029*** (-0.034, -0.024)**
Height	-0.012 (-0.041, 0.018)	-0.012 (-0.002, 0.014)	-0.014 (-0.038, 0.011)	**-0.026*** (-0.037, -0.014)**
Weight	0.015† (-0.002, 0.032)	0.015† (-0.001, 0.032)	0.015† (-0.001, 0.032)	**0.025*** (0.017, 0.033)**
Hb	-0.080 (-0.316, 0.167)	-0.077 (-0.275, 0.121)	-0.087 (-0.278, 0.104)	excluded
Smoking status	-0.080 (-0.463, 0.304)	-0.081 (-0.458, 0.297)	excluded	excluded
Intercept	7.631 (2.143, 13.119)	7.713*** (4.031, 11.395)	8.085*** (4.871, 11.299)	8.351 (6.718, 9.985)
N	53	53	53	177
SEE	0.557	0.551	0.546	0.501
R^2	0.373	0.373	0.371	0.450
Adjusted R^2	0.292	0.307	0.319	0.440

*Note: *Where a significant difference was found in V_A value, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

Supplementary Table 9-35. Predictive models for K_{CO} of non-smokers in the TSGH relatively healthy group

	Regression A	**Regression B**	**Regression C**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**
Sex (male)	-0.023 (-0.932, 0.886)	excluded	excluded
Age	**-0.027** (-0.044, -0.009)**	**-0.027** (-0.043, -0.010)**	**-0.026*** (-0.033, -0.020)**
Height	0.001 (-0.039, 0.040)	0.000003 (-0.034, 0.034)	**-0.017* (-0.033, -0.002)**
Weight	0.004 (-0.018, 0.027)	0.004 (-0.018, 0.026)	**0.021*** (0.010, 0.031)**
Hb	-0.112 (-0.453, 0.229)	-0.117 (-0.366, 0.130)	excluded
Intercept	6.875† (-0.986, 14.737)	7.022* (1.787, 12.257)	7.168*** (4.966, 9.370)
N	33	33	113
SEE	0.573	0.562	0.519
R^2	0.343	0.343	0.438
Adjusted R^2	0.221	0.249	0.422

*Note: *Where a significant difference was found in V_A value, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

Supplementary Table 9-36. Predictive models for K_{CO} of those with a history of smoking in the TSGH relatively healthy group

	Regression A	**Regression B**	**Regression C**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**
Sex (male)	0.059 (-1.286, 1.403)	excluded	excluded
Age	**-0.033* (-0.062, -0.005)**	**-0.032** (-0.051, -0.014)**	**-0.036*** (-0.046, -0.027)**
Height	-0.052† (-0.111, 0.007)	**-0.051* (-0.096, -0.006)**	**-0.044*** (-0.063, -0.024)**
Weight	**0.039* (0.009, 0.069)**	**0.039** (0.010, 0.068)**	**0.036*** (0.022, 0.050)**
Hb	0.087 (-0.346, 0.521)	0.098 (-0.252, 0.447)	excluded
Intercept	10.762† (-0.331, 21.854)	10.354* (4.635, 16.073)	10.990*** (8.132, 13.849)
N	20	20	64
SEE	0.516	0.499	0.459
R^2	0.516	0.516	0.520
Adjusted R^2	0.343	0.387	0.496

*Note: *Where a significant difference found in V_A value, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value was <0.001; † p-value <0.1.*

Supplementary Table 9-37. Predictive models for K_{CO} for females in the TSGH relatively healthy group

	Regression A	**Regression B**	**Regression C**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**
Age	**-0.026* (-0.048, -0.003)**	**-0.025* (-0.047, -0.003)**	**-0.024*** (-0.034, -0.014)**
Height	-0.011 (-0.067, 0.046)	-0.010 (-0.065, 0.045)	**-0.023† (-0.049, 0.004)**
Weight	0.015 (-0.013, 0.043)	0.016 (-0.011, 0.043)	**0.028*** (0.014, 0.042)**
Hb	-0.146 (-0.552, 0.259)	-0.158 (-0.550, 0.234)	excluded
Smoking status	-0.128 (-0.830, 0.573)	excluded	excluded
Intercept	8.429 (-1.898, 18.755)	8.430† (-1.677, 18.536)	7.485 (3.456, 0.042)
N	28	28	75
SEE	0.663	0.650	0.545
R^2	0.266	0.261	0.364
Adjusted R^2	0.099	0.133	0.337

*Note: *Where a significant difference was found in V_A value, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

Supplementary Table 9-38. Predictive models for K_{CO} for males in the TSGH relatively healthy group

	Regression A	**Regression B**	**Regression C**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**
Age	**-0.026* (-0.045, -0.008)**	**-0.027*** (-0.039, -0.014)**	**-0.031*** (-0.037, -0.024)**
Height	-0.018 (-0.058, 0.022)	-0.019 (-0.053, 0.015)	**-0.027* (-0.045, -0.010)**
Weight	0.018 (-0.007, 0.042)	0.018 (-0.005, 0.041)	**0.023*** (0.013, 0.033)**
Hb	0.027 (-0.310, 0.363)	0.024 (-0.296, 0.345)	excluded

Smoking status	-0.019 (-0.614, 0.575)	excluded	excluded
Intercept	6.955 (-1.112, 15. 021)	7.092* (0.394, 13.790)	8.851 (6.027, 11.674)
N	25	25	102
SEE	0.480	0.468	0.472
R^2	0.538	0.538	0.522
Adjusted R^2	0.416	0.445	0.507

*Note: *Where a significant difference was found in V_A value, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

Predictive models for DL_{CO}, V_A and K_{CO} in the RBH groups

Supplementary Table 9-39. Predictive models for DL_{CO} of all participants in the RBH group with normal PFT

	Regression A	**Regression B**	**Regression C**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**
Sex (male)	**3.643***** **(2.498, 4.787)**	**3.539***** **(2.421, 4.658)**	**3.537***** **(2.420, 4.654)**
Age	**-0.169***** **(-0.196, -0.142)**	**-0.167***** **(-0.194, -0.140)**	**-0.168***** **(-0.195, -0.141)**
Height	**0.222***** **(0.161, 0.284)**	**0.230***** **(0.171, 0.290)**	**0.240***** **(0.185, 0.295)**
Weight	0.014 (-0.012, 0.039)	0.011 (-0.014, 0.036)	excluded
Smoking status	-0.378 (-1.205, 0.449)	excluded	excluded
Intercept	-7.210 (-17.380, 2.959)	-8.544† (-18.326, 1.236)	-9.213† (-18.859, 0.433)
N	106	106	106
SEE	2.070	2.060	2.058
R^2	0.865	0.864	0.863
Adjusted R^2	0.859	0.859	0.859

*Note: *Where a significant difference was found in DL_{CO} value, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

Supplementary Table 9-40. Predictive models for DL_{CO} of non-smokers in the RBH group with normal PFT

	Regression A	**Regression B**
	β (95% CI)	**β (95% CI)**
Sex (male)	**3.094***** **(1.509, 4.679)**	**3.087***** **(1.513, 4.661)**
Age	**-0.148***** **(-0.186, -0.112)**	**-0.149***** **(-0.185, -0.112)**
Height	**0.285***** **(0.196, 0.374)**	**0.272***** **(0.063, 0.236)**
Weight	-0.013 (-0.054, 0.028)	excluded
Intercept	-16.658 (-31.187, -2.128)	-15.466* (-29.384, -1.547)
N	54	54
SEE	1.986	1.973
R^2	0.896	0.895
Adjusted R^2	0.888	0.889

*Note: *Where a significant difference was found in DL_{CO} value, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

Supplementary Table 9-41. Predictive models for DL_{CO} of those with a history of smoking in the RBH group with normal PFT

	Regression A	**Regression B**
	β (95% CI)	**β (95% CI)**
Sex (male)	**4.201*** (2.518, 5.884)**	**4.070*** (2.361, 5.779)**
Age	**-0.189*** (-0.231, -0.147)**	**-0.191*** (-0.234, -0.148)**
Height	**0.183*** (0.094, 0.272)**	**0.209*** (0.124, 0.294)**
Weight	0.029† (-0.005, 0.063)	excluded
Intercept	-1.366 (-15.857, 13.125)	-3.119 (-17.744, 11.506)
N	52	52
SEE	2.131	2.174
R^2	0.839	0.828
Adjusted R^2	0.825	0.818

*Note: *Where a significant difference was found in DL_{CO} value, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

Supplementary Table 9-42. Predictive models for DL_{CO} for females in the RBH group with normal PFT

	Regression A	**Regression B**	**Regression C**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**
Age	**-0.144*** (-0.174, -0.113)**	**-0.142*** (-0.172, -0.112)**	**-0.142*** (-0.172, -0.113)**
Height	**0.225*** (0.155, 0.294)**	**0.230*** (0.162, 0.298)**	**0.240*** (0.176, 0.308)**
Weight	0.014 (-0.012, 0.040)	0.011 (-0.014, 0.036)	excluded
Smoking status	-0.385 (-1.356, 0.585)	excluded	excluded
Intercept	-9.006 (-20.718, 2.707)	-9.904† (-21.337, 1.529)	-10.651† (-21.918, 0.615)
N	45	45	45
SEE	1.509	1.502	1.498
R^2	0.856	0.854	0.851
Adjusted R^2	0.841	0.843	0.844

*Note: *Where a significant difference was found in DL_{CO} value, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

Supplementary Table 9-43. Predictive models for DL_{CO} for males in the RBH group with normal PFT

	Regression A	**Regression B**	**Regression C**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**
Age	**-0.191*** (-0.234, -0.147)**	**-0.142*** (-0.172, -0.112)**	**-0.189*** (-0.231, -0.148)**
Height	**0.225*** (0.129, 0.322)**	**0.230*** (0.162, 0.298)**	**0.243*** (0.160, 0.325)**
Weight	0.012 (-0.033, 0.057)	0.011 (-0.014, 0.036)	excluded
Smoking status	-0.251 (-1.517, 1.015)	excluded	excluded
Intercept	-2.790 (-19.589, 14.009)	-9.904 (-21.337, 1.529)	-4.963 (-20.490, 10.563)
N	61	62	62
SEE	2.402	2.371	2.353
R^2	0.740	0.738	0.737

Adjusted R^2	0.721	0.724	0.728

*Note: *Where a significant difference was found in DL_{CO} value, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

Supplementary Table 9-44. Predictive model for the V_A of all participants in the RBH group with normal PFT

	Regression A
	β (95% CI)
Sex (male)	**0.369*** (0.048, 0.690)**
Age	**-0.008*** (-0.016, -0.008)**
Height	**0.097*** (0.080, 0.114)**
Weight	**-0.009*** (-0.016, -0.002)**
Smoking status	**0.374** (0.142, 0.605)**
Intercept	-10.163 (-13.015, -7.312)
N	106
SEE	0.580
R^2	0.796
Adjusted R^2	0.786

*Note: *Where a significant difference was found in DL_{CO} value, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

Supplementary Table 9-45. Predictive models for V_A of non-smokers in the RBH group with normal PFT

	Regression A	**Regression B**	**Regression C**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**
Sex (male)	0.149 (-0.313, 0.611)	excluded	excluded
Age	-0.005 (-0.016, 0.006)	-0.004 (-0.014, 0.006)	excluded
Height	**0.099*** (0.073, 0.125)**	**0.104*** (0.084, 0.124)**	**0.107*** (0.088, 0.126)**
Weight	-0.011† (-0.023, 0.001)	-0.011† (-0.023, 0.001)	-0.011† (-0.023, 0.001)
Intercept	-10.373*** (-14.608, -6.137)	-11.262*** (-14.462, -8.061)	-11.925*** (-14.654, -9.196)
N	54	54	54
SEE	0.579	0.575	0.573
R^2	0.786	0.784	0.781
Adjusted R^2	0.768	0.771	0.772

*Note: *Where a significant difference was found in V_A value, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

Supplementary Table 9-46. Predictive models for V_A of those with a history of smoking in the RBH group with normal PFT

	Regression A	**Regression B**
	β (95% CI)	**β (95% CI)**
Sex (male)	**0.600** (0.158, 1.042)**	**0.629** (0.184, 1.074)**
Age	**-0.014* (-0.026, -0.003)**	**-0.014* (-0.025, -0.003)**
Height	**0.099*** (0.076, 0.123)**	**0.094*** (0.072, 0.116)**
Weight	-0.006	excluded

	(-0.015, 0.003)	
Intercept	-10.226*** (-14.033, -6.419)	-9.842*** (-13.650, -6.034)
N	52	52
SEE	0.560	0.566
R^2	0.827	0.820
Adjusted R^2	0.813	0.809

*Note: *Where a significant difference was found in V_A value, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

Supplementary Table 9-47. Predictive models for V_A for females in the RBH group with normal PFT

	Regression A	**Regression B**	**Regression C**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**
Age	**-0.013* (-0.023, -0.003)**	**-0.013* (-0.023, -0.003)**	**-0.013* (-0.022, 0.003)**
Height	**0.066*** (0.043, 0.090)**	**0.067*** (0.044, 0.089)**	**0.063*** (0.042, 0.084)**
Weight	-0.004 (-0.024, 0.016)	-0.004 (-0.012, 0.004)	excluded
Smoking status	-0.102 (-0.012, 0.005)	excluded	excluded
Intercept	-5.125* (-9.022, -1.229)	-5.152** (-8.926, -1.379)	-4.888*** (-8.612, -1.164)
N	45	45	45
SEE	0.502	0.496	0.495
R^2	0.611	0.611	0.603
Adjusted R^2	0.572	0.583	0.584

*Note: *Where a significant difference was found in V_A value, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

Supplementary Table 9-48. Predictive models for V_A for males in the RBH group with normal PFT

	Regression A	**Regression B**
	β (95% CI)	**β (95% CI)**
Age	-0.007 (-0.018, 0.003)	excluded
Height	**0.121*** (0.098, 0.144)**	**0.125*** (0.102, 0.148)**
Weight	**-0.014* (-0.024, -0.003)**	**-0.013* (-0.024, -0.002)**
Smoking status	**0.654*** (0.349, 0.958)**	**0.653*** (0.346, 0.960)**
Intercept	-13.823*** (-17.864, -9.783)	-14.988*** (-18.704, -11.271)
N	61	61
SEE	0.577	0.583
R^2	0.714	0.704
Adjusted R^2	0.694	0.689

*Note: *Where a significant difference was found in V_A value, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

Supplementary Table 9-49. Predictive model for K_{CO} of all participants in the RBH group with normal PFT

	Regression A
	β (95% CI)
Sex (male)	**0.301* (0.036, 0.566)**
Age	**-0.023*** (-0.029, -0.016)**

Height	**-0.029*** (-0.044, -0.015)**
Weight	**0.009** (0.003, 0.015)**
Smoking status	**-0.297** (-0.489, -0.106)**
Intercept	9.887 (7.531, 12.243)
N	106
SEE	0.480
R^2	0.400
Adjusted R^2	0.370

*Note: *Where a significant difference was found in V_A value, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

Supplementary Table 9-50. Predictive models for K_{CO} of non-smokers in the RBH group with normal PFT

	Regression A	**Regression B**
	β (95% CI)	**β (95% CI)**
Sex (male)	**0.445* (0.059, 0.832)**	**0.449* (0.062, 0.836)**
Age	**-0.022*** (-0.031, -0.013)**	**-0.023*** (-0.031, -0.014)**
Height	**-0.024* (-0.045, -0.002)**	-0.018† (-0.037, -0.001)
Weight	0.006 (-0.004, 0.016)	excluded
Intercept	9.112*** (5.567, 12.657)	8.594*** (1.787, 12.257)
N	54	54
SEE	0.485	0.486
R^2	0.386	0.371
Adjusted R^2	0.336	0.333

*Note: *Where a significant difference was found in V_A value, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

Supplementary Table 9-51. Predictive models for K_{CO} of those with a history of smoking in the RBH group with normal PFT

	Regression A	**Regression B**
	β (95% CI)	**β (95% CI)**
Sex (male)	0.158 (-1.214, 0.530)	excluded
Age	**-0.022*** (-0.031, -0.012)**	**-0.021*** (-0.030, -0.012)**
Height	**-0.034** (-0.054, -0.014)**	**-0.028*** (-0.043, -0.014)**
Weight	**0.010* (0.003, 0.018)**	**0.010* (0.002, 0.017)**
Intercept	10.277*** (7.072, 13.483)	9.413*** (6.948, 11.878)
N	52	52
SEE	0.471	0.470
R^2	0.442	0.433
Adjusted R^2	0.394	0.398

*Note: *Where a significant difference was found in V_A value, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

Supplementary Table 9-52. Predictive models for K_{CO} for females in the RBH group with normal PFT

	Regression A	**Regression B**	**Regression C**	**Regression D**

	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Age	**-0.018** (-0.029, -0.008)**	**-0.018** (-0.029, -0.009)**	**-0.016** (-0.026, -0.007)**	**-0.017** (-0.027, -0.008)**
Height	-0.012 (-0.036, 0.013)	-0.011 (-0.035, 0.012)	excluded	excluded
Weight	0.006 (-0.003, 0.015)	0.006 (-0.003, 0.014)	0.004 (-0.004, 0.012)	excluded
Smoking status	-0.026 (-0.364, 0.313)	excluded	excluded	excluded
Intercept	6.886** (2.798, 10.974)	6.826** (2.867, 10.786)	4.957*** (4.031, 5.884)	5.347*** (4.772, 5.961)
N	45	45	45	45
SEE	0.527	0.520	0.520	0.521
R^2	0.275	0.275	0.258	0.237
Adjusted R^2	0.203	0.222	0.223	0.220

*Note: *Where a significant difference was found in V_A value, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

Supplementary Table 9-53. Predictive model for K_{CO} for males in the RBH group with normal PFT

	Regression A
	β (95% CI)
Age	**-0.025*** (-0.032, -0.017)**
Height	**-0.043*** (-0.060, -0.026)**
Weight	**0.012** (0.004, 0.019)**
Smoking status	**-0.485*** (-0.707, 0.262)**
Intercept	12.553 (9.602, 15.504)
N	61
SEE	0.422
R^2	0.567
Adjusted R^2	0.536

*Note: *Where a significant difference was found in V_A value, these values are shown in bold, *p-value <0.05; ** p-value <0.01; *** p-value <0.001; † p-value <0.1.*

9.2.4 Supplemental tables and figures for simulations of the CFK (1965) and modified CFK (2009) models

Comparison of measured data from the literature with predicted data from the CFK models

Supplementary Table 9-54. Parameters used in the CFK (1965) model and the modified CFK (2009) model

Parameters and values used in the CFK model	
M	250
P_B	760 mmHg
P_{H2O}	47 mmHg
Pc,o_2	100 mmHg
V_{AR}	6813 ml/min
V_{BL}	5180 ml
Vco	0.0085 ml/min
$[O_2Hb]_{max}$	0.25 ml/ml

$[COHb]_0$	0.0018 ml/ml
Parameters and values used in the modified CFK (2009) model	
B_{COHb}^{Max}	1393.56 ml_{CO}
b_{COHb}^{Max}	1.68 ml_{CO}/g_{Hb}
CHb	150 g_{Hb}/L_{blood}
$Endo$	0.0085 ml_{CO}/min
f_R	12 min^{-1}
Initial CO amount (ml)	9.75
k_{CO2}	0.0000333/min
k_{HbS}	0.002/min
k_{Sf}	0.01/min
M	240
P_{O2}	97.5 mmHg
Q_{ALV}	6982 ml_{air}/min
R	2.55 mmHg (ml_{air})/Kelvin(ml_{CO})
T	310 Kelvin
V_{ALV}	582 ml
V_{BL}	5.53 L_{blood}
V_D	168 ml_{air}
V_T	750 ml_{air}

Comparison of CO-rebreathing data with predicted data from the CFK models

PI_{CO} was calculated based on the CO dose in the bloodstream, and the exposure time was assumed to be around two minutes, according following to the protocols of the CO-rebreathing experiment in Section 4.2.2. Take Subject A for example; the calculation is as follows:

$$PI_{CO}\,(ppm) = \frac{CO\ in\ bloodstream(ml)}{Vb(ml)} \times 1{,}000{,}000 = \frac{44.22}{3624} \times 1{,}000{,}000 = 12202$$

