



UNIVERSITE CATHOLIQUE DE LOUVAIN

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EARLY CARE MANAGEMENT AFTER
CARBON MONOXIDE POISONING BASED ON
CARBOXYHEMOGLOBIN TOXICOKINETIC

ON NON-INVASIVE VENTILATION AFTER
ACUTE CARBON MONOXIDE EXPOSURE
ON A SWINE MODEL

A dissertation submitted to the *Université catholique de Louvain* in fulfilment of
the requirement for the degree of Doctor of Medical Science

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APPENDIX: SUPPLEMENTAL DATA

EMERGENCE OF THIS THESIS

The idea for this thesis was born in 2013 of several sources. First, innovations in recent years have contributed significantly to the improvement of therapeutic options for patients with acute respiratory failure - including the improvement of ventilation devices. Prof. F. Thys wrote the first thesis on Non-Invasive Positive Pressure Ventilation (NiPPV) in emergency care in 2003, and shared his knowledge, taking me under his wing in the emergency care unit in Cliniques Universitaires Saint-Luc.

Secondly, I often met Dr Georges Boussignac , an inventor of medical devices with particular patents for his famous CPAP (Continuous Positive Airway Pressure as 'CPAP Boussignac®' or CPAPb) and his Vy-Life® (NiPPV) (Vygon©, France, Ecoen). After validation of his CPAPb for acute pulmonary edema (APE) and parallel NiPPV (Non-Invasive Positive Pressure Ventilation) for chronic obstructive pulmonary disease (COPD) decompensation, we discussed at length other potential medical indices and developed a productive friendship. This new NiPPV ventilation standards mode enhanced ventilation, alveolar recruitment and oxygen supply. Then, CO elimination occurring exclusively by way of lungs, we considered assessing them for the treatment of CO poisoning. Dr Boussignac passed away in 2020, and I had pleasure of presenting an on-line account of his rich life and the intellectual approach behind his patents.

Thirdly, at the same time, a few interested medical practitioners around the world began to propose CPAP treatment for acute CO exposure - but only as case reports[1, 2] or without peer-reviewing[3]. At this time, I was a resident in emergency and pre-hospital care, interested in fauna, flora and environmental toxicity, while simultaneously beginning (2011-2012), a master's in biomedical

science with toxicology orientation, supervised by Prof. Ph. Hantson and Prof. P. Wallemacq (SBIM, UCL). In the course of this I was regularly confronted by a wide range of opinions regarding care or HBO eligibility (proposed by hyperbarist colleagues) in cases of acute CO exposure. I decided to go deeper into this topic myself, discovering in the process a degree of scientific ignorance, particularly as regards potential oxygen toxicity[4]. Thus, linking all these areas, the idea emerged of using Non-invasive ventilation to treat acute CO exposure.

This thesis would not have been possible without the remarkable co-operation of the scientific communities at Cliniques Universitaires Saint-Luc (CUSL) and Université catholique de Louvain (UCL), from professors and biostatisticians to the CHEX laboratory and the St-Luc Foundation, communities dedicated to both promoting better care for patients and to encouraging innovatory treatments. CUSL offers a climate of genuine intellectual excitement, with nurses voluntarily assisting us in and out of the laboratory after their clinical work.

To prove our hypothesis, we opted, for ethical reasons, to develop a laboratory protocol with swine. The Saint-Luc Foundation, Vygon© company and CHEX largely supported the initial experiments. Originally started as a four-year project alongside full-time clinical activity, this evolved into a PhD, with Prof. Dr. Philippe Hantson as the main supervisor and Prof. Dr. Bruno Megarbane as co-promoter. CUSL and the emergency team offered me time off to compose two of the manuscripts. Ethical Committee approval was obtained, an animal laboratory certificate was validated, and the required data collection was made possible through delocalized blood analyser devices generously provided by the Radiometer© company. Experiments began in 2013 and 2014 after research with a plastic artist, Mis Genevieve Periat, who was commissioned to build a special hermetic swine-like latex face mask (Fig. 1). In 2015, we discussed and analysed data with a biostatistician who had a specialised interest in matters toxicokinetic.

Over the two years, we wrote up our two systematic and meta analyse reviews. Research was slowed by a long-planned 2017 project to travel *en famille et en camion* from Brussels to New Caledonia. Nonetheless, I was proud to retain sufficient motivation to dispatch, a year and 40,000 kilometres later, the *Principes* article from the base of Mount Fuji. Stranded by the Covid phenomenon in New Caledonia, (probably the only Covid-free territory of our first 18 months) we were able to publish further systematic reviews from Noumea in 2019 and 2020. We submitted more publications in 2021 from the island of Lifou (Loyalty Islands province) where the thesis was in part concluded. We took advantage of circumstances to alert the native population to the dangers of CO exposure in their traditional tribal houses, and to discuss with them the benefit or threat of the pharmacological properties of fauna and flora. It took periods of hard work and seclusion in Lifou to be able to concentrate on this project. These periods alternated with moments when my professional clinical activities were disrupted by the emergence of Covid-19 in September 2021. Over the last year I have increasingly focused on PhD activity, culminating in today's presentation of this thesis for the degree of Doctor in Science Health.

This thesis is humbly dedicated to the teachers who spent time sharing their knowledge of the certainties and uncertainties of science. They know who they are.

I will end with special thanks to my thesis committee, who have had the kindness, the accessibility, and the patience to accompany me on this long journey.

LIST OF ABBREVIATIONS

AA	Ambiant Air or Atmospheric Air
Atm	Atmosphere
ACLS	Advanced cardiac life support
ACEP	American College of Emergency Physicians
APE	Acute pulmonary edema
ARF	Acute respiratory failure
ATP	Adenosine triphosphate
AUC	Aera under the curve
BIC	Bayesian information criterion
BiPAP	Bilevel positive airway pressure
CcO	Cytochrome C oxydase
CFK(E)	Coburn-Forster-Kane equation
CO₂	Carbon dioxide
CO	Carbon monoxide
CA	Cardiac arrest
CHEX	Experimental Surgery and Transplantation Laboratory
COHb	Carboxyhemoglobin
CO-induced CA	Cardiac arrest following CO poisoning
CA-COP	Cardiac arrest following CO poisoning
COHbt_{1/2}	Carbon monoxide elimination half-life
COMb	Carboxymyoglobin
COPD	Chronic obstructive pulmonary disease
CPAP	Continuous positive airway pressure
CPAPb	CPAP Boussignac device

CPR	Cardiopulmonary resuscitation
DL_{co}	CO diffusion capacity of the lung
DNS	Delayed neurological syndrome
Dm_{co}	CO diffusion coefficients in muscle
ECLS	Extra corporeal life support
ECMO	Extra corporeal membrane oxygenation
ECHM	European Committee for Hyperbaric Medicine
ED	Emergency department
ERC	European Resuscitation Council
HBO	Hyperbaric oxygen
HFCN	High-flow nasal cannula
IPAP	Inspiratory positive airway pressure
EtCO₂	End-tidal CO ₂
EPAP	Expiratory positive airway pressure
FiO₂	Inspired oxygen fraction
Hb	Hemoglobin
HBO(T)	Hyperbaric oxygen(therapy)
H₂O₂	Hydrogen peroxide
Hemo-CD	Synthetic hemoprotein complex
HO	Heme oxygenase
H₂S	Hydrogen sulfide
ICU	Intensive care unit
K_a	Association constant
KCE	Belgian Health Care Knowledge Centre
K_d	Dissociation constant
K_{Eq}	Equilibrium constant
LOC	Lost of consciousness.
Mb	Myoglobin

MV	Mechanical ventilation
Nb	Neuroglobin
NBO(T)	Normobaric oxygen (therapy)
NIV	Non-invasive ventilation
NiPPV	Non-invasive positive pressure ventilation
NiPPV-V or Vy	Non-invasive positive pressure ventilation VYGON [®]
NiPPV-L or Leg	Non-invasive positive pressure ventilation Legendair [®]
NO	Nitric (mono)oxide
NRM	Non-rebreathing mask
OH	Hydroxyl radical
O₂	Oxygen
O₂Hb	Oxyhemoglobin
PaCO₂	Arterial partial pressure of carbon dioxide
P_{co}	Partial pressure of carbon monoxide
PO₂	Partial pressure of oxygen
PEP	Positive expiratory pressure
PEEP	Positive end expiratory pressure
PS(V)	Pressure support (ventilation)
ROS	Reactive oxygen species
ROSC	Return of spontaneous circulation
RR	Respiratory rate
SD	Standard deviation
SFMU	Société Française de Médecine d'Urgence
SpO₂	Transcutaneous SaO ₂ measured from a pulse oxymeter
SaO₂	Arterial oxygen saturation
SpCO[®]	SpCO is a continuous and non-invasive measurement of the carbon monoxide levels in arterial blood.
SMUR	Extrahospital emergency medical team