Supplementary Table 9-55. Derived data used in the CFK and modified CFK model

	Subject A	**Subject B**	**Subject C**
Age	30	42	50
Sex	Female	Female	Male
Height (cm)	169.9	162.2	177.2
Weight (kg)	55.3	51.0	78.0
V_{AR} (ml/min)	4213	4213	6813
V_{BL} (ml)	3624	3745	5667
V_{CO} (ml/min)	0.0073	0.0073	0.0095
Hb (g/dL)	11.0	14.6	14.8
CO in bloodstream (ml)	44.22	44.4	70.41
PI_{CO} (ppm)	12202	11856	12425
$[COHb]_0$ (%)	0.80	0.40	0.83
$[O_2Hb]_{max}$ (ml/ml)	0.18	0.25	0.25
$[COHb]_0$ (ml/ml)	0.0014	0.0010	0.0021
Parameters for the CFK (1965) model			
$P_{C,O2} = Pao_2$ (mmHg)	100	100	100
P_B (mmHg)	760	760	760
P_{H2O} (mmHg)	47	47	47
PL (mmHg)	713	713	713
V_{AR} (ml/min)	4213	4213	6813
M	250	250	250

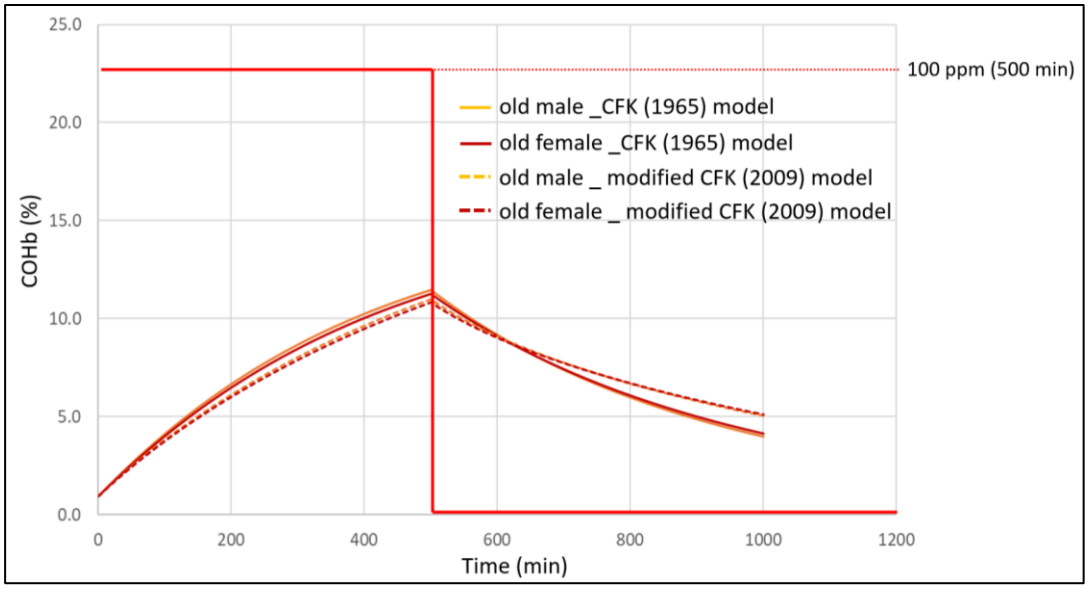
CFK (1965) model			
BSA (m^2)	1.62	1.52	1.96
DL (ml/min/mmHg)	22.4	20.2	31.4
Parameters for the modified CFK (2009) model			
C_{Hb} (g_{Hb} /L_{blood})	110	146	148
V_T (ml_{air})	464	464	750
V_D (ml_{air})	143	143	168
f_R (1/min)	14	14	12
Q_{ALV} (1/min)	4496	4496	6982
V_{ALV} (1/min)	321	321	582
R(mmHg (ml_{air})/Kelvin (ml_{CO}))	2.55	2.55	2.55
M	240	240	240
k_{HbS} (1/min)	0.002	0.002	0.002
$Endo$ (ml_{CO}/min)	0.0073	0.0073	0.0095
k_{Sf} (1/min)	0.01	0.01	0.01
T (Kelvin)	310	310	310
P_{O2} (mmHg)	97.5	94.6	92.7
b_{COHb}^{Max} (ml_{CO}/g_{Hb})	1.68	1.68	1.68
k_{CO2} (1/min)	0.0000333	0.0000333	0.0000333
B_{COHb}^{Max} (ml_{CO})	669.71	918.57	1049.04
Initial CO amount (ml)	5.36	3.67	8.71
Parameters for the modified CFK (2009) model			
DL (ml/min/mmHg)	35.9	33.5	41.2
Parameters for estimated DL_{CO}			
DL (ml/min/mmHg)	28.0	23.7	34.4

Comparison of exhaled CO data of smokers with predicted data from the CFK models

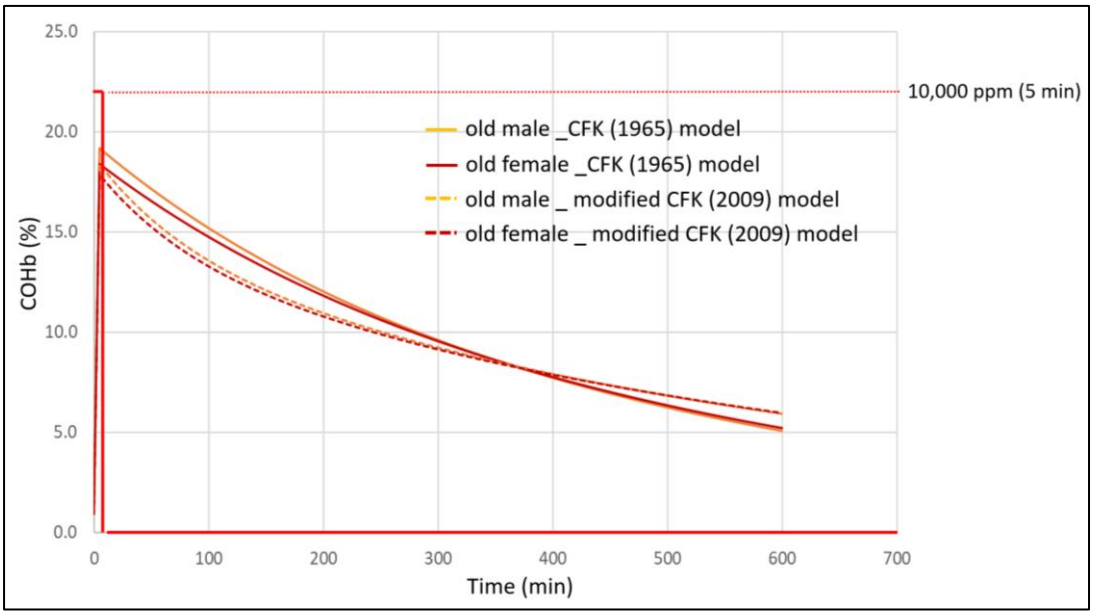
Supplementary Table 9-56. Parameters used in the CFK model and modified CFK model

Parameters and values used in the CFK (1965) model	
P_B (mmHg)	760
Pc,o_2 (mmHg)	100
M	250
$[O_2Hb]_{max}$ (ml/ml)	0.25
[Hb] (g_{Hb}/dL_{blood})	15
Parameters and values used in the modified CFK (2009) model	
V_T (ml_{air})	Male: 750, Female: 464
V_D (ml_{air})	Male: 168, Female: 143
f_R (min^{-1})	Male: 12, Female: 14
Q_{ALV} (ml_{air}/min)	Male: 6982, Female: 4496
V_{ALV} (ml)	Male: 582, Female: 321
R (mmHg (ml_{air})/Kelvin(ml_{CO}))	2.55
M	240
k_{HbS} (min^{-1})	0.002
k_{Sf} (min^{-1})	0.01
T (Kelvin)	310
CHb (g_{Hb}/L_{blood})	150
b_{COHb}^{Max} (ml_{CO}/g_{Hb})	1.68
k_{CO2} (min^{-1})	0.0000333

Comparison of CO uptake and CO elimination between old males and females

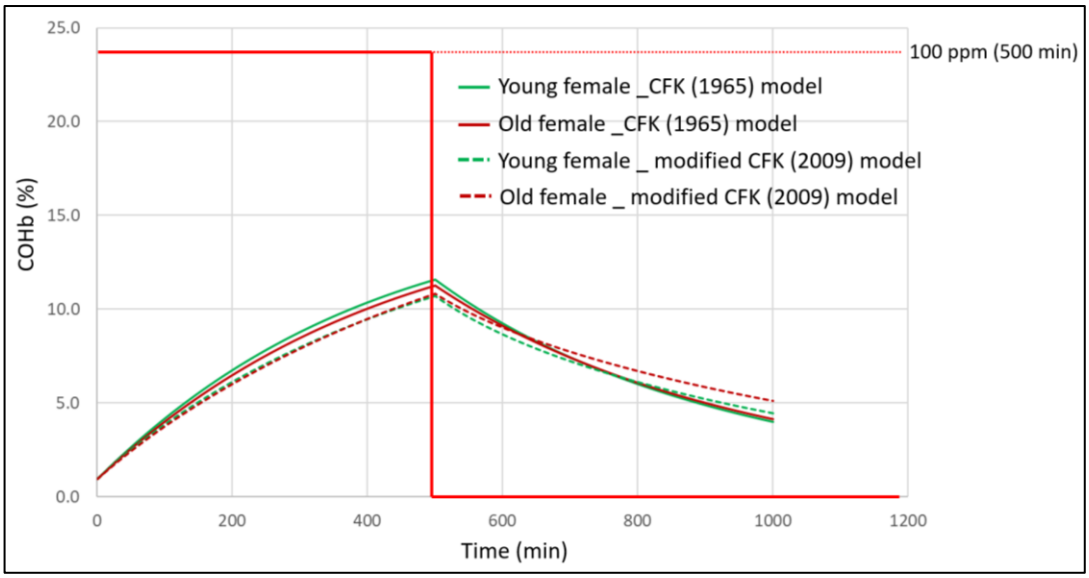


Supplementary Figure 9-10. Simulation for old males and females Scenario A (100 ppm for 500 min)

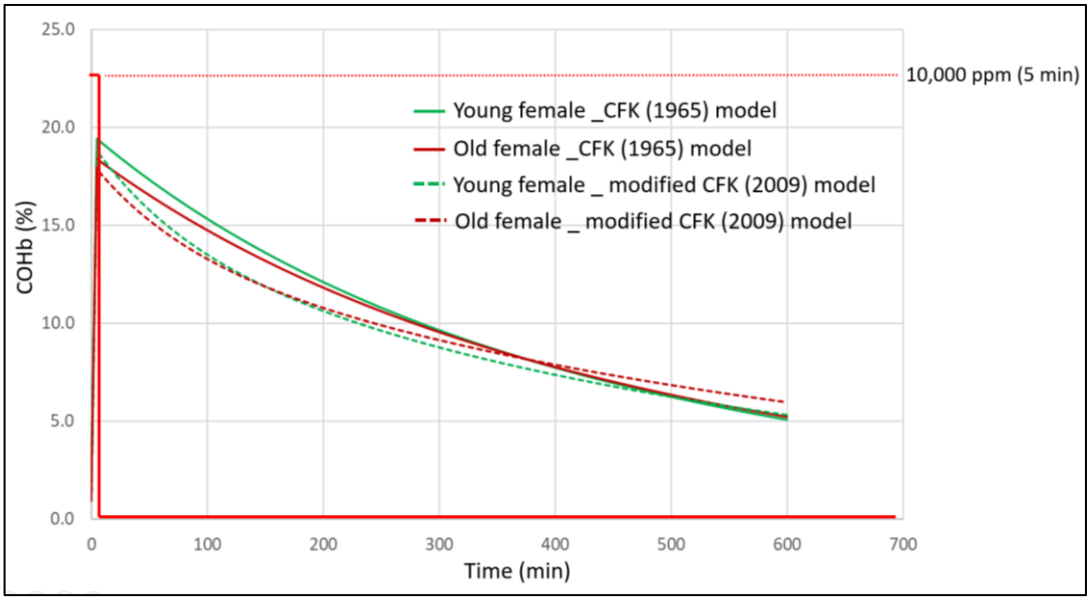


Supplementary Figure 9-11. Simulation for old males and females Scenario B (10,000 ppm for 5 min)

Comparison of CO uptake and CO elimination between young and old females



Supplementary Figure 9-12. Simulation for young and old females Scenario A (100 ppm for 500 min)



Supplementary Figure 9-13. Simulation for young and old females Scenario B (10,000 ppm for 5 min)

9.3Publications

Journal Paper (2019). Prognostic factors of carbon monoxide poisoning in Taiwan: a retrospective observational study. *BMJ Open.* 2019 Nov 18;9(11): e031135.

Journal Paper (2020). Factors Contributing to CO Uptake and Elimination in the Body: A Critical Review. *Int J Environ Res Public Health.* 2020 Jan 14;17(2): 528.

Journal Paper (2021). Can Exhaled Carbon Monoxide Be Used as a Marker of Exposure? A Cross-Sectional Study in Young Adults. *Int J Environ Res Public Health.* 2021 Nov 12;18(22): 11893.

Poster (2019). CO uptake and elimination: a comparison of modelling and pulmonary function observations. UK & Ireland Occupational and Environmental Epidemiology and Exposure Science meeting, 01-02 April, Edinburgh, UK

Poster presentation (2020). Modified CO uptake and elimination model compared with pulmonary function observations. Association for Respiratory Technology & Physiology (ARTP) conference, 16-17 Jan, Birmingham, UK

Oral presentation (2020). Factors related to CO uptake and elimination of smokers and comparison with CO model. UK & Ireland Occupational and Environmental Epidemiology Conference, 16 Mar, Bristol, UK

Poster (2020). Modification of CO models with factors related to CO uptake and elimination and comparison of the simulations with experimental observations. 32^{ed} Annual Conference of the International Society for Environmental Epidemiology (ISEE), August 24-27, Washington, D.C., US. (fully virtual due to COVID-19)

Poster (2021). Empirical validation and simulation of existing CO exposure models with hospital pulmonary function datasets. 33rd Annual Conference of the International Society for Environmental Epidemiology (ISEE), August 23-26, New York, US. (fully virtual due to COVID-19)

Poster (2021). Comparison of factors affecting pulmonary function parameters between Taiwan and UK. Annual Congress of Taiwan Society of Pulmonary and Critical Care Medicine (TSPCCM), December 11-12, Taichung, Taiwan

BMJ Open

Prognostic factors of carbon monoxide poisoning in Taiwan: a retrospective observational study

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ABSTRACT

Objectives To identify the risk factors related to the prognosis of carbon monoxide (CO)-poisoned patients in the hospital.

Design Retrospective observational study.

Setting Tri-Service General Hospital, Taiwan.

Methods We conducted a review of the medical records of 669 CO-poisoned patients, who were admitted to the Department of Emergency, Tri-Service General Hospital, Taiwan, from 2009 to 2014. Demographic, clinical and laboratory data were collected for analysis. In the study, the end points for poor outcome were patients who either still had sequelae, were bedridden or died after treatment. The independent t-test, χ^2 test and binary logistic regression were used to identify the association between the prognostic factors and the outcomes.

Results The logistic regression analysis confirmed that the Glasgow Coma Scale (GCS) score (p=0.008) and blood urea nitrogen (BUN) (p=0.002) were related to poor outcomes. Furthermore, the receiver operating characteristic (ROC) curve showed that the cut-off point of intubation days was 1.5 days (area under the ROC curve [AUC]=0.793) for all patients and 2.5 days (AUC=0.817) for patients with intubation when predicting poor outcomes.

Conclusion We identified the factors that most strongly predict the prognosis of CO poisoning, including the GCS score, serum BUN and intubation days. Moreover, the number of hyperbaric oxygen treatments seems to have impact of the outcome.

Strengths and limitations of this study

- ► Most biochemical factors were considered in the study.
- ► Most of the carbon monoxide (CO)-poisoned patients received the hyperbaric oxygen therapy in the hospital.
- ► The rate of recovery was similar to other studies.
- ► Some data missing of the patients transferred from other hospitals.
- ► Give the specific indications for clinical research work and clinical practice with CO-poisoned patients.

INTRODUCTION

Carbon monoxide (CO) poisoning is a global health issue. In a study by Mott *et al*, 116 703 people died from non-fire CO poisoning in the USA from 1968 to 1998, with around 10 deaths per day.[1] In an UK-based study, 2463 CO poisoning admissions were noted from 2001 to 2010, most of which were preventable.[2] The incidents of CO poisoning usually have a higher rate in winter, because people tend to use heaters and close the windows when the weather is cold.[2–5] The causes of CO poisoning include defective heaters, fires, cooking appliances, the exhaust of vehicles, smoke, waterpipe smoking and so on.[6–8] Besides accidental CO poisoning, the number of deliberate CO intoxications increased due to the increased suicide rate by facing higher stress in their lives now than the past.[9] The suicide rate has increased in Taiwan from around 15 to 35 per 100 000 for males and from 8 to 16 per 100 000 for females from 1992 to 2006. Most of them used charcoal as the source of CO.[10 11]

CO poisoning causes cellular hypoxia by reducing oxygen delivery to tissues and decreasing the dissociation of oxygen from haemoglobin (Hb) to the cells. Energy depletion is the direct cause of CO-induced cell damage, as oxidative phosphorylation is suppressed when cytochrome a3 in the inner membrane of mitochondria is inhibited by CO.[12 13] The most vulnerable organs are the heart and the brain because of their high oxygen demand. The symptoms of acute CO poisoning are headache, fatigue, nausea, vomiting, convulsion and death.[14 15] Although there are some discussions on the use of hyperbaric oxygen (HBO) therapy in treating CO poisoning,[16 17] it has been shown to enhance CO elimination and reverse cytochrome a3 inhibition, resulting in a lower severity of neurological sequelae after CO poisoning.[18] The neuropsychological sequelae include neurological deficits,

cognitive impairments and affective disorders,[19 20] which may cause a drastic impact on the quality of life.

The majority of patients with CO poisoning are around 25–45 years of age in Taiwan representing the most productive group in the society.[10 21] If the factors related to the prognosis were known, then more effective treatments could have been offered. Some predictive factors have been proposed, including hydrogen ion, serum lactate, myocardial injury, Glasgow Coma Scale (GCS) score, leucocytosis and troponin I (TnI).[6 18 19 22–25] However, the clinical indications are still controversial for predicting the outcomes in patients with CO poisoning.[26] In the present study, we tried to find factors for predicting the prognosis of CO poisoning and providing indications for further clinical research work.

METHODS

Study design

A retrospective observational study was conducted at the Hyperbaric Oxygen Therapy Centre, Tri-Service General Hospital, Taipei, Taiwan. Data were collected on all CO-poisoned patients admitted to the hospital and were coded with CO poisoning (International Classification of Diseases, Ninth Revision, Clinical Modification Diagnosis Code 986-Toxic Effect of Carbon Monoxide) from September 2009 to August 2014. There were 669 patients in the study and data were also retrieved from the medical records (paper and digital records) and online database of the hospital, including demographic data, clinical data and laboratory data. Patient data that were missing clinical information or laboratory information or did not have defined outcome information were excluded from the study.

Data collection

For patients included in the study, the following variables were collected and analysed: CO source; height; weight; body mass index (BMI); sex; suicide attempt; habits of smoking and drinking; chronic diseases of the patients, including psychosis, diabetes and hypertension; the initial GCS score on arriving at the emergency department (ED); times of HBO treatment for patients since they arrived at the ED; the number of days for which the patients were intubated, stayed in the intensive care unit (ICU) and were hospitalised; whether they used benzodiazepines (BZD) and also the clinical symptoms of poisoning, including metabolic acidosis, rhabdomyolysis and myocardial injury.

The initial laboratory data after they arrived at the ED in the Tri-Service General Hospital were recorded in the study, including the COHb level, arterial blood gas data, white blood cell (WBC) and platelet count, Hb level, creatine kinase (CK), creatine kinase-myocardial band (CKMB), TnI, serum levels of glucose, blood urea nitrogen (BUN), serum creatinine (Cre), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and base excess (BE).

Patient and public involvement

Patients were involved in this study.

Definition

The clinical criteria were defined as follows: when a patient's BE was lower than −2 mmol/L, then the patient was assumed to be in metabolic acidosis; rhabdomyolysis was assumed in those who had CK >5000 units/L; patients who have CKMB >25 units/L and those with TnI of >1.5 ng/mL were described to have myocardial injury.

Patients were separated into two groups (poor outcome and non-poor outcome). Patients in the poor-outcome group were those who either still had sequelae, were bedridden or died after treatment.

Statistical analysis

For the two groups in the study (ie, poor outcome and non-poor outcome), factors that may relate to the outcomes were analysed using the t-test for continuous variables (eg, age, height and concentration of CK) and χ^2 test for categorical variables (eg, sex, psychosis and myocardial injury). A multivariate logistic regression model with adjusting variables was applied to find the factors that are related to the outcome. A p value <0.05 was considered to be statistically significant, and all p values were two-sided. The IBM SPSS Statistics 24 statistical software (IBM, Armonk, New Y, USA) was used for data management and modelling.

RESULT

Characteristics of the study group

In the study, there were three different categories of CO poisoning: deliberate (336 patients), accidental fire-related (31 patients) and accidental non-fire-related (ANFR, 273 patients). The sources of CO poisoning in the ANFR case (29 patients had no data) were categorised into different types. The primary sources of CO poisoning in Taiwan were charcoal burning (43, 15.7%) and gas boilers or water heaters (216, 79.1%). These two sources accounted for 95% of the all CO poisoning cases, whereas car exhaust (1, 0.4%) and other factors (13, 4.8%) accounted for <10% of the data.

Variables related to CO poisoning outcomes

The variables related to the CO poisoning outcome of patients are shown in tables 1–3. Eighteen per cent of all patients (116 patients) were in the poor-outcome group. The mean age of all patients was 37.40±16.79 years, with 46.7% (269 patients) males and 53.3% (338 patients) females. The category 'deliberate' accounted for around half of all patients. The percentages of chronic diseases in the patients were about 30% with psychosis, 6% with diabetes mellitus and 10% with hypertension. The key findings were as follows:

- Patients with poor outcomes were older than those who did not have poor outcomes (p<0.001).

Table 1 Demographic characteristics related to the CO poisoning outcome

Variables	Total n (%)/mean±SD	Non-poor outcome (n=518) n (%)/mean±SE	Poor outcome (n=116) n (%)/mean±SE	P value
Age	37.40±16.79	35.77±0.73	43.14±1.56	<0.001
Height	162.11±14.85	161.53±0.78	164.00±0.86	0.035
Weight	59.63±15.60	59.11±0.77	60.06±1.58	0.608
BMI	22.43±3.99	22.26±0.19	22.70±0.42	0.357
Sex				0.319
Men	296 (46.7)	237 (45.8)	59 (50.9)	
Women	338 (53.3)	281 (54.2)	57 (49.1)	
Deliberate*				<0.001
No	309 (49.8)	276 (54.5)	33 (28.9)	
Yes	311 (50.2)	230 (45.5)	81 (71.1)	
Smoking†				0.161
No	283 (58.6)	230 (60.2)	53 (52.5)	
Yes	200 (41.4)	152 (39.8)	48 (47.5)	
Drinking†				0.115
No	349 (72.6)	282 (74.2)	67 (66.3)	
Yes	132 (27.4)	98 (25.8)	34 (33.7)	
Psychosis‡				<0.001
No	428 (69.8)	365 (73.0)	63 (55.8)	
Yes	185 (30.2)	135 (27.0)	50 (44.2)	
Diabetes mellitus‡				0.002
No	566 (93.9)	467 (95.3)	99 (87.6)	
Yes	37 (6.1)	23 (4.7)	14 (12.4)	
Hypertension‡				<0.001
No	539 (89.4)	451 (92.0)	88 (77.9)	
Yes	64 (10.6)	39 (8.0)	25 (22.1)	

*Patients exposed to CO intentionally or by accident.
†Patients' habits.
‡Patients' chronic diseases.
BMI, body mass index; CO, carbon monoxide.

- Suicidal patients had a higher chance of poor outcomes compared with those who were exposed to CO by accident (p<0.001).
- Patients with rhabdomyolysis, myocardial injury and metabolic acidosis may be apt to have poor outcomes.
- Patients who had chronic diseases may tend to have poor outcomes.
- The patients' weight, BMI, gender and habits (smoking and drinking) had no significant correlation with the outcomes.

The clinical characteristics exhibited more significant results (table 2). The mean score of the GCS was around 11. The average number of HBO treatment sessions was about five for all patients. The percentages after exposure to CO were approximately 40% with metabolic acidosis, 8% with rhabdomyolysis and 21% with myocardial injury. In the poor-outcome group, the patients had a lower initial GCS score than those in the non-poor-outcome group (p<0.001). In the study, there were 646 patients who received HBO therapy, excluding those who did not receive HBO therapy or had missing data. The average number of HBO treatment sessions was around nine in the poor-outcome group and 3.5 in the non-poor-outcome group. The intubation days, the days for which the patient stayed in the ICU and the days of hospitalisation were greater in the poor-outcome group than in the non-poor-outcome group (p<0.001). Moreover, 42% of the patients took BZD in the poor-outcome group compared with 22.3% in the non-poor-outcome group (p<0.001). When patients were exposed to CO and then suffered from metabolic acidosis, rhabdomyolysis or myocardial injury, they had a higher chance of a poor outcome (p<0.001).

Table 2 Clinical characteristics related to CO poisoning outcomes

Variables	Total n (%)/mean±SD	Non-poor outcome (n=518) n (%)/mean±SE	Poor outcome (n=116) n (%)/mean±SE	P value
GCS	10.66±5.01	11.5±0.21	7.45±0.44	<0.001
HBO therapy sessions	4.69±5.29	3.50±0.16	9.39±0.77	<0.001
Intubation days*	2.22±8.64	0.50±0.06	10.08±1.78	<0.001
ICU days	1.93±6.75	0.59±0.06	7.99±1.39	<0.001
Hospitalisation days	6.91±16.92	3.31±0.28	22.84±3.17	<0.001
Metabolic acidosis†				<0.001
No	353 (59.4)	307 (63.4)	46 (41.8)	
Yes	241 (40.6)	177 (36.6)	64 (58.2)	
BZD				<0.001
No	459 (74.2)	393 (77.7)	66 (58.4)	
Yes	160 (25.8)	113 (22.3)	47 (41.6)	
Rhabdomyolysis‡				<0.001
No	550 (91.5)	463 (94.9)	87 (77.0)	
Yes	51 (8.5)	25 (5.1)	26 (23.0)	
Myocardial injury§				<0.001
No	457 (78.8)	399 (84.9)	58 (52.7)	
Yes	123 (21.2)	71 (15.1)	52 (47.3)	

*Intubation days: days for which the patient underwent intubation.
†Metabolic acidosis:base excess <−2 mmol/L.
‡Rhabdomyolysis:creatine kinase > 5000 U/L.
§Myocardial injury:creatine kinase-myocardialband >25 U/L or troponin I >1.5 ng/mL.
BZD, benzodiazepines; CO, carbon monoxide; GCS, Glasgow Coma Scale; HBO, hyperbaric oxygen; ICU, intensive care unit.

Table 3 shows the laboratory data. With the exception of Hb, platelets, the level of pH and plasma bicarbonate concentration (HCO_3^-), all other variables exhibited abnormal values from the reference values. The concentration of WBCs, Hb, platelets, CKMB, the level of pH and the arterial oxygen tension (PO_2) showed no significant difference between the poor-outcome group and the non-poor-outcome group. Patients had a higher concentration of CK in the poor-outcome group than in the non-poor-outcome group (p=0.031 in men, p<0.001 in women). The concentrations of TnI, glucose, BUN, creatinine, AST and ALT were higher in the poor-outcome group than in the non-poor-outcome group. However, the pressure of arterial carbon dioxide tension (PCO_2), HCO_3^-, the concentration of COHb and BE were lower in the poor-outcome group than in the non-poor-outcome group.

Table 4 shows the variables that may predict the outcome of CO-poisoned patients after they underwent the HBO treatments. The variables in the model included psychosis, diabetes mellitus, metabolic acidosis, hypertension, GCS score, CK, TnI, glucose, BUN, Cre and AST. Age and sex were used as adjusting variables in the model. The OR of GCS was 0.932 (95% CI 0.872 to 0.997). When the patients had a higher score of GCS, they had a lower chance of having a poor outcome. The OR of BUN was 1.089 (95% CI 1.031 to 1.150). If the patients had a higher concentration of BUN, they had a higher chance of having a poor outcome. The remaining variables in the model showed no statistically significant relationship with the outcomes.

Intubation days and CO poisoning outcomes

For cases of acute respiratory failure, patients' consciousness and haemodynamic variables were evaluated every hour. Ventilator management and weaning were performed by respiratory therapists according to the protocol of this hospital. Extubation criteria included stable haemodynamic variables, able to protect airway and spontaneously breathing for 30 min with reliable respiratory effort and oxygen saturation. Figures 1 and 2 show the receiver operating characteristic (ROC) curve of intubation days, including all patients (figure 1) and patients who were intubated (figure 2). The area under the ROC curve (AUC) was 0.757 for the intubation days of all patients (615 patients) and 0.817 for intubated patients (188 patients). The cut-off point was 1.5 intubation days in all patients and 2.5 intubation days in intubated patients. Therefore, for all patients, if their intubation days were >1.5 days, they may have a higher chance of having a poor outcome after treatment; for

Table 3 Laboratory data related to CO poisoning outcomes

Variables	Non-poor outcome (n=518) Mean±SE	Poor outcome (n=116) Mean±SE	P value	Reference value
WBC	13.54±0.74	15.06±0.58	0.327	4.0–8.0
Hb				
Men	148.71±1.07	147.49±3.22	0.649	135–176
Women	126.23±1.01	127.70±2.72	0.557	113–152
Platelet*	244.40±2.89	242.44±7.39	0.777	150.0–350.0
CK				
Men	1121.20±212.91	6231.71±2297.13	0.031	57–197
Women	668.36±147.27	2656.16±645.47	<0.001	32–180
CKMB	26.96±2.09	35.09±3.29	0.077	<25
TnI	0.53±0.09	2.90±0.78	0.003	<0.5
Glucose†	128.14±2.44	156.24±6.60	<0.001	70–110
BUN	15.09±0.33	20.07±0.95	<0.001	9–21
Cre	0.89±0.02	1.23±0.07	<0.001	0.2–0.9
AST	39.18±2.84	140.81±26.26	<0.001	6–43
ALT	46.61±14.99	228.25±72.84	0.031	11–33
pH	7.40±0.003	7.40±0.01	0.556	7.40±0.07
PCO_2	37.75±0.37	34.65±0.93	0.002	40±4
PO_2	225.34±7.99	256.37±15.67	0.079	95±7
HCO_3–	22.88±0.18	20.63±0.43	<0.001	24±2
COHb	12.52±0.18	8.22±1.12	0.001	<5
BE	–1.88±0.23	–3.66±0.44	0.001	0±2

*Platelet (10^3/μL).
†Glucose (mg/dL).
ALT, alanine aminotransferase (U/L); AST, aspartate aminotransferase (U/L); BE, base excess (mmol/L); BUN, blood urea nitrogen (mg/dL); CK, creatine kinase (U/L); CKMB, creatine kinase-myocardial band (U/L); CO, carbon monoxide; COHb, COHb concentration (%); Cre, serum creatinine (mg/dL); Hb, haemoglobin (g/dL); HCO_3–, plasma bicarbonate concentration (mmol/L); PCO_2, arterial carbon dioxide tension (mm Hg); pH, potential of hydrogen; PO_2, arterial oxygen tension (mm Hg); TnI, troponin I (mg/dL); WBC, white blood cell (10^3/μL).

intubated patients, if their intubation days were >2.5 days, they may have a higher chance of having a poor outcome.

DISCUSSION

The epidemiology of CO poisoning differs from one country to another because of the weather, geographical and cultural variations. The main sources of CO poisoning in Taiwan are gas boilers or water heaters (accidental) and charcoal burning (intentional). The charcoal burning cases accounted for more than the half of the cases. The results were very different in Western countries. For example, the main sources of CO poisoning were heaters' and vehicles' exhaust in the UK and the USA.[1 4 27 28] Charcoal burning accounted for around 23% of all suicides in Asia, a percentage that is 10 times higher than in Western countries.[10] The mean age of patients in this study was 37.4 years. The age of CO-poisoned patients was around 40 years in Turkey, the USA and Italy.[5 19 26] The reason for the lower age in Taiwan may be the type of suicide prevalent in Taiwan. Recently, charcoal burning has become one of the main methods of suicide and has increased both in urban and in rural areas.[10 11] Most of the suicides are in the group of young adults. An early prognostic factor evaluation is more important for this group of patients, because young adults may impose a heavier burden to their family and to the medical society if they have a worse neurological treatment outcome. Our data revealed that patients exposed to CO intentionally have a significantly worse treatment outcome than those by accident. This further stresses the importance of early diagnosis and management of this group of patients. Clinicians should refer patients who attempted suicide to psychiatrists to minimise the incidence of poor outcome.

The poor-outcome group in the study represented around 18% of the total. This treatment outcome was similar to those in the studies by Kao *et al* in Taiwan.[22] In other countries, Weaver *et al* reported a poor treatment outcome of 25.0% in CO-poisoned patients treated with

Table 4 Factors related to CO poisoning outcomes

	Model		
Variable	OR	95% CI	P value
Psychosis	1.467	0.793 to 2.714	0.222
Diabetes mellitus	1.352	0.412 to 4.444	0.619
Metabolic acidosis	1.435	0.761 to 2.703	0.264
Hypertension	1.285	0.491 to 3.362	0.609
GCS	***0.932***	***0.872 to 0.997***	***0.039***
CK	1.000	1.000 to 1.000	0.685
TnI	1.078	0.975 to 1.191	0.141
Glucose	1.000	0.995 to 1.005	0.989
BUN	***1.089***	***1.031 to 1.150***	***0.002***
Cre	0.850	0.402 to 1.797	0.671
AST	1.003	0.999 to 1.007	0.205

Adjusting variables, age and sex.
AST, aspartate aminotransferase; BUN, blood urea nitrogen ; CK, creatine kinase; CO, carbon monoxide; Cre, serum creatinine; GCS, Glasgow Coma Scale ; TnI, troponin I.

HBO and of 46.1% in those with normobaric oxygen treatment alone.[18] Pepe *et al* also found that 34 patients out of 141 (24%) had delayed neuropsychological sequelae (DNS) after they left the hospital by 1 month.[19] The better treatment outcome in Taiwan might be due to the high medical accessibility for CO poisoning in this country. Patients can reach hospitals with treatment facilities within 2 hours and the hospitals could quickly initiate HBO therapy or transfer to other treatment facilities in another 2 hours. Moreover, the treatment outcome in this study may be underestimated because of the high disease severity in our patients. This hospital is equipped with HBO therapy facilities for critically ill patients using ventilator and provides emergency treatments on an all year basis. Sixty per cent of our patients were transferred from other hospital for HBO therapy and 40% among them were using ventilator on arrival. For patients with such high severity, the treatment outcome could have been worse than it was analysed in this study. Certainly, other factors such as short duration of outcome assessment might also contribute to the lower incidence of poor outcome in this study. DNS usually occur around 20 days after poisoning.[18] We evaluated the outcome based on the medical records that were available when the patients left the hospital or when they made return visits to the hospital 1 week later, so some patients may not have had later sequelae detected.

The GCS, an index of severity of neurological awareness, may be also an indicator of some injuries to other organs. An acute CO poisoning may severely suppress central drive of respiration, causing acute respiratory failure. However, a deeply comatous consciousness could be a consequence of overdose of sedatives, which happens frequently in patients attempting to suicide.

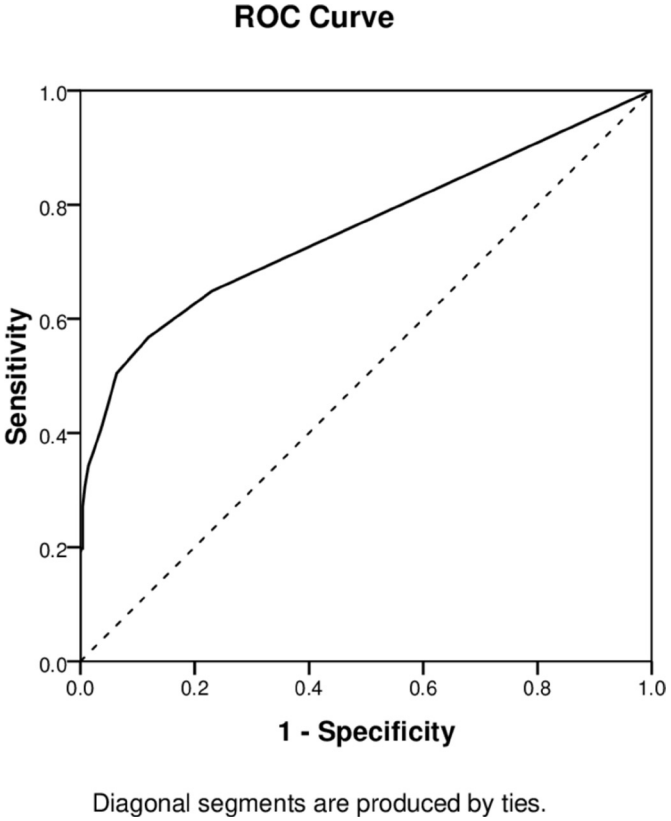

Figure 1 Receiver operating characteristic (ROC) curves for the intubation days of all patients (n=615).

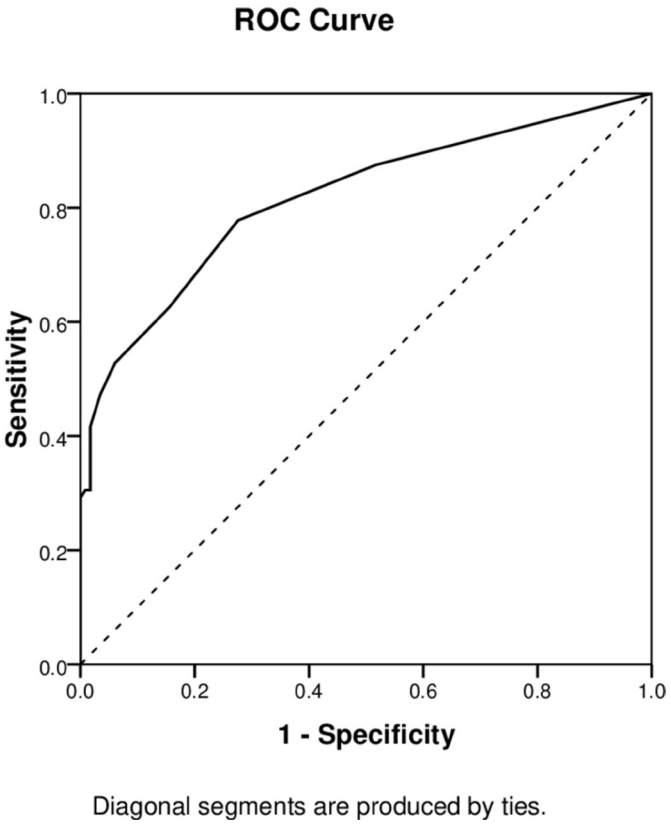

Figure 2 Receiver operating characteristic (ROC) curves for the intubation days of patients with intubation treatment (n=188).

The intubation days of patients, on the other hand, indicate their unconscious time and show the severity of the patients' illnesses.[29] Our previous study also showed that the duration of mechanical ventilation is a predictor of CO poisoning severity.[25] In this study, the intubation days were used as a predictor of the outcome of CO poisoning. The results show that the AUC was around 0.757–0.817 in all patients and in intubated patients. Cha *et al* showed that GCS may be indicative of myocardial injury in CO-poisoned patients.[30] Patients with a myocardial injury are usually more severely affected, so they may be more likely to have poor outcomes.[31 32] In this study, myocardial injury and GCS were closely related to the outcome of CO poisoning. These results are compatible with several other studies,[19 22 23 33 34] suggesting that these two prognostic factors could be used to predict the treatment outcome of CO-poisoned patients. Some studies interpreted their results that people who suffered from a myocardial injury might have been exposed to CO longer than others.[19 30 35 36] However, there has been no report that could clearly identify the exposure duration. Our results showed that elevation of serum BUN in the first blood test is highly associated with poor outcome (table 4). For patients with a normal renal function, serum BUN could be a reliable indicator of body fluid status. Patients with CO poisoning cannot drink any water until the medical intervention, so the concentration of BUN may be related to the time between exposure to CO and presenting at the hospital. Therefore, elevation of BUN might be an indicator to predict the exposure time of CO poisoning and could be a predictor of poor treatment outcome.