TK	Toxicokinetic
UHMS	Undersea and Hyperbaric Medical Society

EXECUTIVE SUMMARY

Carbon monoxide (CO) is a colorless, odorless, non-irritating gas produced by the incomplete combustion of hydrocarbons. CO poisoning from exogenous exposition is probably born with volcanoes and fire domestication in closed places for cooking including smoking food technic, de-worming primitive tribal houses and heating. After, from Greeks to Romans, using coal in terms, in Indian's tipis to smoke meat for conservative reasons or later, the use of lighting gas containing a part of pure CO before any combustion or gases combustion in industrialized countries since the end of the 18th century, the story of the world in all civilizations has had probably to accommodate of this gas and this story is far from over.

CO is the main cause of accidental, intentional, and fired related poisoning deaths over the world. More specifically, for the last twenty years, there are annually, ± 50.000 emergency department visits (ED) in United States, ± 5.000 in France and ± 1.300 in Belgium (Fig1). The overall mortality seems not modified by any treatment, with approximately 3% fatalities for medically treated CO poisoning and from 2.6 to 3.9% fatalities with the use of hyperbaric oxygen therapy (HBO). Up to 40% of the survivors will suffer from permanent or transient neurocognitive and/or affective disorders named Delayed Neurological Syndrome (DNS).

CARBON MONOXIDE			
	US	France	Belgium
» (ED) visits/year	50,000	5,000	1,308
» Accidental exposure	±17,500	±3,000	±652
/10 ⁵ population	7,0	4,5	5,58
» COP deaths	1,555	150	33
/10 ⁵ population	0,52	0,16	0,29

Fig 1: Annually emergency department (ED) visits for accidental, intentional and fire-related CO poisoning. For the recent years, accidental exposure reported and, /10⁵ population. CO poisoning deaths (non-fired related) declined from 1999 to 2014, averaging annually 1.555 (US) (1245 in 2014), +-150 (F) (78 in 2008), <33 (B) (11 in 2017) and /10⁵ population.

Despite 150 years of research on CO, physiopathology understanding remains partial which leads to controversy on treatment strategy. Up to now, oxygen therapy seems to be the key stone of the treatment but studies on doses and means to provide it failed to fix strong evidence to avoid DNS. Moreover, for years studies highlighted the oxygen potential toxicity. Therefore clinicians and physiologists continue to investigate other ways to treat acute CO poisoning and we have decided to play a part in this process.

While for methodological reasons, clinical studies struggle to demonstrate the effectiveness of one treatment over another, we decided to study the experimental and toxicokinetic side of CO poisoning to provide objective answers in order to improve the management of these intoxications in the first hours of care. This thesis discusses the toxicokinetic aspect of carboxyhemoglobin after acute CO

exposure including new ventilatory treatment. For ethic reasons, needing to control a maximum of parameters during CO exposure, we focused on swine model. Our first aim was to respond to important following: Is Non-Invasive Ventilation efficient on CO elimination compared to standard treatment? In view to discuss extrapolation from our swine data to human, we try to provide elements to answer the following questions: Is affinity of hemoglobin for CO compared to O₂ comparable between swine and human? Secondly, CO also binding to myoglobin, muscles could be a CO storage which could explain in part some toxicity process and heterogeneity in CO poisoning severity which is not always clinically apparent. Unfortunately, this storage is unevaluable in clinical practice. So, extended our experimental procedure we want to find indirect arguments to investigate this storage as to observe a bi-compartmental COHb elimination profile and/or a COHb rebound after treatment stopped. For those, we have first to demonstrate if COHbt_{1/2} profile fit the best with a mono- or bi-compartmental model to resolve the controversy on this point? It is also the starting point before any evaluation of COHbt_{1/2} toxicokinetic interest as a potential CO poisoning severity criterion. The aim being to improve a more homogenous distribution of patients in studies comparing treatment strategy? Second, once COHb backed to 5% after treatment, if a significant rebound occurred, it could result from a CO storage release from extravascular tissue. Moreover, considering patient discharge, if COHb rebound from muscles compartment is observed, it could encourage to ongoing oxygen treatment duration? At the end, we had the opportunity to observe a very short unexpected COHb elimination during a resuscitation on CO poisoning, highlighted by all our thesis discussions, we published a case report where we discoursed about the origins of this surprising result.