In this study, almost all of the patients received HBO treatment. Patients were exposed to 100% oxygen at 3 atmospheres absolute for the first chamber session and then to 100% oxygen at 2.5 atmospheres absolute for the second and third chamber sessions. All chamber sessions consisted of three 25 min oxygen-breathing periods with two 5 min air breaks. Due to the fact that most of our patients were referred from other hospitals, their conditions were more severe and they were more in need of HBO treatment than in other local hospitals. In a recent study, Rose *et al* found that HBO treatment may reduce acute and 1-year mortality.[26] They revealed that older age, being a male, respiratory distress and elevated TnI may relate to 1-year mortality, which could be considered as a poor outcome. Compared with the study by Rose *et al*, our results showed that all the above-mentioned factors, except gender, affect the outcome of CO-poisoned patients. It is interesting to note that in our study the poor outcome is associated with a more HBO therapy sections (table 2). In the regression analysis, the patients who received more HBO therapy sessions may have a higher opportunity to be poor outcome. This raise two concerns about the HBO therapy in CO-poisoned patients. First, could HBO therapy by itself cause poor outcome of CO poisoning? Patients in this study were initially treated with HBO therapy for three sessions. After the patient arrived our ED, the first hyperbaric chamber session was provided as soon as possible; and the third section was ideally to be performed <24 hours after the first chamber section. If it was possible, the interval between each section was around 6–12 hours.[25] If the patients' conditions improved, the HBO therapy would stop; otherwise, they would receive more HBO therapy sections. Therefore, we would not interpret these data as that HBO therapy causes poor outcome of CO poisoning, but that patients need more sessions of HBO therapy had a higher disease severity. Second, is a multiple sections of HBO therapy necessary? The HBO therapy protocol adapted in this hospital was following the Undersea Hyperbaric Medical Society treatment indications of acute tissue injuries, such as crush injury, arterial insufficiency or thermal burn injury. In a nationwide population-based cohort study using Taiwan National Health Insurance Research Database (TNHIRD), Huang *et al* reported that CO-poisoned patients who received HBO therapy had a lower mortality rate[37] but a higher risk for neurological sequelae.[38] It is interesting to note that the patient number in the without HBO therapy group was about 3 times of that in the HBO therapy group. In Taiwan, CO poisoning is a health insurance payable indication for HBO therapy and patients are supposed to receive HBO therapy in the hospital or be transferred to other treatment facilities, unless their disease severity did not fulfil treatment criteria. Therefore, the only explanation for lower risk of the without HBO therapy group is their lower severity of poisoning. Unfortunately, data from TNHIRD provide little information about disease severity of CO poisoning. In a small case series, Lo *et al* reported that 8–40 sections of HBO therapy significantly reduced the neurological and image abnormalities in CO-poisoned patients.[39] Therefore, it remains unclear whether HBO therapy or multisection treatment will produce a better outcome than a conservative management.

Due to the fact that this study was retrospective in nature, there are some limitations. One is the incomplete data (eg, the total time of exposure to CO, the initial COHb of patients, the time from exposure to presenting at the hospital and the duration of loss of consciousness), which may affect the outcomes of CO poisoning. Another limitation is that >60% of the patients in this study were transferred from another hospital. This may result in a lack of the initial data, which were recorded in the previous hospital and ambulance.

CONCLUSION

In summary, in the present study, we tried to find the factors related to the poor prognosis of CO poisoning. The factors best at predicting outcomes were a high GCS score, high BUN and more intubation days. Even HBO therapy is paid by insurance in Taiwan, the benefit of repetitive HBO therapy on the treatment outcome remains to be elucidated. Our results in this retrospective study could give the indications for clinical research work in the future.

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REFERENCES

1 Mott JA, Wolfe MI, Alverson CJ, *et al*. National vehicle emissions policies and practices and declining us carbon monoxide-related mortality. *JAMA* 2002;288:988–95.
2 Ghosh RE, Close R, McCann LJ, *et al*. Analysis of hospital admissions due to accidental non-fire-related carbon monoxide poisoning in England, between 2001 and 2010. *J Public Health* 2016;38:76–83.
3 Clower JH, Hampson NB, Iqbal S, *et al*. Recipients of hyperbaric oxygen treatment for carbon monoxide poisoning and exposure circumstances. *Am J Emerg Med* 2012;30:846–51.
4 Hamilton-Farrell MR. British hyperbaric association carbon monoxide database, 1993-96. *J Accid Emerg Med* 1999;16:98–103.
5 Iqbal S, Law H-Z, Clower JH, *et al*. Hospital burden of unintentional carbon monoxide poisoning in the United States, 2007. *Am J Emerg Med* 2012;30:657–64.
6 Turner M, Esaw M, Clark RJ. Carbon monoxide poisoning treated with hyperbaric oxygen: metabolic acidosis as a predictor of treatment requirements. *J Accid Emerg Med* 1999;16:96–8.
7 Ilano AL, Raffin TA. Management of carbon monoxide poisoning. *Chest* 1990;97:165–9.
8 Eichhorn L, Michaelis D, Kemmerer M, *et al*. Carbon monoxide poisoning from waterpipe smoking: a retrospective cohort study. *Clin Toxicol* 2018;56:264–72.
9 Tsai C-W, Gunnell D, Chou Y-H, *et al*. Why do people choose charcoal burning as a method of suicide? an interview based study of survivors in Taiwan. *J Affect Disord* 2011;131:402–7.
10 Chang S-S, Gunnell D, Wheeler BW, *et al*. The evolution of the epidemic of charcoal-burning suicide in Taiwan: a spatial and temporal analysis. *PLoS Med* 2010;7:e1000212.
11 Liu KY, Beautrais A, Caine E, *et al*. Charcoal burning suicides in Hong Kong and urban Taiwan: an illustration of the impact of a novel suicide method on overall regional rates. *J Epidemiol Community Health* 2007;61:248–53.
12 R Oliveira S, Queiroga CSF, Vieira HLA. Mitochondria and carbon monoxide: cytoprotection and control of cell metabolism - a role for $Ca^{(2+)}$? *J Physiol* 2016;594:4131–8.
13 Queiroga CSF, Almeida AS, Vieira HLA. Carbon monoxide targeting mitochondria. *Biochem Res Int* 2012;2012:1–9.
14 Croxford B, Leonardi GS, Kreis I. Self-Reported neurological symptoms in relation to CO emissions due to problem gas appliance installations in London: a cross-sectional survey. *Environ Health* 2008;7.
15. Weaver LK. Clinical practice. carbon monoxide poisoning. *N Engl J Med* 2009;306:1217–25.
16 Annane D, Chadda K, Gajdos P, *et al*. Hyperbaric oxygen therapy for acute domestic carbon monoxide poisoning: two randomized controlled trials. *Intensive Care Med* 2011;37:486–92.
17 Buckley NA, Juurlink DN, Isbister G, *et al*. Hyperbaric oxygen for carbon monoxide poisoning. *Cochrane Database Syst Rev* 2011.
18 Weaver LK, Hopkins RO, Chan KJ, *et al*. Hyperbaric oxygen for acute carbon monoxide poisoning. *N Engl J Med* 2002;347:1057–67.
19 Pepe G, Castelli M, Nazerian P, *et al*. Delayed neuropsychological sequelae after carbon monoxide poisoning: predictive risk factors in the emergency department. A retrospective study. *Scand J Trauma Resusc Emerg Med* 2011;19:16.
20 Sykes OT, Walker E. The neurotoxicology of carbon monoxide - Historical perspective and review. *Cortex* 2016;74:440–8.
21 Pan Y-J, Liao S-C, Lee M-B. Suicide by charcoal burning in Taiwan, 1995-2006. *J Affect Disord* 2010;120:254–7.
22 Kao H-K, Lien T-C, Kou YR, *et al*. Assessment of myocardial injury in the emergency department independently predicts the short-term poor outcome in patients with severe carbon monoxide poisoning receiving mechanical ventilation and hyperbaric oxygen therapy. *Pulm Pharmacol Ther* 2009;22:473–7.
23 Grieb G, Simons D, Schmitz L, *et al*. Glasgow coma scale and laboratory markers are superior to COHb in predicting CO intoxication severity. *Burns* 2011;37:610–5.
24 Benaissa ML, Mégarbane B, Borron SW, *et al*. Is elevated plasma lactate a useful marker in the evaluation of pure carbon monoxide poisoning? *Intensive Care Med* 2003;29:1372–5.
25 Shen C-H, Peng C-K, Chou Y-C, *et al*. Predicting duration of mechanical ventilation in patients with carbon monoxide poisoning: a retrospective study. *J Crit Care* 2015;30:19–24.
26 Rose JJ, Nouraie M, Gauthier MC, *et al*. Clinical outcomes and mortality impact of hyperbaric oxygen therapy in patients with carbon monoxide poisoning. *Crit Care Med* 2018;46:e649–55.
27 Lynch R, Laden G, Grout P. Carbon monoxide poisoning: correlation of neurological findings between accident and emergency departments and a hyperbaric unit. *Emerg Med J* 2001;18:95–8.
28 Weaver LK, Howe S, Hopkins R, *et al*. Carboxyhemoglobin half-life in carbon monoxide-poisoned patients treated with 100% oxygen at atmospheric pressure. *Chest* 2000;117:801–8.
29 Hampson NB, Hauff NM. Risk factors for short-term mortality from carbon monoxide poisoning treated with hyperbaric oxygen. *Crit Care Med* 2008;36:2523–7.
30 Cha YS, Cha KC, Kim OH, *et al*. Features and predictors of myocardial injury in carbon monoxide poisoned patients. *Emerg Med J* 2014;31:210–5.
31 Henry TD, Lesser JR, Satran D. Myocardial fibrosis from severe carbon monoxide poisoning detected by cardiac magnetic resonance imaging. *Circulation* 2008;118:792.
32 Satran D, Henry CR, Adkinson C, *et al*. Cardiovascular manifestations of moderate to severe carbon monoxide poisoning. *J Am Coll Cardiol* 2005;45:1513–6.
33 Henry CR, Satran D, Lindgren B, *et al*. Myocardial injury and long-term mortality following moderate to severe carbon monoxide poisoning. *JAMA* 2006;295:398–402.
34 Lippi G, Rastelli G, Meschi T, *et al*. Pathophysiology, clinics, diagnosis and treatment of heart involvement in carbon monoxide poisoning. *Clin Biochem* 2012;45:1278–85.
35 Kalay N, Ozdogru I, Cetinkaya Y, *et al*. Cardiovascular effects of carbon monoxide poisoning. *Am J Cardiol* 2007;99:322–4.
36 Weaver LK, Valentine KJ, Hopkins RO. Carbon monoxide poisoning: risk factors for cognitive sequelae and the role of hyperbaric oxygen. *Am J Respir Crit Care Med* 2007;176:491–7.
37 Huang C-C, Ho C-H, Chen Y-C, *et al*. Hyperbaric oxygen therapy is associated with lower short- and long-term mortality in patients with carbon monoxide poisoning. *Chest* 2017;152:943–53.
38 Huang C-C, Ho C-H, Chen Y-C, *et al*. Impact of hyperbaric oxygen therapy on subsequent neurological sequelae following carbon monoxide poisoning. *J Clin Med* 2018;7.
39 Lo C-P, Chen S-Y, Chou M-C, *et al*. Diffusion-tensor MR imaging for evaluation of the efficacy of hyperbaric oxygen therapy in patients with delayed neuropsychiatric syndrome caused by carbon monoxide inhalation. *Eur J Neurol* 2007;14:777–82.

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Review

Factors Contributing to CO Uptake and Elimination in the Body: A Critical Review

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Abstract: Background: Carbon monoxide (CO) poisoning is an important public health issue around the world. Research indicates that many factors may be related to the rate of CO uptake and elimination in the human body. However, some factors related to CO uptake and elimination are considered controversial. Relatively little attention has been devoted to review and synthesis of factors affecting CO uptake and elimination. Purpose: This paper provides a critical scoping review of the factors and divides them into four aspects, including environmental, demographic, physiological and treatment factors. Methods: We searched the scientific databases for research that has proposed a mathematical equation as a synthesis of quantities related to CO poisoning, CO elimination, CO uptake, CO half-life, CO uptake and elimination and their relationships. After excluding the studies that did not meet the study criteria, there were 39 studies included in the review and the search was completed before 16 December 2019. Results and conclusion: This review discusses most of the factors that impact the rate of CO uptake and elimination. Several factors may be related to CO uptake and elimination, such as CO concentration, the duration of exposure to CO, age, sex, exercise, minute ventilation, alveolar ventilation, total haemoglobin mass and different treatments for CO poisoning. Although some potential factors were not included in the review, the findings are useful by presenting an overview for discussing factors affecting CO uptake and elimination and provide a starting point for further study regarding strategies for CO poisoning and the environmental standard of CO.

Keywords: carbon monoxide; CO uptake; CO elimination

1. Introduction

Exogenous carbon monoxide (CO) results from the incomplete combustion of carbon-containing molecules, and endogenous CO is formed within the body by metabolic processes [1]. CO is a neurotransmitter in the brain and peripheral autonomic nervous system but is also a poison in high enough quantities [2]. Here, we consider uptake to be due to breathing in exogenous CO, and excretion to include both exogenous and endogenous sources. CO is transported across the lungs into the bloodstream and binds preferentially to haemoglobin in the blood, forming carboxyhaemoglobin (COHb); the affinity of haemoglobin for CO is around 210 times greater than that for oxygen [3,4]. Inhaling excess CO can lead to a situation where there is inadequate oxygen transported by haemoglobin,

and the human body will then suffer from hypoxia [3,5]. CO poisoning results in symptoms that range from headache to unconsciousness, depending on the dose.

Once exposure to exogenous CO ceases, the body's mechanisms for excreting CO can return the COHb level to baseline. The typical baseline level of people unexposed to exogenous CO is around 0.8% COHb. For this process, many studies have shown that the half-life for COHb in the body is about 4 h [6].

CO enters the human body through the lungs, is transported via the blood system and enters the tissue/muscle system. Since the CO partial pressure is higher in the vascular system than in tissue, CO enters and can be stored in the tissue/muscle system. This CO transport process is reversible. If the partial pressure of CO is lower in the ambient environment than in the vascular system, then CO is released from the tissue to the blood and then to the lungs to be exhaled [7]. However, due to the stronger affinity of CO for Hb, there is a baseline COHb concentration in the blood.

Several factors are known to relate to CO uptake and excretion, including minute ventilation rate (V_E), alveolar ventilation rate (V_A), arterial oxygen tension, haem mass and haemoglobin mass. V_E is the total rate of ventilation, and V_A is the rate of the gas exchange via the alveolar surface during normal breathing. There is a relationship between V_E and V_A. The equation used is $V_A = V_E - fV_D$, where f is the respiration rate (1/min), and V_D is the dead space (mL) [1,8–10].

Haemoglobin is the main oxygen carrier in the human body. It contains a haem prosthetic group that has an iron atom, and it binds to oxygen to form oxyhaemoglobin. By this method, the haemoglobin takes the oxygen through the body [11]. In physiology, CO affects the oxygen–haemoglobin dissociation curve (ODC). Because CO has such a high affinity with haemoglobin, it decreases the blood oxygen concentration significantly [12].

Although several factors relating to CO uptake and elimination in the human body have been described, we did not find an overview of the situation worldwide. This review aimed to summarise the literature on factors that relate to CO uptake and elimination in the human body. Furthermore, we divided the factors into different dimensions to present a clear relationship between each factor. If we understand the factors that affect the rate of CO uptake and elimination, we will be able to predict the CO concentration in the human body and may be able to give suggestions for more effective treatment of CO poisoning.

In this paper, several factors are described that relate to the rate of CO uptake and elimination, which include environmental, demographic, physiological and treatment factors. The related factors contain different dimensions, from physical exposure to physiological metabolism.

2. Materials and Methods

2.1. Scope and Search Strategy

Scientific databases, including PubMed, EMBASE and Web of Science, were searched for studies. The search strategy used a combination of keywords related to carbon monoxide poisoning and elimination, carbon monoxide poisoning and uptake, carbon monoxide poisoning and half-life and carbon monoxide poisoning and equation. We also manually searched the references of every primary study and review article for further publications to make sure all relevant publications were included.

2.2. Inclusion and Exclusion Criteria

In the literature review, certain study inclusion and exclusion criteria were applied. Particularly, the review only included data-based studies on human subjects that appeared in peer-reviewed journals in the English language that had the full text available. Theses, dissertations or presentation abstracts that were not published in peer-reviewed journals were excluded. Also, the authors screened the titles and abstracts to exclude irrelevant publications.

2.3. Search Results and Study Characteristics

The initial search identified 394 studies by the keywords. The references from the papers were checked to see if there were papers that needed to be considered. After deleting duplicative papers and screening the titles and abstracts, 39 studies met the criteria (Figure 1). We identified 39 studies published since 1945 and the search was completed on 16 December 2019.

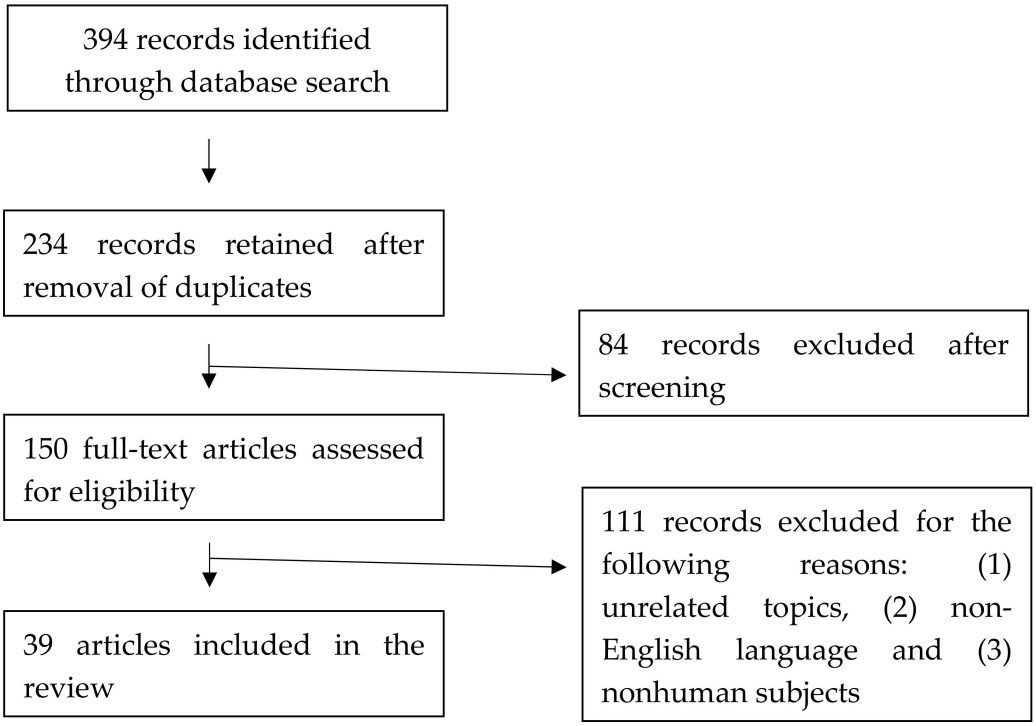

Figure 1. Summary of review process.

3. Results and Discussion

These 39 studies were divided into four aspects, including environmental, demographic, physiological and treatment factors.

3.1. Environmental Factors

When measuring the rate of CO uptake and elimination, the first point to consider is the dose of CO to which the subjects are exposed. There are several environmental factors related to CO exposure, including CO concentration in the ambient air, the duration of CO exposure, the oxygen concentration in the ambient air and altitude.

3.1.1. CO Concentration in Ambient Air and Duration

From the literature, the main factor that may relate to the rate of CO uptake and elimination is ambient CO concentration. In Forbes et al.'s study, the authors obtained more than 100 observations from seven healthy male laboratory workers. When the concentration of CO increased in the inspired air, the rate of CO uptake would also rise [13]. Moreover, Peterson and Stewart created an experiment for 22 subjects. Two subjects (subjects 21 and 32) breathed in 200 ppm CO, and two other subjects (subjects 1 and 12) breathed in 100 ppm CO. As a result, for subjects who breathed in 200 ppm CO, their COHb reached 10% in around 60 min. However, for the subjects who breathed in 100 ppm CO, their COHb reached 10% by 200 min later [8].

The duration of CO exposure affects CO uptake and elimination. In a multicompartment model, researchers tried to predict the CO washout time from different durations. Bruce and Bruce matched

the simulation model with the measured data from Benignus et al.'s study and found that the model predicted COHb concentration well [14,15]. They simulated the same dose of CO through two different scenarios of CO exposure. One was exposed to 10,000 ppm CO for 5 min, and the other was exposed to 1250 ppm CO for 40 min. The result for the elimination time of the long duration was slower than for the short duration [15].

3.1.2. Oxygen Concentration in Ambient Air

In Forbes et al.'s study, the authors made subjects breathe CO in the air environment and also in a pure oxygen environment. Then, they compared the rate of CO uptake of the subjects. The ratio of CO uptake rate in the pure oxygen environment compared with in air was around 0.77 during rest and 0.62 during hard work. The reason is that there would be much more oxygen competing with CO if the subjects breathe CO in oxygen than in air. Therefore, the CO uptake will also be slower in oxygen than in air [13].

3.1.3. Altitude

Some researchers found that altitude may be a factor that governs the rate of CO uptake and elimination [13,16]. Collier and Goldsmith modified the Coburn–Forster–Kane (CFK) equation by adding altitude as a factor affecting CO uptake and elimination [16]. For example, the partial pressure of oxygen decreases when the altitude increases. Therefore, when people breathe the same amount of CO, it may cause a higher CO concentration at higher altitudes than at sea level. The reason may be due to the lower partial pressure of oxygen at high altitudes, which means there is less oxygen to compete with CO and the COHb half-life increases. Moreover, altitude may also affect the ODC to the left, which increases haemoglobin's affinity to bind to oxygen [16]. However, Forbes et al. recorded the CO uptake of three subjects at sea level, 16,000 ft and 40,000 ft. The results showed that the CO uptake rate increased by increasing the altitude due to higher V_E [13].

All the factors described above are mainly divided into three parts, namely, the CO concentration, the duration of CO exposure and the partial pressure of CO. When people are exposed to high concentrations of CO or high partial pressure of CO, the rate of CO uptake increases. However, when considering the duration of CO exposure, even though subjects are exposed to a lower concentration of CO, they have a longer elimination time if the exposure time is increased. Both the CO concentration and duration of CO exposure are critical environmental factors related to the rate of CO uptake by, and washout from, the human body.

3.2. Demographic Factors

In many disease-related studies, we could find demographic factors that may be relevant to the disease [17,18]. In some studies, age and sex were reported to relate to the rate of CO uptake and elimination [19,20].

3.2.1. Age

In Klasner et al.'s study, the authors focused on CO poisoning in the paediatric population. Compared with previous studies, they found that children had a shorter COHb half-life than adults. For 26 children, the mean half-life of COHb was 44.0 min on 100% oxygen at 1 atm. However, the half-life of COHb in adults was around 80 min in the same situation. The authors assumed that the reason for this was the difference in minute ventilation between children and adults. Although children have a smaller tidal volume than adults, they have faster respiratory rates, which leads to an increase in their V_E [19,21].

Moreover, there are still several factors that may change with age, including the volume of haem, blood volume, muscle myoglobin mass and lung function. Therefore, further studies need to be done to understand the age effects.

3.2.2. Sex

Tracing back to Pace et al.'s study, they found a sex-related difference in the half-life of COHb. The half-life of COHb elimination by breathing 100% oxygen at 2.5 atmosphere absolute (ATA) was 22.3 min for men and 15.1 min for women. However, the authors did not explain the reason for the sex-related difference [22]. Although some studies showed a sex-related difference, there were still other studies that found no sex-related difference in the rate of CO uptake and elimination [9,23]. In a large natural experiment, 184 people were exposed to CO in a public high school for around 2.5 h. The researchers gave questionnaires to the victims and analysed the data. They found no differences between ages, sexes and smokers and nonsmokers [23]. Moreover, Weaver et al. found that sex did not have a significant influence on half-life [9].

Zavorsky et al. did find a sex-related difference for the half-life of CO elimination and revealed the factors behind this effect. The results showed that women had a shorter half-life of CO elimination than men. The factors found to influence the rate of CO elimination were V_A and total haemoglobin mass [20].

3.2.3. Smoking

When someone smokes a cigarette, the smoker is likely to be exposed to CO concentrations of around 400–500 ppm and experience a higher COHb concentration than a nonsmoker. The COHb concentration is usually less than 5% in nonsmokers and more than 5% in smokers [24]. Another study also showed that COHb is different between smokers and nonsmokers in London. Smokers have COHb levels of around 5%–8% compared with nonsmokers, who have COHb levels of about 1%–3% [25]. This is in contrast with the study of Burney et al. where no differences were observed [23].

3.2.4. Exercise

The level of exercise or activity of subjects may have some influence on the rate of CO uptake and elimination. In Forbes et al.'s study, the rate of CO uptake in the subjects was higher during hard work than during rest [13]. Filley et al. also found that the rate of CO uptake was different between subjects at rest and exercise. When the subjects increased the level of exercise, the minute ventilation and the rate of CO uptake also rose [26]. However, the rate of CO uptake was not significantly different between either a low (~45 W) or moderate (~90 W) power output measured by a cycle ergometer in an experiment involving 29–37-year-old subjects [27].

Demographic factors, such as age, sex and exercise, are related to the rate of CO uptake and elimination. However, the physiological factors of minute ventilation, alveolar ventilation and total haemoglobin mass likely explain the demographic observations.

3.3. Physiological Factors

When people breathe in CO, the CO gas enters the lungs and then transfers via the alveoli into the vascular system. Through the blood circulation, most of the CO binds to haemoglobin and is transferred from the arterial to the venous blood. Besides the blood, some of the CO also crosses into the tissue and binds to it, leading to the formation of carboxymyoglobin [7]. Consequently, like lung and cardiovascular functions, muscle function may play a role in CO circulation in the human body and it is related to the rate of CO uptake and elimination. In Penney's book, he stated that the two main physiological factors that affect the rate of CO uptake and elimination are the ventilation and diffusion rates of CO [28].

3.3.1. Lung Function

Ventilation Rate

Many studies have discovered that the ventilation rate may affect the rate of CO uptake and elimination [1,13,19,26,29,30]. When people breathe at a high ventilation rate, they tend to absorb more CO into the lungs and blood. However, a high ventilation rate can also exhale more CO than a low ventilation rate over the same duration [1,13]. In a study by Zavorsky et al. (2014), the results showed that men have a more prolonged washout time of CO than women, and the authors tried to explain the result. After they tested different factors in the subjects, they found that the alveolar ventilation and total haemoglobin mass may be the reasons that explain the difference in the CO half-life. When people have increased alveolar ventilation, the CO elimination time decreases [20].

However, in Bruce and Bruce's model (2006), they found that the half-life of COHb has a higher correlation (r = 0.714) with Vb/VAwo (blood volume/ventilation during washout) than ventilation alone. Because the CO is exhaled directly from the lungs and carried by the blood, the limiting factor may be this ratio [15].

Diffusion Capacity of CO (DL_{CO})

Between the alveolar and pulmonary capillaries, gas passes the pulmonary membrane by simple diffusion. The diffusion capacity "is the ability of the lungs to transfer gas from inhaled air to the red blood cells in pulmonary capillaries" [26]. The diffusion capacity is affected by molecular species, body size, rate of work, temperature and pressure [8]. The DL_{CO} is widely used to test patients' lung function in hospitals nowadays [31]. The mean values for DL_{CO} were found to be 28.05 ± 5.07 mL/min/mmHg for men and 20.79 ± 4.03 mL/min/mmHg for women [32]. The CFK equation, an equation for the study of the endogenous production of CO, CO distribution, CO uptake and elimination, contains the pulmonary diffusing capacity as a factor that may affect the rate of CO uptake and elimination. When the diffusion capacity is higher, it means that CO has a great ability to pass through the membrane, and the rate of the CO uptake and elimination is increased [1,33,34]. However, in Filley et al.'s study, the authors found that the ventilation rate may play a more important role in the rate of CO uptake and elimination [26].

Chronic Obstructive Pulmonary Disease (COPD)

COPD is defined as an obstruction of the airways that makes it hard to breathe. COHb levels were found to be significantly higher in COPD patients compared with the normal population [35,36]. Some COPD patients have a lower diffusing capacity for CO in the lungs [37–39]. In Crowley et al.'s study, their data suggested that the half-life of COHb is around 6.5 h in COPD patients compared with healthy subjects, who have a COHb half-life of about 2–5 h [40]. Therefore, COPD patients may have a slower rate of CO elimination than healthy people due to the lower gas exchange and poor respiratory mechanics. However, Crowley et al. explained that there was no dramatic difference of COHb half-life between COPD patients and normal subjects, so it might be the sedentary life of COPD patients that causes the longer COHb half-life [40].

3.3.2. Cardiovascular Function

Blood Volume

When CO enters the vascular system, most of the CO combines with haemoglobin as COHb. At the end of CO exposure, most of the CO stays in the blood. Consequently, the blood volume may be an important factor that relates to CO uptake and elimination. In Pugh's study, the average blood volume was around 78 mL/kg [41]. In the CFK equation, blood volume is one of the factors affecting the rate of CO uptake and elimination [1]. Furthermore, in Bruce and Bruce's study, their model predicted

that if people have a large blood volume, they carry more CO in the body and have an increased rate of CO uptake and elimination [15].

Haemoglobin Mass

Haemoglobin is the crucial factor that determines the maximum amount of oxygen uptake. The average haemoglobin mass is about 11.6 g/kg [41]. However, when compared with oxygen, CO has around 210 times greater affinity for haemoglobin [4]. In Zavorsky et al.'s study, the authors suggested that the total haemoglobin mass affects the rate of CO uptake and elimination [20]. However, the effects of total haemoglobin mass on the rate of CO uptake and elimination require further investigation.

Diffusion Rate of CO Flux from Blood to Muscle Compartment

The blood-to-muscle diffusion coefficient (Dmco) refers to the diffusion rate of the CO entering the muscle compartment. In a multicompartment model, Bruce and Bruce (2006) set the Dmco to zero and tried to determine how it would influence the half-life for CO washout. When the Dmco was set to zero, the half-life increased. Therefore, this means that no CO entered the muscle compartment, and all the CO decreased by exhalation from the lungs [15]. Moreover, in 2008, the authors tested the model with experimental data, including human and animal data [14,23,42,43]. They found their model could fit well with the experimental data when changing the Dmco in different conditions [44].

Muscle Mass

Not only can haemoglobin bind to CO in muscle cells, but myoglobin also contains haem, to which CO can bind. Muscle tissue can take up CO over a prolonged period, even after the end of exposure. For a young adult male, the muscle compartment may account for about 41% of the total body mass [45]. In their study, Möller and Sylvén assumed that every gram wet weight of muscle would contain about 4.7 mg of myoglobin [46]. Take a 70 kg man, for example, who may, approximately, have 135 g of myoglobin. Each myoglobin molecule contains a haem molecule that could bind up to 178 mL of CO. Therefore, the muscle compartment could be an essential place to store CO and increase the half-life of CO elimination. Although muscle may be a factor, it is less critical for the half-life of COHb. The reason is that the volume of CO removed from muscle is less than the volume of CO removed from the blood [15].

Anaemia

Anaemia refers to a low haemoglobin level or low red blood cell count in the blood or increased destruction of red blood cells. In Woehlck et al.'s study (2001), the authors predicted that patients would have more severe CO poisoning according to the haematocrit level. They explained that patients with low haemoglobin tend to have a higher COHb concentration than people with normal haemoglobin after exposure to CO. When the subjects breathed in the same CO concentration, the rate of COHb increased more rapidly in the subjects with a lower haematocrit level than a higher haematocrit level [47].

Among physiological factors, besides ventilation rate and diffusion capacity (which have been emphasised for a long time), there are still many factors that need to be considered. For example, our review indicates that blood volume, total haemoglobin mass, muscle mass and disease may influence the rate of CO uptake and elimination. However, the physiology of the human body is known to be complicated. Some factors may have a relationship with other factors. Isolating the role of any specific factor will require careful study.

3.4. Treatment Factors

The most common treatment for CO poisoning is breathing 100% oxygen as soon as possible. Moreover, high-pressure oxygen or hyperbaric oxygen (HBO) therapy also has been used in several

countries as a solution for CO poisoning. The rate of CO elimination may relate to the atmospheric pressure or percentage of oxygen. Higher atmospheric pressure and percentage of oxygen result in a faster CO elimination rate [19,29].

3.4.1. 100% Oxygen

Weaver et al. (2000) conducted a study to understand which factors may influence the COHb half-life. Through their retrospective chart review from 1985 to 1995, they showed that the half-life of COHb decreases with the increase of arterial oxygen tension. As a result, they found that the half-life of COHb was around 74 min for patients treated with 100% oxygen at atmospheric pressure, which was shorter than for patients only breathing in air [9].

There are several methods to provide 100% oxygen to patients, such as a rebreathing reserve mask, high-flow nasal cannula (HFNC) oxygen and oxygen therapy with continuous positive airway pressure (CPAP) [48–51]. In Kim et al.'s (2019) study, HFNC did not reduce the CO half-life compared with a rebreathing reserve mask [49]. When comparing normobaric oxygen therapy with 1 h of CPAP therapy, Bal et al. discovered that patients receiving CPAP therapy had a shorter CO half-life than those receiving normobaric oxygen therapy. The authors assumed that CPAP therapy increases the gas exchange area and improves ventilation due to the positive pressure going into the alveoli [50].

3.4.2. HBO Therapy

Treating CO-poisoned patients with HBO therapy is still controversial [6,52,53]. There are different policies in different countries. However, Pace et al. found that high-pressure oxygen could increase CO elimination in CO-poisoned patients [22]. In Ernst and Zibrak's study, they found that the half-life of COHb would be approximately 4 h on air, 1.5 h on oxygen and less than 20 min during HBO therapy [54].

3.4.3. Carbogen

Carbogen is a mixture of carbon dioxide and oxygen gas. Usually, CO_2 is set at 5%–10% in O_2 [55,56]. When patients breathe in carbogen, their brain CO_2 sensor detects that more CO_2 is stored in the body, and as a result, the brain sends a signal to increase alveolar ventilation, thus decreasing the half-life of COHb [56,57].

The 100% oxygen and HBO therapies are based on the theory that the alveolar partial pressures of oxygen would be affected by the inhaled partial pressure of oxygen. When increasing the partial pressure of oxygen, there is more oxygen that can compete with CO to bind with haemoglobin. Then, the rate of CO elimination would be raised. However, using HBO therapy for CO-poisoned patients is still controversial. Although HBO therapy is not recommended for CO-poisoned patients in the United Kingdom, it is a treatment for CO-poisoned patients that is widely used in Taiwan.

4. Conclusions

In the literature review, some environmental, demographic, physiological and treatment factors were found to have an impact on the rate of CO uptake and elimination (Tables 1 and 2). Among environmental factors, the rate of CO uptake increases by raising the CO concentration or reducing the oxygen concentration of the inhaled gas. Moreover, the altitude can alter the rate of CO uptake due to the different partial pressure of oxygen at different altitudes. The duration of CO exposure is an important factor. If people were exposed to CO for a long time, even if the concentration of CO were low, it would also have an adverse impact and reduce the rate of CO elimination. That is why attention is needed not only for acute CO poisoning but also chronic CO poisoning, which is often ignored.

Table 1. The factors related to CO uptake.

Field	Factor	Results	Experiment	Control	Reference
Environment	CO concentration increase	CO uptake rate increase	Range: 0.01%–0.2% CO		Forbes et al. (1945)
			Range: 0–523 CO ppm		Peterson and Stewart (1970)
			Range: 8.7–1000 CO ppm		Peterson and Stewart (1975)
	Duration of exposure longer	CO uptake amount increase	Range: 0–270 min		Forbes et al. (1945)
			Range: 15–480 min		Peterson and Stewart (1970)
			Range: 0–1440 min (50 CO ppm)		Benignus et al. (1994)
	O_2 concentration increase	CO uptake rate decrease	Oxygen	Air	Forbes et al. (1945)
	Altitude increase	CO uptake rate increase	16,000 ft; 40,000 ft	0 ft	Forbes et al. (1945)
	Exercise increase	CO uptake rate increase	Hard work	Rest	Forbes et al. (1945)
		CO uptake rate increase	Light exercise; moderate exercise	Resting	Filley et al. (1954)
		No difference	Moderate exercise	Low exercise	Tikuisis et al. (1992)
Physiology	Ventilation rate increase	CO uptake rate increase	Range: 6–30 L/min		Forbes et al. (1945)
		CO uptake rate increase	Range: 5.8–105 L/min		Filley et al. (1954)
	Diffusion capacity of CO (DL_{CO}) increase	CO uptake rate increase	36.3 cm^3/min/mmHg	16.9 cm^3/min/mmHg	Filley et al. (1954)
		CO uptake rate increase	Range: 5–30 mL/min/torr		Bruce and Bruce (2003)
		CO uptake rate increase	-	-	Gosselin et al. (2009)
	Blood volume increase	CO uptake rate increase	-	-	Coburn et al. (1965)
	Diffusion rate of CO flux from blood to muscle compartment in crease	CO uptake rate increase	Range: 0–100 mL/min/torr		Bruce et al. (2008)
	Muscle mass	Less important	-	-	Bruce and Bruce (2006)
	Anaemia	CO uptake rate increase	Haematocrits of 18% and 30%	Haematocrits of 42% and 60%	Woehlck et al. (2001)

Note: 1 torr = 1 mmHg, a unit of pressure based on an absolute scale; 1 cm^3 = 1 mL.

Table 2. The factors related to CO elimination.

Field	Factor	Results	Experiment	Control	Reference
Environment	CO concentration increase	CO half-life longer	200.8 CO ppm for 60 min	51.6 CO ppm for 60 min	Peterson and Stewart (1970)
	Duration of exposure longer	CO half-life longer	1250 CO ppm for 40 min (same CO dose in two groups)	10,000 CO ppm for 5 min	Bruce and Bruce (2006)
	O2 concentration increase	CO half-life shorter	100% oxygen	-	Weaver et al. (2000)
			2.5 atm, 100% oxygen (HBO)	-	Pace et al. (1950)
Demography	Age increase	No difference	Range: 9–86 years old		Burney et al. (1982)
			>40 years old	<40 years old	Weaver et al. (2000)
		CO half-life shorter	4–12 years old	-	Klasner et al. (1998)
	Sex	No difference	Female	Male	Burney et al. (1982)
			Female	Male	Weaver et al. (2000)
		CO half-life shorter	Female	Male	Pace et al. (1950)
			Female	Male;	Zavorsky et al. (2014)
	Smoking	No difference	Smokers	Nonsmokers	Burney et al. (1982)

Table 2. *Cont.*

Field	Factor	Results	Experiment	Control	Reference
Physiology	Ventilation rate increase	CO half-life shorter	Range: 4–10 L/min		Coburn et al. (1965)
			15 and 30 L/min	3 and 6 L/min	Selvakumar et al. (1993)
			Range: 5–20 L/min		Kreck et al. (2001)
			Range: 4–40 L/min		Zavorsky et al. (2014)
	Chronic obstructive pulmonary disease (COPD)	No difference/CO half-life slightly longer	COPD patients	Normal subjects	Crowley et al. (1989)
	Blood volume increase	CO half-life shorter	-	-	Coburn et al. (1965)
			Range: 0.3–0.7 (Vb/VAwo)		Bruce and Bruce (2006)
	Haemoglobin mass increase	CO half-life longer	Male	Female	Zavorsky et al. (2014)
	Diffusion rate of CO flux from blood to muscle compartment increase	CO half-life shorter	Range: 0–2 mL/min/torr		Bruce et al. (2003)
	Muscle mass	Less important	-	-	Bruce and Bruce (2006)
	Anaemia	CO half-life shorter	Anaemia	Polycythaemia	Zavorsky et al. (2014)
Treatment	100% oxygen	CO half-life shorter	100% oxygen	-	Weaver et al. (2000)
	High-flow nasal cannula (HFNC)	No difference	100% oxygenwith high flow	100% oxygen	Kim et al. (2019)
	Continuous positive airway pressure (CPAP)	CO half-life shorter	100% oxygenwith positive pressure	100% oxygen	Bal et al. (2019)Caglar et al. (2019)
	Hyperbaric oxygen (HBO) therapy	CO half-life shorter	2.5 atm, 100% oxygen	-	Pace et al. (1950)
			3 atmosphere absolute (ATA), 100% oxygen	1 ATA, 100% oxygen	Peterson and Stewart (1970)
	Carbogen	CO half-life shorter	Hyperventilation(6% CO_2 in O_2)	Without isocapnia	Sein Anand et al. (2017)

Note: 1 torr = 1 mmHg, a unit of pressure based on an absolute scale.