In order to have the data required to answer these research questions, first, an interventional prospective animal study on nine swine including 21 experiments, was set up in the Department of Health Sciences, Institute for Experimental and Clinical, Experimental Surgery and Transplantation (CHEX), Université catholique de Louvain, Brussels, Belgium. We assessed data with Integrated Pharmacometrics, pharmacogenomics and pharmacokinetics (PMGK) research group Louvain Drug Research Institute (LDRI) Université catholique de Louvain, Brussels, Belgium. On the same experimental procedure, we investigated separately the efficiency of CPAP and NiPPV on COHbt_{1/2}, the global COHb elimination profile being mono- or bi-compartmental and the potential COHb rebound. Second, we set up the first systematic review and meta-analyse on Hb affinity constant for CO compared to O₂ for human and in the animal world. Focusing on this COHb elimination during the thesis process, we were concerned by an unexpected COHb elimination observed during a cardiac arrest on CO resuscitation.

Thus, we have endeavoured to formulate an answer to the following five research topics:

Topic 1

Does new ventilatory or support device used and available in emergency department from 2004 to treat COPD decompensation or APE, as called NiPPV or CPAP, improve the elimination of COHb during the first hours after CO exposure?

We compared treatment to non-intervention and to standard treatment with (NRM). Which treatment has the higher impact to shorten COHb half-life (COHbt_{1/2}) after mild to moderate CO poisoning? We have determined that, on swine, Pressure Support Ventilation (PSV) simulating NiPPV, could be effective to shorten COHbt_{1/2} compared to classical NRM. The closed PSV-Leg system (NiPPV-Leg) gave

the shortest COHbt_{1/2} (median 58 vs. 85min with NRM) and the lowest AUC for COHb (median 63 vs. 79min with NRM). The results of this pilot study suggest that non-invasive ventilation, such as the closed PSV-Leg system, can be more efficient than NRM at eliminating CO when HBO is unavailable or non-indicated and supports the design of a clinical study to evaluate this hypothesis.

Topic 2

Does the COHb toxicokinetic fits with a mono- or bi-compartmental model?

Controversy exists between human retrospective clinical study and animal prospective experimental study in favour of mono- or bi-compartmental model respectively. Concept of bi-compartmental model is in accordance with a CO body burden in the extravascular compartment and could afford to estimate more accurately the severity of the intoxication. First, in our Princeps study we assessed toxicokinetic with a mono-compartmental model to be compared to COHbt_{1/2} from clinical study. In our second study on toxicokinetic, we refined the assessment's toxicokinetic which fitted with a bi-compartmental model according with another experimental study and with mathematical model and, in opposite with clinical study. Moreover, this bi-compartmental model confirmed our first result using a mono-compartmental model on the efficiency of ventilation with NiPPV-Leg (PSV-Leg). Moreover, multiple toxicokinetic approaches highlighted NiPPV effects on COHbt_{1/2} α - and β -phase.

Topic 3

A bi-compartmental model expressed a distribution in multiple compartments in addition to hemoglobin compartment. CO bind all metalloprotein as hemoglobin but also myoglobin representing 40% of the body weight. **When COHb backed to**

infra-clinic level at 5% after treatment, is it a COHb rebound from other compartments with potential new intoxication? We demonstrated that COHb after stopping treatment ceased to decrease and even increased lightly. This could be also in favour of multi-compartmental model and might suggested to ongoing normobaric oxygen therapy up to 6 to 12 hours. However, we remain cautious about the interpretation of this rebound and its potential clinical impact. Its magnitude should also be confronted with other experimental or clinical protocols.