Demographic factors, such as age, sex, smoking and exercise, are not direct factors relating to the rate of CO uptake and elimination. Included in these factors may be physiological factors, such as minute ventilation, alveolar ventilation and total haemoglobin mass, which are direct factors affecting the rate of CO uptake and elimination. Other physiological factors, including muscle mass and diffusion capacity, can also influence the rate of CO uptake and elimination. Consider the treatments, for example: the three treatments for CO poisoning increase the rate of CO elimination by raising the partial pressure of oxygen and the ventilation rate. Therefore, these treatments are based on the physiological reactions that have been tested in past studies.

This review discussed most of the factors that impact the rate of CO uptake and elimination. Information remains limited and there are numerous other potentially important factors that could influence CO update and elimination, such as genetics, disease, vulnerable groups, children, the elderly, weight and so forth. Thus, there may be different treatment strategies for groups with different characteristics. Further studies focused on this field may find better ways to increase the rate of CO elimination in CO-poisoned patients.

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References

1. Coburn, R.F.; Forster, R.E.; Kane, P.B. Considerations of the physiological variables that determine the blood carboxyhemoglobin concentration in man. *J. Clin. Investig.* **1965**, *44*, 1899–1910. [CrossRef] [PubMed]
2. Barañano, D.E.; Snyder, S.H. Neural roles for heme oxygenase: Contrasts to nitric oxide synthase. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 10996–11002. [CrossRef] [PubMed]
3. Dolan, M.C. Carbon monoxide poisoning. *CMAJ* **1985**, *133*, 392–399. [PubMed]
4. Blumenthal, I. Carbon monoxide poisoning. *J. R. Soc. Med.* **2001**, *94*, 270–272. [CrossRef]
5. Kao, L.W.; Nanagas, K.A. Carbon monoxide poisoning. *Emerg. Med. Clin. North. Am.* **2004**, *22*, 985–1018. [CrossRef]
6. Weaver, L.K.; Hopkins, R.O.; Chan, K.J.; Churchill, S.; Elliott, C.G.; Clemmer, T.P.; Orme, J.F., Jr.; Thomas, F.O.; Morris, A.H. Hyperbaric oxygen for acute carbon monoxide poisoning. *N. Engl. J. Med.* **2002**, *347*, 1057–1067. [CrossRef]
7. Erupaka, K.; Bruce, E.N.; Bruce, M.C. Prediction of Extravascular Burden of Carbon Monoxide (CO) in the Human Heart. *Ann. Biomed. Eng.* **2010**, *38*, 403–438. [CrossRef]
8. Peterson, J.E.; Stewart, R.D. Predicting the carboxyhemoglobin levels resulting from carbon monoxide exposures. *J. Appl. Physiol.* **1975**, *39*, 633. [CrossRef]
9. Weaver, L.K.; Howe, S.; Hopkins, R.; Chan, K.J. Carboxyhemoglobin half-life in carbon monoxide-poisoned patients treated with 100% oxygen at atmospheric pressure. *Chest* **2000**, *117*, 801–808. [CrossRef]
10. Brochu, P.; Brodeur, J.; Krishnan, K. Derivation of cardiac output and alveolar ventilation rate based on energy expenditure measurements in healthy males and females. *J. Appl. Toxicol.* **2012**, *32*, 564–580. [CrossRef]
11. Hsia, C.C.W. Respiratory Function of Hemoglobin. *N. Engl. J. Med.* **1998**, *338*, 239–248. [CrossRef] [PubMed]
12. Morgan, T.J. The oxyhaemoglobin dissociation curve in critical illness. *Crit. Care Resusc.* **1999**, *1*, 93–100. [PubMed]
13. Forbes, W.H.; Sargent, F.; Roughton, F.J.W. The rate of carbon monoxide uptake by normal men. *Am. J. Physiol.* **1945**, *143*, 594–608. [CrossRef]
14. Benignus, V.A.; Hazucha, M.J.; Smith, M.V.; Bromberg, P.A. Prediction of carboxyhemoglobin formation due to transient exposure to carbon monoxide. *J. Appl. Physiol.* **1994**, *76*, 1739. [CrossRef] [PubMed]
15. Bruce, M.C.; Bruce, E.N. Analysis of factors that influence rates of carbon monoxide uptake, distribution, and washout from blood and extravascular tissues using a multicompartment model. *J. Appl. Physiol.* **2006**, *100*, 1171. [CrossRef]

16. Collier, C.R.; Goldsmith, J.R. Interactions of carbon monoxide and hemoglobin at high altitude. *Atmos. Environ.* **1983**, *17*, 723–728. [CrossRef]
17. Bueno, V.; Sant'Anna, O.A.; Lord, J.M. Ageing and myeloid-derived suppressor cells: Possible involvement in immunosenescence and age-related disease. *Age* **2014**, *36*, 9729. [CrossRef]
18. Aziz, A.; Hansen, H.S.; Sechtem, U.; Prescott, E.; Ong, P. Sex-Related Differences in Vasomotor Function in Patients With Angina and Unobstructed Coronary Arteries. *J. Am. Coll. Cardiol.* **2017**, *70*, 2349–2358. [CrossRef]
19. Klasner, A.E.; Smith, S.R.; Thompson, M.W.; Scalzo, A.J. Carbon monoxide mass exposure in a pediatric population. *Acad. Emerg. Med.* **1998**, *5*, 992–996. [CrossRef]
20. Zavorsky, G.S.; Tesler, J.; Rucker, J.; Fedorko, L.; Duffin, J.; Fisher, J.A. Rates of carbon monoxide elimination in males and females. *Physiol. Rep.* **2014**, *2*, e12237. [CrossRef]
21. Peterson, J.E.; Stewart, R.D. Absorption and Elimination of Carbon Monoxide by Inactive Young Men. *Arch. Environ. Health Int. J.* **1970**, *21*, 165–171. [CrossRef] [PubMed]
22. Pace, N.; Strajman, E.; Walker, E.L. Acceleration of Carbon Monoxide Elimination in Man by High Pressure Oxygen. *Science* **1950**, *111*, 652. [CrossRef] [PubMed]
23. Burney, R.E.; Wu, S.-C.; Nemiroff, M.J. Mass carbon monoxide poisoning: Clinical effects and results of treatment in 184 victims. *Ann. Emerg. Med.* **1982**, *11*, 394–399. [CrossRef]
24. Marshall, M.D.; Kales, S.N.; Christiani, D.C.; Goldman, R.H. Are reference intervals for carboxyhemoglobin appropriate? A survey of Boston area laboratories. *Clin. Chem.* **1995**, *41*, 1434. [CrossRef]
25. Castleden, C.M.; Cole, P.V. Carboxyhaemoglobin levels of smokers and non-smokers working in the City of London. *Br. J. Ind. Med.* **1975**, *32*, 115–118. [CrossRef]
26. Filley, G.F.; MacIntosh, D.J.; Wright, G.W. Carbon monoxide uptake and pulmonary diffusing capacity in normal subjects at rest and during exercise. *J. Clin. Investig.* **1954**, *33*, 530–539. [CrossRef]
27. Tikuisis, P.; Kane, D.M.; McLellan, T.M.; Buick, F.; Fairburn, S.M. Rate of formation of carboxyhemoglobin in exercising humans exposed to carbon monoxide. *J. Appl. Physiol.* **1992**, *72*, 1311. [CrossRef]
28. Penney, D.G. *Carbon Monoxide*, 1st ed.; CRC Press: Boca Raton, FL, USA, 1996.
29. Selvakumar, S.; Sharan, M.; Singh, M.P. A Mathematical Model for the Elimination of Carbon Monoxide in Humans. *J. Theor. Biol.* **1993**, *162*, 321–336. [CrossRef]
30. Kreck, T.; Shade, E.; Lamm, W.; McKinney, S.; Hlastala, M. Isocapnic Hyperventilation Increases Carbon Monoxide Elimination and Oxygen Delivery. *Am. J. Respir. Crit. Care Med.* **2001**, *163*, 458–462. [CrossRef]
31. Bowley, N.B.; Hughes, J.M.B.; Steiner, R.E. The chest X-ray in pulmonary capillary haemorrhage: Correlation with carbon monoxide uptake. *Clin. Radiol.* **1979**, *30*, 413–417. [CrossRef]
32. Park, J.O.; Choi, I.S.; Park, K.O. Normal Predicted Values of Single-Breath Diffusing Capacity of the Lung in Healthy Nonsmoking Adults. *Korean J. Intern. Med.* **1986**, *1*, 178–184. [CrossRef] [PubMed]
33. Bruce, E.N.; Bruce, M.C. A multicompartment model of carboxyhemoglobin and carboxymyoglobin responses to inhalation of carbon monoxide. *J. Appl. Physiol.* **2003**, *95*, 1235. [CrossRef] [PubMed]
34. Gosselin, N.H.; Brunet, R.C.; Carrier, G. Determination of carboxyhaemoglobin in humans following low-level exposures to carbon monoxide. *Inhal. Toxicol.* **2009**, *21*, 1077–1091. [CrossRef] [PubMed]
35. Özgür Dogan, N.; Corbacioglu, S.; Bildik, F.; Kilicaslan, I.; Gunaydın, G.; Günaydın, G.P.; Cevik, Y.; Ülker, V.; Hakoglu, O.; Gökcen, E. Determining the diagnostic value of endogenous carbon monoxide in Chronic Obstructive Pulmonary Disease exacerbations. *J. Pak. Med. Assoc.* **2014**, *64*, 1037–1041.
36. Nambu, A.; Zach, J.; Schroeder, J.; Jin, G.Y.; Kim, S.S.; Kim, Y.-I.L.; Schnell, C.; Bowler, R.; Lynch, D.A. Relationships between diffusing capacity for carbon monoxide (DLCO), and quantitative computed tomography measurements and visual assessment for chronic obstructive pulmonary disease. *Eur. J. Radiol.* **2015**, *84*, 980–985. [CrossRef] [PubMed]
37. Sandek, K.; Bratel, T.; Lagerstrand, L. Effects on diffusing capacity and ventilation–perfusion relationships of budesonide inhalations for 2 months in chronic obstructive pulmonary disease (COPD). *Respir. Med.* **2001**, *95*, 676–684. [CrossRef]
38. Murphy, T.F.; Sethi, S. Chronic Obstructive Pulmonary Disease. *Drugs Aging* **2002**, *19*, 761–775. [CrossRef]
39. O'Donnell, D.E.; Laveneziana, P.; Webb, K.; Neder, J.A. Chronic Obstructive Pulmonary Disease: Clinical Integrative Physiology. *Clin. Chest Med.* **2014**, *35*, 51–69. [CrossRef]
40. Crowley, T.J.; Andrews, A.E.; Cheney, J.; Zerbe, G.; Petty, T.L. Carbon monoxide assessment of smoking in chronic obstructive pulmonary disease. *Addict. Behav.* **1989**, *14*, 493–502. [CrossRef]

41. Pugh, L.G.C.E. Blood volume changes in outdoor exercise of 8-10 hour duration. *J. Physiol.* **1969**, *200*, 345–351. [CrossRef]
42. Roughton, F.J.W.; Root, W.S. The fate of co in the body during recovery from mild carbon monoxide poisoning in man. *Am. J. Physiol. Leg. Content* **1945**, *145*, 239. [CrossRef] [PubMed]
43. Prommer, N.; Schmidt, W. Loss of CO from the intravascular bed and its impact on the optimised CO-rebreathing method. *Eur. J. Appl. Physiol.* **2007**, *100*, 383–391. [CrossRef] [PubMed]
44. Bruce, E.N.; Bruce, M.C.; Erupaka, K. Prediction of the rate of uptake of carbon monoxide from blood by extravascular tissues. *Respir. Physiol. Neurobiol.* **2008**, *161*, 142–159. [CrossRef] [PubMed]
45. Lee, R.C.; Wang, Z.; Heo, M.; Ross, R.; Janssen, I.; Heymsfield, S.B. Total-body skeletal muscle mass: Development and cross-validation of anthropometric prediction models. *Am. J. Clin. Nutr.* **2000**, *72*, 796–803. [CrossRef]
46. Möller, P.; Sylvén, C. Myoglobin in human skeletal muscle. *Scand. J. Clin. Lab. Investig.* **1981**, *41*, 479–482. [CrossRef]
47. Woehlck, H.J.; Mei, D.; Dunning 3rd, M.B.; Ruiz, F. Mathematical Modeling of Carbon Monoxide Exposures from Anesthetic Breakdown Effect of Subject Size, Hematocrit, Fraction of Inspired Oxygen, and Quantity of Carbon Monoxide. *Anesthesiology* **2001**, *94*, 457–460. [CrossRef]
48. Ozturan, I.U.; Yaka, E.; Suner, S.; Ozbek, A.E.; Alyesil, C.; Dogan, N.O.; Yilmaz, S.; Pekdemir, M. Determination of carboxyhemoglobin half-life in patients with carbon monoxide toxicity treated with high flow nasal cannula oxygen therapy. *Clin. Toxicol.* **2019**, *57*, 617–623. [CrossRef]
49. Kim, Y.-M.; Shin, H.-J.; Choi, D.-w.; Kim, J.-M.; Lee, S.-W.; Jeong, S.-H.; Kim, H. Comparison of high-flow nasal cannula oxygen therapy and conventional reserve-bag oxygen therapy in carbon monoxide intoxication: A pilot study. *Am. J. Emerg. Med.* **2019**. Publish Ahead of Print. [CrossRef]
50. Bal, U.; Sönmez, B.M.; Inan, S.; Iscanli, M.D.; Yilmaz, F. The efficiency of continuous positive airway Press. therapy in carbon monoxide poisoining in the emergency department. *Eur. J. Emerg. Med.* **2019**. Publish Ahead of Print. [CrossRef]
51. Caglar, B.; Serin, S.; Yilmaz, G.; Torun, A.; Parlak, I. The Impact of Treatment with Continuous Positive Airway Pressure on Acute Carbon Monoxide Poisoning. *Prehospital Disaster Med.* **2019**, *34*, 588–591. [CrossRef]
52. Buckley, N.A.; Juurlink, D.N.; Isbister, G.; Bennett, M.H.; Lavonas, E.J. Hyperbaric oxygen for carbon monoxide poisoning. *Cochrane Database Syst. Rev.* **2011**, *4*, CD002041. [CrossRef] [PubMed]
53. Pan, K.-T.; Shen, C.-H.; Lin, F.-G.; Chou, Y.-C.; Croxford, B.; Leonardi, G.; Huang, K.-L. Prognostic factors of carbon monoxide poisoning in Taiwan: A retrospective observational study. *BMJ Open* **2019**, *9*, e031135. [CrossRef] [PubMed]
54. Ernst, A.; Zibrak, J.D. Carbon monoxide poisoning. *N. Engl. J. Med.* **1998**, *339*, 1603–1608. [CrossRef] [PubMed]
55. Douglas, T.A.; Lawson, D.D.; Ledingham, I.M.; Norman, J.N.; Sharp, G.R.; Smith, G. Carbogen in Experimental Carbon-monoxide Poisoning. *Br. Med. J.* **1961**, *2*, 1673. [CrossRef] [PubMed]
56. Sein Anand, J.; Schetz, D.; Waldman, W.; Wiśniewski, M. Hyperventilation with Maintenance of Isocapnia. An Old New Method in Carbon Monoxide Intoxication. *PLoS ONE* **2017**, *12*, e0170621. [CrossRef] [PubMed]
57. Fisher, J.A.; Iscoe, S.; Fedorko, L.; Duffin, J. Rapid elimination of CO through the lungs: Coming full circle 100 years on. *Exp. Physiol.* **2011**, *96*, 1262–1269. [CrossRef]

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Can Exhaled Carbon Monoxide Be Used as a Marker of Exposure? A Cross-Sectional Study in Young Adults

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Abstract: Carbon monoxide (CO) poisoning is a major public health issue worldwide. People are exposed to CO in their daily lives, with one of the common sources of CO being cigarette smoking. Inhalation of CO leads to elevated carboxyhaemoglobin (COHb) levels in the blood and also in exhaled CO concentration. Several factors have been shown to affect COHb concentration and COHb half-life. However, factors affecting exhaled CO concentration and exhaled CO half-life are not well understood. The present study aimed to investigate the potential factors related to baseline exhaled CO concentration and exhaled CO half-life among smokers. A cross-sectional study was conducted between 26 January and 30 June 2019, and young adults were recruited into the study. A total of 74 participants (mean age: 27.1 years, 71.6% males and 28.4% females) attended the study. They were invited to complete a questionnaire, including demographic, physiological, and behavioural factors. Then, exhaled CO measurements were taken. These measurements were taken before and after smoking a single cigarette for smokers and only once for non-smokers. The average baseline exhaled CO concentration was 6.9 $\pm$ 4.9 ppm for smokers and 1.9 $\pm$ 0.5 ppm for non-smokers. The mean of exhaled CO half-life was around 273.3 min (4.6 h) for smokers. No difference was seen in exhaled CO half-life between light smokers and heavy smokers in the smoking group. Gender and cigarettes smoked weekly affected baseline exhaled CO in smokers. Even though height seemed to positively associate with exhaled CO half-life, the relationship disappeared when adjusting by gender and weight. Therefore, exhaled CO could be used as a marker of CO exposure, but we cannot ignore the factors mentioned in the study. For future study, considering factors related to smoking habits and smoking style are recommended as these may affect total inhaled CO.

Keywords: carbon monoxide; CO half-life; CO elimination; cigarette; smoking

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1. Introduction

Carbon monoxide (CO) is an odourless, tasteless, colourless, and poisonous gas produced from the incomplete combustion of organic compounds [1,2]. In many countries, CO was the leading cause of the fatal poisonings reported [3]. It behaves similarly to oxygen in the body, but has around 200–260 times higher affinity to haemoglobin (Hb) and forms as carboxyhaemoglobin (COHb) in the blood [2,4]. Exposure to high amounts of CO may result in hypoxia and produce a series of adverse health effects, such as headaches, nausea, fatigue, respiratory dysfunction, tissue damage and even death [1,5,6]. In the United States, there were a total of 24,890 CO poisoning deaths (including unintentional and intentional) from 1999 to 2014 (annual death rate of 0.5/100,000) [7]. In the WHO European Region report, CO-related deaths were recorded at a total of 140,490 between 1980 to 2008 (annual death rate of 2.2/100,000) [8].

The treatment guide for CO poisoning is to help patients to eliminate CO as soon as possible. The COHb half-life has been estimated as approximately 4 h in room air [5,9] and approximately 30 min with Hyperbaric oxygen (HBO) therapy [9]. Several factors have been shown to affect COHb elimination, such as severity and duration of exposure to CO, ventilation rate, age, gender, and blood volume [10–13]. However, the effects of cigarette smoking on CO uptake and elimination remains controversial [14–16]. In an observational study of a CO poisoning incident in a public high school, Burney et al. investigated the factors related to COHb half-life and found cigarette smoking did not impact COHb half-life [14]. However, Cronenberger et al.'s study showed that smokers have a longer COHb half-life than non-smokers [16].

Smoking prevalence varies by country, ranging from 43.4% in Greece to 14.7% in Iceland from Our World in Data [17]. It is the major source of CO exposure. For smokers, smoking exposes people to a high concentration of CO [18]. In the WHO report, the CO concentration in tobacco smoke is around 4.5% (45,000 ppm), and smokers inhale air with a concentration of about 400–500 CO ppm during smoking [19]. Therefore, smokers usually have a higher concentration of COHb in the blood, around 6% to 9% of COHb, compared to 1% to 3% of COHb in non-smokers [20,21]. Exhaled CO concentration has been shown to be highly correlated with COHb concentration, especially in healthy smokers [21–23]. The use of devices to monitor CO in breath has increased in research settings and clinics to diagnose CO exposure [24–26]. Generally, without potential air pollution, the exhaled CO concentration would be expected in a range of 1–4 ppm in non-smokers and 2–18 ppm in smokers [24]. Suppose the exhaled CO concentration of the participants and patients was higher than expected, in that case, they might be exposed to CO. Breath CO monitors have provided a non-invasive, relatively low-cost and quicker way to measure CO concentrations compared to the blood COHb test.

However, factors affecting exhaled CO as a marker of CO exposure are not well characterised. Even though Jarvis et al. reported that exhaled CO measurement could distinguish smokers from non-smokers, they mentioned that a few smokers could not be identified due to not inhaling the smoke very deeply [27]. In 2020, Ghorbani et al. indicated that breath sampling may also have an impact on exhaled CO concentration [28]. Moreover, Chatrchaiwiwatana and Ratanasiri stated that the cut-off point of differentiating exhaled CO concentration between smokers and non-smokers might be affected by age [29]. Therefore, factors affecting the exhaled CO concentration and exhaled CO half-life are worth exploring and addressing. The poor quantitative characterisation of the effect of demographic, physiological factors, and smoking behaviour on exhaled CO limits its value for modelling CO exposure and documenting its health effects.

In the present study, breath CO monitors were used to measure CO concentration from the participants. The primary aim of this study was to explore the factors, including demographic, physiological and behavioural factors, and smoking status, that affect baseline exhaled CO concentration and exhaled CO half-life.

2. Materials and Methods

2.1. Study Design and Participant Recruitment

The present study was a cross-sectional study conducted between 26 January and 30 June 2019. The participants were recruited through physical posters placed at University College London (UCL) and Goodenough College. The participants were young, healthy, aged 18 to 34 years old, university students or their friends, with no pregnancy and no history of illness related to lung function changes. Participants were categorised as "smokers" if they had smoked more than 100 cigarettes through their entire life till the present [30,31]. "Light smokers" were defined as those who smoked less than ten cigarettes per day, and "heavy smokers" were those who smoked equal to or more than ten cigarettes per day [32,33]. In the study, the sample size was calculated using data from a previous study [24]. The sample size was calculated using STATA software by setting 80% for the power and 0.05 for the significance value. As a result, the researcher estimated that at

least 13 participants were needed for each group, including smokers (light smokers and heavy smokers) and non-smokers. This study was approved by the UCL Research Ethics Committee (REC) (Project ID: 14201/001).

2.2. Data Collection Procedure

On the day participants attended the study, non-smokers were excluded if they had smoked before attendance (n = 1), and smokers if they could not properly follow the protocol of exposure measurement (n = 9). The study protocol contained two parts, including questionnaires and exposure measurements. After recruitment, participants were invited to fill out the consent and questionnaire. The questionnaire included age, gender, height, weight, BMI, ethnicity, diet, menstrual cycle and smoking habits, such as years of smoking, type of cigarettes, number of cigarettes smoked daily and weekly and time since the last cigarette. Participants were also asked if they had exercise or had been exposed to CO (ex. Exposure to secondhand smoke, gas fire, cars exhaust, etc.) before attendance for the study measurements, and their responses were recorded.

2.3. Exposure Measurement

In the exposure measurement part, baseline exhaled CO concentration was measured in all participants. After their baseline exhaled CO concentration had been recorded, smokers were asked to smoke one control cigarette with the same brand and type (Seven Stars, Japan Tobacco, Tokyo, Japan). Then, the researcher (K.-T.P.) measured exhaled CO concentration immediately after smoking and at 30 min, 60 min, 90 min and 120 min after smoking. Moreover, smokers were asked not to smoke for at least four hours before attending the study [34,35]. This period of four hours was based on the half-life of COHb in people breathing natural air [5], aiming to minimise the effects of the last cigarette. The researcher recorded the time since the last cigarette before the exhaled CO test of each participant.

The exhaled CO half-life was calculated from the formula below. The method was described by Weaver et al. and Ozturan et al. [15,36]. In the equation, if concentration 1 (c1) and concentration 2 (c2) are the levels of exhaled CO concentration taken at time 1 (t1) and time 2 (t2) during CO 'wash-out' time, then the half-life of exhaled CO can be calculated. The exhaled CO half-life is also calculated as follows:

$$\text{CO half} - \text{life} = (t2 - t1) \times \ln(2)/[\ln(c1/c2)]$$

a. t1 is time point 1
b. t2 is time point 2
c. c1 is the concentration of exhaled CO in t1
d. c2 is the concentration of exhaled CO in t2.

Exhaled CO concentration was monitored by a breath CO monitor, the 'Micro^{+}™ Smokerlyzer®' (Bedfont Scientific Ltd., Medical manufacturer, Maidstone, UK). The participants were asked to hold their breath for 20 s and then blow continuously and slowly into the Smokerlyzer mouthpiece, following the procedure described in the manual of Smokerlyzer. The researcher stayed with the participants and instructed them about the protocol at the time of their attendance for the study.

2.4. Statistical Analysis

Analyses were conducted using Microsoft Excel, IBM SPSS Statistics 26 (IBM, Armonk, NY, USA) and Stata IC 15 (TX: StataCorp LLC, College Station, TX, USA). Descriptive statistics were computed and reported as mean ± standard deviation (SD) for age, gender, height, weight, BMI and exhaled CO at each time point. Univariable analysis was then conducted to describe the relationship of each variable with baseline exhaled CO concentration and exhaled CO half-life. Mean differences between the two groups, such as gender and smoking status, were compared by the Student's t-test. If variables had more than two

groups, such as ethnicity, analysis of variance (ANOVA) was performed to understand the difference across each group. When the number of participants was less than 10, the nonparametric Mann–Whitney U test or the Kruskal-Wallis H test was applied to compare median values. The chi-square test was applied when analyzing the relationship by gender, ethnicity, smoking status, etc. (categorical variable data). A Pearson's correlation was used to study the relationship between baseline exhaled CO concentration and age, height, weight, etc. (two quantitative and continuous variables). A backward stepwise multivariable regression was then applied to investigate the factors related to baseline exhaled CO concentration and the exhaled CO half-life. A standardised beta coefficient was used to rank the most important variables in the stepwise multivariable regression model presented. A p-value of <0.05 was considered to be statistically significant, and all p-values were given for two-sided tests.

3. Results

A total of 84 participants were recruited for the study. After exclusion, exhaled CO concentrations were assessed for 74 participants, including 48 smokers (28 light smokers and 20 heavy smokers) and 26 non-smokers.

Table 1, part (A) displays the basic demographics of the study participants. The mean age was 27.1 ± 4.0 with a mean height of 173.0 ± 9.3 and weight of 69.1 ± 13.5. Twenty-one participants were female, and the majority of ethnicities were Asian or White/Caucasian in both smokers and non-smokers. Around 30% of the participants were exposed to CO or exercised before attending the study. When comparing the characteristics between smokers and non-smokers, smokers had a higher concentration of baseline exhaled CO than non-smokers (6.9 ± 4.9 vs. 1.9 ± 0.5, p-value < 0.001), and a higher mean of weight and BMI. Also, compared to non-smokers, there was a higher percentage of males among smokers, and more smokers exercised before attending the study.

Table 1. (**A**). Demographics, physiological and baseline exhaled CO of the study participants by smoking status. (**B**). Demographics, physiological, smoking-related and baseline exhaled CO characteristics of light smokers and heavy smokers.

(A)				
Characteristics	**Total**	**Smokers**	**Non-Smokers**	***p*-Value**
	(n = 74)	**(n = 48)**	**(n = 26)**	
Age (years)	27.1 ± 4.0	26.6 ± 4.5	27.9 ± 2.7	0.202
Height (cm)	173.0 ± 9.3	174.3 ± 8.1	170.6 ± 10.9	0.100
Weight (kg)	69.1 ± 13.5	72.1 ± 13.8	63.2 ± 11.1	0.007 **
BMI (kg/m^2)	23.1 ± 3.3	23.6 ± 3.6	21.8 ± 2.3	0.026 *
Baseline exhaled CO (ppm)	5.2 ± 4.6	6.9 ± 4.9	1.9 ± 0.5	<0.001 **
Gender				0.013 *
Male	53 (71.6)	39 (81.3)	14 (53.9)	
Female	21 (28.4)	9 (18.7)	12 (46.2)	
Ethnicity				0.507
Asian	45 (60.8)	27 (56.3)	18 (69.2)	
Black/Africa American	2 (2.7)	1 (2.1)	1 (3.9)	
Hispanic/Latino	4 (5.4)	2 (4.2)	2 (7.7)	
White/Caucasian	21 (28.4)	16 (33.3)	5 (19.2)	
Mixed Ethnicity	2 (2.7)	2 (4.2)	0 (0)	
Exposure CO before the study				0.199

Table 1. *Cont.*

(A)				
Characteristics	**Total**	**Smokers**	**Non-Smokers**	***p*-Value**
	(n = 74)	**(n = 48)**	**(n = 26)**	
None	53 (71.6)	32 (66.7)	21 (80.8)	
Yes	21 (28.4)	16 (33.3)	5 (19.2)	
Exercise before study				0.047 *
None	52 (70.3)	30 (62.5)	22 (84.6)	
Yes	22 (29.7)	18 (37.5)	4 (15.38)	
(**B**)				
Characteristics		**Light Smokers**	**Heavy Smokers**	***p*-Value**
		(n = 28)	**(n = 20)**	
Age (years)		27.2 ± 4.4	25.9 ± 4.6	0.302
Height (cm)		173.4 ± 8.8	175.5 ± 7.0	0.386
Weight (kg)		70.9 ± 11.2	73.9 ± 16.8	0.456
BMI (kg/m^2)		27.2 ± 4.4	25.9 ± 4.6	0.302
Baseline exhaled CO (ppm)		4.8 ± 2.6	10.0 ± 5.8	<0.001 **
Years of smoking (year)		8.6 ± 4.7	9.0 ± 5.0	0.783
Time since last cigarette (hour ago)		34.3 ± 69.4	7.6 ± 3.7	0.093
Cigarettes smoked (daily)		3.2 ± 2.0	12.6 ± 4.0	<0.001 **
Cigarettes smoked (weekly)		23.1 ± 16.6	89.6 ± 28.6	<0.001 **
Puffs		12.4 ± 4.3	11.3 ± 3.9	0.368
Smoking duration (min)		3.6 ± 0.8	3.3 ± 1.3	0.250
Gender				0.039 *
Male		20 (71.4)	19 (95.0)	
Female		8 (28.6)	1 (5.0)	
Ethnicity				0.304
Asian		14 (50.0)	13 (65.0)	
Black/Africa American		0 (0)	1 (5.0)	
Hispanic/Latino		2 (7.1)	0 (0)	
White/Caucasian		10 (35.7)	6 (30.0)	
Mixed Ethnicity		2 (7.1)	0 (0)	
Exposure CO before the study				0.301
None		17 (60.7)	15 (75.0)	
Yes		11 (39.3)	5 (25.0)	
Exercise before study				0.762
None		18 (64.3)	12 (60.0)	
Yes		10 (35.7)	8 (40.0)	
Type of cigarette				0.883
Factory-made cigarette		19 (67.9)	14 (70.0)	
Hand-rolled cigarette		7 (25.0)	4 (20.0)	
Both		2 (7.1)	2 (10.0)	

Data are reported as the mean ± standard deviation or number (percentage). Where a significant difference between groups was found, the *p*-values are highlighted: * *p*-value < 0.05; ** *p*-value < 0.01.

Table 1, part (B) describes the demographics and smoking-related characteristics between light smokers and heavy smokers. The baseline exhaled CO was 4.8 ± 2.6 ppm in light smokers and 10.0 ± 5.8 ppm in heavy smokers (p-value < 0.001). Light smokers had fewer cigarettes smoked daily and weekly compared to heavy smokers. A higher percentage of males were in the heavy smokers' group than light smokers (95.0% vs. 71.4%, p-value = 0.039). The majority of ethnicities were Asian or White/Caucasian with a similar distribution of light smokers and heavy smokers (p-value = 0.304). Other factors, such as age, height, weight, BMI, years of smoking, time since the last cigarette, puffs, smoking duration, ethnicity, and type of cigarettes used to smoke, showed no significant difference between light smokers and heavy smokers.

Figure 1 presents the exhaled CO concentration for light smokers and heavy smokers at different time points. The average exhaled CO concentrations changed following the same pattern in both smoking groups (light smokers and heavy smokers) through the time points, and heavy smokers had a higher exhaled CO concentration than light smokers at all time points.

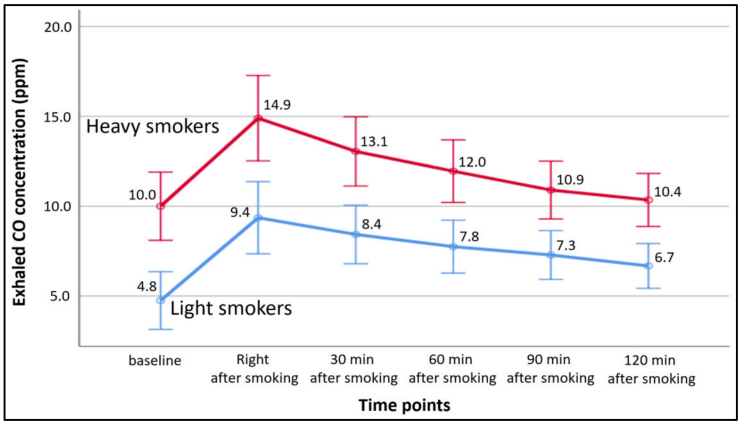

Figure 1. Exhaled CO concentration for light smokers and heavy smokers at different time points. Error bar—means ± 95% CI (Confidence Interval).

Table 2 reports that baseline exhaled CO concentration and exhaled CO half-life showed a significant difference between males and females. In contrast, the exhaled CO half-life showed no significant difference between light smokers and heavy smokers. The average exhaled CO half-life among the smokers was 273.3 ± 95.6 min (4.6 ± 1.6 h).

Table 2. (**A**). Comparison of baseline exhaled CO concentration between different groups in smokers. (**B**). Comparison of exhaled CO half-life between different groups in smokers.

(A)		
Variable (n = 48)	**Baseline Exhaled CO (ppm)**	***p*-Value**
	Mean ± SD [1]	
Total (n = 48)	5.2 ± 4.6	
Gender		0.002 **
Male (n = 39)	7.7 ± 5.1	
Female (n = 9)	3.6 ± 2.1	
Smoking status		<0.001 **
Light smokers (n = 28)	4.8 ± 2.6	
Heavy smokers (n = 20)	10.0 ± 5.8	
Ethnicity		0.264
Asian (n = 27)	7.9 ± 5.9	

Table 2. *Cont.*

(A)		
Variable (n = 48)	**Baseline Exhaled CO (ppm)**	***p*-Value**
	Mean ± SD [1]	
Black/African-American (n = 1)	9	
Hispanic/Latino (n = 2)	5.0 ± 4.2	
White/Caucasian (n = 16)	5.9 ± 3.0	
Mixed ethnicity (n = 2)	2.5 ± 0.7	
Type of cigarette		0.744
Factory-made cigarette (n = 33)	7.3 ± 1.0	
Hand-rolled cigarette (n = 11)	5.7 ± 0.8	
Both (n = 4)	7.0 ± 1.4	
Exposure to CO before the study		0.094
None (n = 32)	7.8 ± 1.0	
Yes (n = 16)	5.3 ± 0.7	
Exercise before study		0.586
None (n = 30)	6.6 ± 0.8	
Yes (n = 18)	7.4 ± 1.4	
(**B**)		
Variable (n = 45)	**CO Half-Life (Minutes)**	***p*-Value**
	Mean ± SD [1]	
Total (n = 45)	273.3 ± 95.6	
Gender		0.010 *
Male (n = 36)	288.1 ± 96.1	
Female (n = 9)	213.9 ± 70.4	
Smoking status		0.396
Light smokers (n = 25)	262.3 ± 90.5	
Heavy smokers (n = 20)	287.0 ± 22.9	
Ethnicity		0.462
Asian (n = 25)	282.8 ± 101.8	
Black/African-American (n = 1)	314.4	
Hispanic/Latino (n = 2)	205.8 ± 52.8	
White/Caucasian (n = 15)	272.7 ± 95.4	
Mixed Ethnicity (n = 2)	206.1 ± 35.6	
Type of cigarette		0.848
Factory-made cigarette (n = 31)	272.3 ± 93.6	
Hand-rolled cigarette (n = 10)	280.1 ± 123.2	
Both (n = 4)	264.5 ± 27.1	
Exposure CO before the study		0.281
None (n = 29)	284.8 ± 106.5	
Yes (n = 16)	252.4 ± 70.2	
Exercise before the study		0.486
None (n = 29)	280.8 ± 94.4	
Yes (n = 16)	259.7 ± 99.3	

[1] SD—standard deviation. Where a significant difference between groups was found, the *p*-values are highlighted: * *p*-value <0.05; ** *p*-value <0.01.

Table 3, part (A) indicates that there was a moderate relationship between cigarettes smoked daily (r = 0.394, *p*-value = 0.006)/ weekly (r = 0.417, *p*-value = 0.003) and the baseline exhaled CO concentration, which means the number of cigarettes smoked daily/weekly was positively associated with the concentration of baseline exhaled CO. Table 3, part (B) shows a weak relationship between height and exhaled CO half-life (r = 0.357, *p*-value = 0.016), indicating that height was positively associated with exhaled CO half-life.

Table 3. (**A**). Correlation of baseline exhaled CO concentration with demographics, physiological and smoking habits in smokers. (**B**). Correlation of exhaled CO half-life with demographics, physiological and smoking habits in smokers.

(A)		
Variable	**Correlation Coefficient**	***p*-Value**
Age (years)	0.163	0.267
Height (cm)	0.061	0.681
Weight (kg)	0.136	0.356
BMI (kg/m^2)	0.132	0.373
Years of smoking (year)	−0.089	0.553
Time since last cigarette (hour ago)	−0.269	0.067
Cigarettes smoked (daily)	0.394	0.006 **
Cigarettes smoked (weekly)	0.417	0.003 **
Puffs	−0.239	0.101
Smoking duration (min)	−0.130	0.379
(B)		
Variable	**Correlation Coefficient**	***p*-Value**
Age (years)	0.007	0.965
Height (cm)	0.357	0.016 *
Weight (kg)	0.292	0.051
BMI (kg/m^2)	0.159	0.297
Years of smoking (year)	0.051	0.714
Time since last cigarette (hour ago)	0.032	0.835
Cigarettes smoked (daily)	0.033	0.828
Cigarettes smoked (weekly)	−0.062	0.688
Puffs	−0.199	0.189
Smoking duration (min)	0.025	0.872

Where a significant correlation was found, the *p*-values are highlighted: * *p*-value < 0.05; ** *p*-value < 0.01.

Tables 4 and 5 show the factors that affect the baseline exhaled CO concentration and exhaled CO half-life of smokers. The final models only included significant and borderline significant factors. The results showed that gender (β = −5.491, *p*-value = 0.020) and cigarettes smoked weekly (β = 0.051, *p*-value = 0.004) affect the baseline exhaled CO concentration. Height and age showed borderline significance. If a person was older or smoked more cigarettes weekly, the baseline CO concentration increased. Height affects the time of exhaled CO half-life (β = 4.878, *p*-value = 0.007). If a person was taller, the exhaled CO half-life time increased. However, once the results were adjusted by gender and weight, the impact of height disappeared. Gender, height and weight did not affect the exhaled CO half-life in the regression analysis.

Table 4. Factors affecting baseline CO concentration in smokers.

Variable [1] (n = 47)	R^2 = 0.349, Adjusted R^2 = 0.287			
	β [2]	Beta [3]	95% CI [4]	*p*-Value
Gender (female/ male)	−5.491	−0.439	(−10.071, −0.911)	0.020
Cigarettes smoked (weekly)	0.051	0.407	(0.017, 0.084)	0.004
Height (cm)	−0.193	−0.310	(−0.417, 0.030)	0.088
Age (year)	0.287	0.260	(−0.0001, 0.573)	0.050

[1] Variables included when running backwards stepwise regression: age, gender, height, weight, BMI, exposure CO, exercise, type of cigarette, cigarettes smoked weekly, years of smoking, time since the last cigarette, number of puffs and smoking duration, [2] β—un-standardised coefficient, [3] Beta–standardised coefficient, [4] 95% CI—95% Confidence Interval.

Table 5. (**A**). Factors affecting exhaled CO half-life in smokers. (**B**). Factors affecting exhaled CO half-life for smokers.

(**A**)				
Variable [1] (n = 45)	**R^2 = 0.163, Adjusted R^2 = 0.143**			
	β [2]	**Beta [3]**	**95% CI [4]**	***p*-Value**
Height (cm)	4.878	0.403	(1.431, 8.326)	0.007
(**B**)				
Variable (n = 45)	**R^2 = 0.141, adjusted R^2 = 0.078**			
	β [1]	**Beta [2]**	**95% CI [3]**	***p*-Value**
Height (cm)	2.483	0.209	(−3.141, 8.109)	0.378
Gender (female/male)	−26.893	−0.114	(−125.814, 72.028)	0.586
Weight	0.718	0.106	(−1.837, 3.273)	0.573

(**A**) [1] Variables included when running backwards stepwise regression: age, gender, height, weight, BMI, exposure CO, exercise, type of cigarette, cigarettes smoked weekly, years of smoking, time since the last cigarette, number of puffs and smoking duration, [2] β—un-standardised coefficient, [3] Beta—standardised coefficient, [4] 95% CI—95% Confidence Interval. (**B**) [1] β—un-standardised coefficient, [2] Beta—standardised coefficient, [3] 95% CI—95% Confidence Interval.