Topic 4

From 1895 to 2016, estimations of M ranging from 140 to more than 400 were published. Given that COHb rates vary directly in relation to M , using a wide variable M value may introduce a margin for error in determining COHb. In recent decades, researchers considered M values ranging from 210 to 245, but in most instances without justifying their choice. **Which is the current value of human relative affinity for hemoglobin for CO compared to O₂ (M-value) ?** Our meta-analysis and systematic review provided up-to-date data on relative affinity value reference in human at physiological conditions for adult and for fetal life. A M-value at 37°C (M_{37}) of 218±8 is confirmed in humans at any pH and under physiological M conditions for adult hemoglobin (HbA), higher than for fetal hemoglobin (HbF) at 175. This value should become the reference when carbon monoxide is investigated. We also considered critical questions of temperature and pH. Moreover, **which is the current value of swine relative affinity for hemoglobin for CO compared to O₂ (M-value) to be able to compare to human and to animal world?** We performed the largest review of M-values collecting data in the animal world. In the different mammals explored, M_{37} is between 109 and 215 under physiological conditions. Sheep are the most studied species but far less commonly

at a physiological temperature. There is also some intraspecies variability, especially among mice. Our analysis did not provide evidence for the existence of a species-specific Hb that would approximate the human M-value sufficiently enough to propose it as a replacement for human blood in relevant or future studies. Only two articles investigated M for swine, both without mentioning the pH. In the first, the M_{15} was 220, and in the second, the M_{35-40} was 130.

Topic 5

Aware about toxicokinetic CO topic, we managed a CO cardiac arrest and observed an unexpected $COHbt_{1/2}$ during cardiopulmonary resuscitation which was declined as a case report. $COHbt_{1/2}$ found was 40.3min while on HBO therapy, the $COHbt_{1/2}$ is estimated to be around 23.3 min or even >43min. The mortality rate of CO-induced CA may be as high as 100%, thus, we found important to pay attention to toxicokinetic understanding to discuss on care management to improve prognosis. Considering the lack of clinical evidence for HBO, the lack of treatment guidelines on the subgroup of CO-induced CA, the complex access to HBO facilities in these extreme cases, **we discussed our unexpected short $COHbt_{1/2}$ during cardiopulmonary resuscitation found based on our COHb toxicokinetic reviewing.**

We have demonstrated the relevance of these new findings with four illustrations or recommendations:

Firstly, we were able to propose PSV (NiPPV) for clinical study for ethical approbation. Furthermore, we discouraged the use of CPAPb to treat CO poisoning. In case of only NRM applying, we confirmed the necessity to optimize the FiO_2 managing the leak around the mask to maximize $\text{COHbt}_{1/2}$.

Secondly, human M value is 218 and should become the reference in CO studies. M value on swine is found at 130 but seems to be insufficiently studied at physiological conditions. Our analysis did not provide evidence for the existence of a species-specific Hb that would approximate the human M-value sufficiently enough to propose it as a replacement for human blood in relevant or future studies.

Thirdly, we confirmed that toxicokinetic clinical or experimental studies on COHb should be assessed with bi-compartmental model. Bi-compartmental toxicokinetic and COHb rebound fit with an extravascular CO storage, probably in muscles. Rebound seems to be limited in our protocol but, waiting for further studies, in case of no residual symptoms, it could be better to discharge patient when COHb backed to 5% and after 6 to 12 hours of NBO depending on CO exposure time and CO exposure level estimation. In case of imprecision, a better $\text{COHbt}_{1/2}$ estimation case by case using bi-compartmental model (based on several samples and including early ones from extra hospital first care) could reflect the severity of the acute CO poisoning. This opens up new fields of investigation. We argue that this $\text{COHbt}_{1/2}$ criterion has to be assess in future prospective studies as the reflect of CO poisoning severity criteria potentially allowing to homogenise cohorts in studies comparing treatment effectiveness.

Finally, scientific societies having to homogenize protocol for ACR CO, we encourage multi assessment of COHb if possible during resuscitation to confirm our unexpected COHbt_{1/2} and to guide management case by case, independently of the decision to use HBO secondary.

These illustrations demonstrate that gaining more insight in CO toxicokinetic understanding after CO exposure could be necessary to guide treatment management. And importantly, this will lead to new ethical acceptable investigations on non-invasive ventilation and/or on treatment duration to treat acute CO exposure. We argue that COHbt_{1/2} studies are one of the paths to question global CO elimination and to reach the ultimate goal of research on CO which is to prevent or decrease Delayed Neurologic Syndromes (DNS).