4. Discussion

To date, non-invasive monitors for CO assessment have been widely used. This study is the first study to use a breath CO monitor to calculate exhaled CO half-life and explore factors affecting baseline exhaled CO concentration and exhaled CO half-life. Our results using exhaled CO were relatively similar to those from studies using COHb from blood as an exposure marker, where half-life is about 4–5 h [2,5]. The average age of the participants was 27 years old since the inclusion criteria were 18–34 years old. Therefore, the potential effects of ageing of the lungs were eliminated [37,38]. In the study, the difference of baseline exhaled CO concentration between smokers and non-smokers was around 5 ppm (6.9 ppm vs. 1.9 ppm), which was similar to the data from Kozienice in Maga et al.'s study, in which the average baseline exhaled CO concentration was 6.5 ppm in smokers and 1.1 ppm in non-smokers [24]. However, the baseline exhaled CO concentration was less than the study by Maga et al., based in Krakow (smokers vs. non-smokers, 12.3 ppm vs. 7.0 ppm) and Warsaw (smokers vs. non-smokers, 14.4 ppm vs. 5.1 ppm) [24]. Another study also showed a higher baseline exhaled CO concentration than our study, and the mean exhaled CO concentration was 3.6 ppm for non-smokers and 17.1 ppm for smokers [26]. The lower baseline CO concentration in the study may be related to the lower number of heavy smokers, lower background CO concentration, shorter years of smoking, and the mean of time since the last cigarette, which was much longer than other studies [24,26].

The baseline CO concentration of the smokers was between 1 ppm to 24 ppm. It showed that some of the smokers' baseline exhaled CO concentration was similar to non-smokers, which was around 1.9 ppm. The possible reason for the low exhaled CO baseline

concentration in smokers might be the long period since the last cigarette. In our study, the average time since the last cigarette was around 23 h. The COHb half-life for a healthy person breathing air is approximately 4 h [5]. If a person stops smoking for a sufficiently long period, the exhaled CO concentration could be similar to non-smokers. Besides, some studies reported that smokers could lower their CO exposure by reducing the puff volume, the puffs smoked and the tendency and depth of inhaling [18,39–42]. In terms of puffs, males generally tended to have a higher puff volume, a longer puff duration and shorter intervals between puffs than females [42]. Above all, these may be highly related to smoking habits and hard to control. Therefore, this might be a reason for the big variation of exhaled CO concentration within and between different studies [20,24,26,43]. Even though the exposure of CO from smoking may be highly affect by smoking habits and hard to control, smoking is the major source of CO exposure in the population. Future studies should consider the possible ways to measure the actual amount of CO that goes into the body while smoking.

Moreover, some studies showed that cigarettes themselves might play a role in CO exposure in smoking, such as paper porosity, filter, cigarette CO level, cigarette nicotine level and type of cigarettes [18,40,44]. Laugesen et al.'s study reported that even though the increased CO ppm was similar in hand-rolled cigarettes and factory-made cigarettes, the CO ppm increase per g of tobacco burnt was higher in hand-rolled cigarettes than in factory-made cigarettes [44]. Therefore, the cigarettes in the present study were controlled to being the same brand and type to avoid the effects of the properties of different cigarettes.

In the regression model, gender and cigarettes smoked weekly affected baseline exhaled CO concentration. The gender effect may be due to more heavy smokers in the male group, as heavy smokers tend to have a higher concentration of COHb [3,24,45]. Moreover, some studies showed that females may have lower exhaled CO concentrations during menstruation due to loss of blood, which has a high affinity with CO [46]. The baseline exhaled CO concentration was positively associated with the number of cigarettes smoked daily and weekly, similar to other studies [20,24,26,39,43]. Some studies also reported that exhaled CO concentration is higher for participants who smoke and inhale more deeply [39,43]. In our study, the concentration of exhaled CO showed no difference before and after smoking in a few participants. Some of them claimed that they did not inhale the smoke into their lungs, while some of the participants said they did inhale deeply. The same situation was also found in Jarvis et al.'s study [27].

The average COHb half-life in smokers was 4.5 h in our study, similar to other studies [2,5]. Light smokers and heavy smokers showed no significance in exhaled CO half-life. Similar findings were also demonstrated in the studies [14,15]. However, Cronenberger et al. (2008) have reported the median (range) COHb half-life was 30.9 h (7.13–367) in adult smokers [16], which was longer compared with the results from exhaled CO half-life in our study (median, 4.1 h). The possible reason that COHb half-life was longer in Cronenberger et al.'s study than in the present study might be the younger age of participants in the present study (age range: 18–34) compared to the participants in Cronenberger et al.'s study (age range: 21–63). Moreover, even though some studies showed that cigarette smoking might affect lung function and reduce gas exchange efficiency [47,48], the effects may be reduced due to only young and healthy participants being recruited.

Moreover, there were only 45 participants in the regression. The reason was that in three participants, the exhaled CO concentration did not decrease after 120 min after smoking. Therefore, their exhaled CO half-life could not be calculated. Besides the equipment error for the three participants, the reason for exhaled CO concentration without decreasing after 120 min after smoking might be the longer exhaled CO half-life of smokers than non-smokers [16]. Therefore, it is hard to detect the decrease of exhaled CO concentration within 120 min.

Gender and height showed their effects on exhaled CO half-life in the correlation and univariable test. Height was also found to have a positive association with exhaled CO half-life in smokers in multivariable regression. However, when controlling for gender and

weight (significant and borderline significant factors in the univariable test), height, gender and weight together showed no significant effects on exhaled CO half-life in the regression model. Gender has been postulated to affect COHb half-life in studies [11,49]. Female smokers had a shorter exhaled CO half-life compared to male smokers, which may be due to females having a lower Hb mass and higher alveolar ventilation than males [11,49]. Some studies have suggested that alveolar ventilation and total Hb mass, more than gender, may play a critical role in COHb elimination and half-life [11–13]. Besides gender and height, weight showed a slightly positive association with exhaled CO half-life with a borderline significance (Table 3, part (B)). Generally, heavier people have increased blood volume and have a longer COHb half-life [12,13].

Study limitations. Firstly, the participants smoked a controlled cigarette in their usual manner. The number of puffs, interval time between puffs and the depth of smoking were hard to control and may affect exhaled CO concentration. Fortunately, the puffs and smoking duration were recorded, and the researcher recruited more participants than estimated in each group to reduce the effects of the big variation in exhaled CO concentration on the analysis. Moreover, different CO exposure methods could be used in future studies, such as the DL_{CO} test and CO-rebreathing experiment, which are safer and utilise a known dose of CO exposure under clinical and medical staff control. Secondly, many females tended to reject the study and were not willing to report their smoking status when recruiting participants. This situation resulted in there being more males than females involved in the study. Also, the lower number of female participants makes it hard to see if the menstrual cycle would affect the exhaled CO concentration and exhaled CO half-life. Thirdly, the backward stepwise regression was applied to find the factors affecting baseline CO concentration and exhaled CO half-life. However, this method was only based on statistical results without evidence from the literature. Different approaches could be considered in the future. Fourthly, breath CO monitors are most used for healthy participants due to the protocol of breath-holding for 20 s might be hard to perform for patients with certain conditions, such as lung illness and chest pain. Finally, the participants smoked outdoors due to the smoking regulations at the university and did the exhaled CO experiment indoors. Even though there may be a delay after smoking to the exhaled CO measurement, the exact times recorded in the study were much less than the exhaled CO half-life. Therefore, this time delay is not expected to affect the study significantly.

5. Conclusions

This is the first study to calculate exhaled CO half-life using a breath CO monitor and showed relatively similar results compared to the COHb half-life measured in blood, especially in young healthy adults. Therefore, exhaled CO could be used as a marker of CO exposure. For example, patients presenting with an exhaled CO concentration suggest CO exposure above what is expected in smokers, pointing to the need to search for CO sources of exposure different from smoking. However, some factors, such as gender and cigarettes smoked weekly, might influence the value of exhaled CO as a marker of exposure. Those factors should be considered when interpreting the results. Further research should consider additional factors related to smoking habits, such as type/brand of cigarettes, interval time between puffs and the depth of smoking. Moreover, the effect of the menstrual cycle, alveolar ventilation and total Hb mass on exhaled CO concentration and COHb half-life could be explored in the future.

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References

1. Rose, J.J.; Wang, L.; Xu, Q.; McTiernan, C.F.; Shiva, S.; Tejero, J.; Gladwin, M.T. Carbon Monoxide Poisoning: Pathogenesis, Management, and Future Directions of Therapy. *Am. J. Respir. Crit. Care Med.* **2017**, *195*, 596–606. [CrossRef]
2. Dolan, M.C. Carbon monoxide poisoning. *Can. Med. Assoc. J.* **1985**, *133*, 392–399.
3. Raub, J.A.; Mathieu-Nolf, M.; Hampson, N.B.; Thom, S.R. Carbon monoxide poisoning—A public health perspective. *Toxicology* **2000**, *145*, 1–14. [CrossRef]
4. Blumenthal, I. Carbon monoxide poisoning. *J. R. Soc. Med.* **2001**, *94*, 270–272.
5. Kao, L.W.; Nanagas, K.A. Carbon monoxide poisoning. *Emerg. Med. Clin. N. Am.* **2004**, *22*, 985–1018.
6. Weaver, L.K. Carbon Monoxide Poisoning. *N. Engl. J. Med.* **2009**, *360*, 1217–1225.
7. Hampson, N.B. U.S. Mortality Due to Carbon Monoxide Poisoning, 1999–2014. Accidental and Intentional Deaths. *Ann. Am. Thorac. Soc.* **2016**, *13*, 1768–1774.
8. Braubach, M.; Algoet, A.; Beaton, M.; Lauriou, S.; Héroux, M.-E.; Krzyzanowski, M. Mortality associated with exposure to carbon monoxide in WHO European Member States. *Indoor Air* **2013**, *23*, 115–125. [CrossRef]
9. Ernst, A.; Zibrak, J.D. Carbon monoxide poisoning. *N. Engl. J. Med.* **1998**, *339*, 1603–1608.
10. Pan, K.-T.; Leonardi, G.S.; Croxford, B. Factors Contributing to CO Uptake and Elimination in the Body: A Critical Review. *Int. J. Environ. Res. Public Health* **2020**, *17*, 528. [CrossRef]
11. Zavorsky, G.S.; Tesler, J.; Rucker, J.; Fedorko, L.; Duffin, J.; Fisher, J.A. Rates of carbon monoxide elimination in males and females. *Physiol. Rep.* **2014**, *2*, e12237. [CrossRef]
12. Coburn, R.F.; Forster, R.E.; Kane, P.B. Considerations of the physiological variables that determine the blood carboxy-hemoglobin concentration in man. *J. Clin. Investig.* **1965**, *44*, 1899–1910.
13. Gosselin, N.H.; Brunet, R.C.; Carrier, G. Determination of carboxyhaemoglobin in humans following low-level exposures to carbon monoxide. *Inhal. Toxicol.* **2009**, *21*, 1077–1091. [CrossRef]
14. Burney, R.E.; Wu, S.-C.; Nemiroff, M.J. Mass carbon monoxide poisoning: Clinical effects and results of treatment in 184 victims. *Ann. Emerg. Med.* **1982**, *11*, 394–399. [CrossRef]
15. Ozturan, I.U.; Yaka, E.; Suner, S.; Ozbek, A.E.; Alyesil, C.; Dogan, N.O.; Yilmaz, S.; Pekdemir, M. Determination of carboxyhemoglobin half-life in patients with carbon monoxide toxicity treated with high flow nasal cannula oxygen therapy. *Clin. Toxicol.* **2019**, *57*, 617–623. [CrossRef]
16. Cronenberger, C.; Mould, D.R.; Roethig, H.-J.; Sarkar, M. Population pharmacokinetic analysis of carboxyhaemoglobin concentrations in adult cigarette smokers. *Br. J. Clin. Pharmacol.* **2008**, *65*, 30–39. [CrossRef]
17. Tsigaris, P.; Silva, J.A.T.D. Smoking Prevalence and COVID-19 in Europe. *Nicotine Tob. Res.* **2020**, *22*, 1646–1649. [CrossRef]
18. Robinson, J.C.; Forbes, W.F. The Role of Carbon Monoxide in Cigarette Smoking. *Arch. Environ. Health Int. J.* **1975**, *30*, 425–434. [CrossRef]
19. Raub, J. *Environmental Health Criteria 213: Carbon Monoxide*; World Health Organization: Geneva, Switzerland, 1999.
20. Castleden, C.M.; Cole, P.V. Carboxyhaemoglobin levels of smokers and non-smokers working in the City of London. *Occup. Environ. Med.* **1975**, *32*, 115–118. [CrossRef]
21. Wald, N.J.; Idle, M.; Boreham, J.; Bailey, A. Carbon monoxide in breath in relation to smoking and carboxyhaemoglobin levels. *Thorax* **1981**, *36*, 366–369. [CrossRef]
22. Jarvis, M.; Russell, M.A.; Saloojee, Y. Expired air carbon monoxide: A simple breath test of tobacco smoke intake. *BMJ* **1980**, *281*, 484–485. [CrossRef]
23. Jarvis, M.; Belcher, M.; Vesey, C.; Hutchison, D.C. Low cost carbon monoxide monitors in smoking assessment. *Thorax* **1986**, *41*, 886–887. [CrossRef]
24. Maga, M.; Janik, M.K.; Wachsmann, A.; Chrząstek-Janik, O.; Koziej, M.; Bajkowski, M.; Maga, P.; Tyrak, K.; Wójcik, K.; Gregorczyk-Maga, I.; et al. Influence of air pollution on exhaled carbon monoxide levels in smokers and non-smokers. A prospective cross-sectional study. *Environ. Res.* **2017**, *152*, 496–502. [CrossRef]
25. Al-Sheyab, N.; Kheirallah, K.A.; Mangnall, L.J.T.; Gallagher, R. Agreement Between Exhaled Breath Carbon Monoxide Threshold Levels and Self-Reported Cigarette Smoking in a Sample of Male Adolescents in Jordan. *Int. J. Environ. Res. Public Health* **2015**, *12*, 841–854. [CrossRef]

26. Deveci, S.; Deveci, F.; Açik, Y.; Ozan, A. The measurement of exhaled carbon monoxide in healthy smokers and non-smokers. *Respir. Med.* **2004**, *98*, 551–556. [CrossRef]
27. Jarvis, M.; Tunstall-Pedoe, H.; Feyerabend, C.; Vesey, C.; Saloojee, Y. Comparison of tests used to distinguish smokers from nonsmokers. *Am. J. Public Health* **1987**, *77*, 1435–1438. [CrossRef]
28. Ghorbani, R.; Blomberg, A.; Schmidt, F.M. Impact of breath sampling on exhaled carbon monoxide. *J. Breath Res.* **2020**, *14*, 047105. [CrossRef]
29. Chatrchaiwiwatana, S.; Ratanasiri, A. Exhaled Carbon Monoxide Levels among Tobacco Smokers by Age. *Southeast Asian J. Trop. Med. Public Health* **2017**, *48*, 429–437.
30. Sargent, J.D.; Dalton, M. Does Parental Disapproval of Smoking Prevent Adolescents from Becoming Established Smokers? *Pediatrics* **2001**, *108*, 1256–1262. [CrossRef]
31. Starr, G.; Rogers, T.; Schooley, M.; Porter, S.; Wiesen, E.; Jamison, N. *Key Outcome Indicators for Evaluating Comprehensive Tobacco Control Programs, in Preventing Initiation of Tobacco Use Among Young People*; Centers for Disease Control and Prevention: Atlanta, GA, USA, 2005.
32. Biener, L.; Albers, A.B. Young Adults: Vulnerable New Targets of Tobacco Marketing. *Am. J. Public Health* **2004**, *94*, 326–330. [CrossRef]
33. Husten, C.G. How should we define light or intermittent smoking? Does it matter? *Nicotine Tob. Res.* **2009**, *11*, 111–121. [CrossRef]
34. Warner, D.O. Preoperative Smoking Cessation: The Role of the Primary Care Provider. *Mayo Clin. Proc.* **2005**, *80*, 252–258. [CrossRef]
35. Najeeb, H.M. Lung Diffusing Capacity for Carbon Monoxide (DLco-SB): The Influence of Cigarette Smoking. *Iraqi Postgrad. Med. J.* **2010**, *9*, 328–334.
36. Weaver, L.K.; Howe, S.; Hopkins, R.; Chan, K.J. Carboxyhemoglobin half-life in carbon monoxide-poisoned patients treated with 100% oxygen at atmospheric pressure. *Chest* **2000**, *117*, 801–808.
37. Bowdish, D.M.E. The Aging Lung: Is Lung Health Good Health for Older Adults? *Chest* **2019**, *155*, 391–400.
38. Windsor-Shellard, B.; Horton, M.; Scanlon, S.; Manders, B. *Adult Smoking Habits in the UK, in Cigarette Smoking among Adults Including the Proportion of People Who Smoke Including Demographic Breakdowns, Changes over Time, and e-Cigarettes*; Office for National Statistics and Public Health England: London, UK, 2019.
39. Vogt, T.M.; Selvin, S.; Hulley, S.B. Comparison of biochemical and questionnaire estimates of tobacco exposure. *Prev. Med.* **1979**, *8*, 23–33. [CrossRef]
40. Weinhold, L.L.; Stitzer, M.L. Effects of puff number and puff spacing on carbon monoxide exposure from commercial brand cigarettes. *Pharmacol. Biochem. Behav.* **1989**, *33*, 853–858. [CrossRef]
41. Strasser, A.A.; Lerman, C.; Sanborn, P.M.; Pickworth, W.B.; Feldman, E.A. New lower nicotine cigarettes can produce compensatory smoking and increased carbon monoxide exposure. *Drug Alcohol Depend.* **2007**, *86*, 294–300. [CrossRef]
42. Muhammad-Kah, R.; Liang, Q.; Frost-Pineda, K.; Mendes, P.E.; Roethig, H.J.; Sarkar, M. Factors affecting exposure to nicotine and carbon monoxide in adult cigarette smokers. *Regul. Toxicol. Pharmacol.* **2011**, *61*, 129–136. [CrossRef]
43. Zhang, Q.; Li, L.; Smith, M.; Guo, Y.; Whitlock, G.; Bian, Z.; Kurmi, O.; Collins, R.; Chen, J.; Lv, S.; et al. Exhaled carbon monoxide and its associations with smoking, indoor household air pollution and chronic respiratory diseases among 512 000 Chinese adults. *Int. J. Epidemiol.* **2013**, *42*, 1464–1475. [CrossRef]
44. Laugesen, M.; Epton, M.; Frampton, C.M.; Glover, M.; Lea, R.A. Hand-rolled cigarette smoking patterns compared with factory-made cigarette smoking in New Zealand men. *BMC Public Health* **2009**, *9*, 194. [CrossRef]
45. Prockop, L.D.; Chichkova, R.I. Carbon monoxide intoxication: An updated review. *J. Neurol. Sci.* **2007**, *262*, 122–130. [CrossRef]
46. Antczak, A.; Ciebiada, M.; Kharitonov, S.A.; Gorski, P.; Barnes, P.J. Inflammatory Markers: Exhaled Nitric Oxide and Carbon Monoxide During the Ovarian Cycle. *Inflammation* **2012**, *35*, 554–559. [CrossRef]
47. Frans, A.; Stănescu, D.C.; Veriter, C.; Clerbaux, T.; Brasseur, L. Smoking and pulmonary diffusing capacity. *Scand. J. Respir. Dis.* **1975**, *56*, 165–183.
48. Van Ganse, W.F.; Ferris, B.G.; Cotes, J.E. Cigarette smoking and pulmonary diffusing capacity. (Transfer factor). *Am. Rev. Respir. Dis.* **1972**, *105*, 30–41. [CrossRef]
49. Pace, N.; Strajman, E.; Walker, E.L. Acceleration of Carbon Monoxide Elimination in Man by High Pressure Oxygen. *Science* **1950**, *111*, 652–654. [CrossRef]