CHAPTER 1

BACKGROUND

AIMS AND HYPOTHESES

MATERIALS AND METHODS

INTRODUCTION

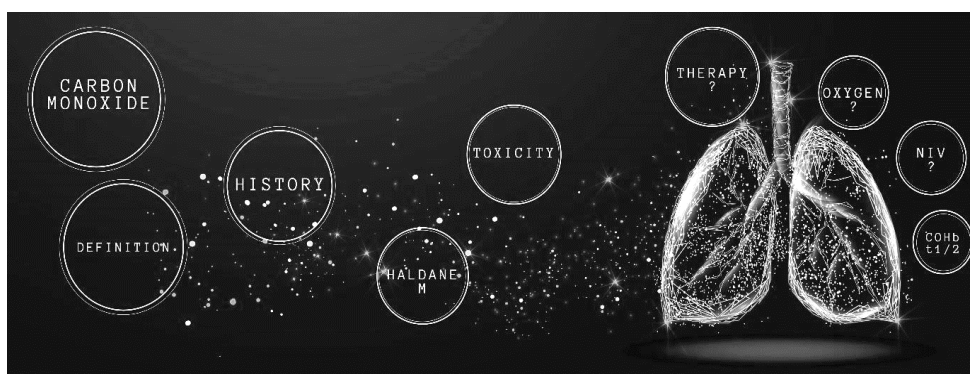
Carbon monoxide (CO) is a colorless, odorless, non-irritating gas produced by the incomplete combustion of hydrocarbons. CO poisoning is the main cause of unintentional, intentional and fire-fume poisoning deaths over the world. The overall mortality is not modified by any treatment, with approximately 3% fatalities for medically treated CO poisoning and from 2.6 to 3.9% fatalities with the use of hyperbaric oxygen therapy (HBO). Up to 40% of the survivors will suffer from permanent or transient neurocognitive and/or affective disorders. Even with current therapy, 15–40% of patients will experience long-term neurocognitive sequelae which could be delayed from 3 to 6 weeks after the CO poisoning [1]. Despite abundant literature on the mechanisms of injury of CO poisoning, the pathophysiology explaining this delayed neurological syndrome (DNS) still remains imperfectly understood. Moreover, accidental, non-fire-related CO poisoning in the US appears to account for over \$1.3 billion in acute medical expenses and lost earnings annually [2].

At present, waiting for a specific antidote which has been recently developed using animals, oxygen (O₂) seems to be the cornerstone of the treatment, but doses, means of administration and protocols are still more deductive than evidence-based [3]. Current evidence only shows that O₂ therapy decreases blood carbon monoxide half-life elimination (COHbt_{1/2}) but with variable influence on neurological outcome. Otherwise, normobaric oxygen therapy (NBO) and hyperbaric oxygen therapy (HBO), although described as the reference treatment, are compared in meta-analyses of seven *randomized* control trials and fail to demonstrate a net benefit for HBO. Even in positive studies, neurological

impairment, although improved, remains substantive [4, 5]. There is clearly an unmet clinical need for better therapies for CO poisoning. In the worst hypothesis, HBO could even be deleterious [6]. Despite the clear effectiveness of HBO for some authors, there still exists a substantial portion of survivors with permanent neurocognitive and affective sequelae (estimated 10.000–20.000 new cases per year). This illustrates the need for investigation of new therapies [7, 8]. Furthermore, at this time, no specific biomarker or clinical variables seem to identify poisoned patients for whom HBO was most likely to provide benefit [9]. Thus, the first question is how to foster COHb elimination in another way, if possible, with techniques or devices currently available, familiar in emergency rooms and ambulances, and, avoiding potential O₂ toxicity. Secondly, carboxyhemoglobin (COHb) level not being a determinant factor in predicting the severity of CO poisoning, it will be necessary to find other (bio)marker, clinical or toxicokinetic variables which could predict more accurately the severity of CO poisoning to assess treatment protocols' effectiveness as NBO, HBO or further antidotes. The main aim of this thesis is to assess the impact of new therapies on COHb_{t1/2}, with reflections on CO body storage distribution and elimination which could have direct clinical impact on our practice in an emergency care context.

Publishing a CO review in 1950, Lilienthal thanked Mrs. Mary Crawford Folk for giving him access to her meticulous collection, already considerable for the time, of over 3000 references on CO [10]. This illustrates the abundant literature on CO, and in part, the complexity and time-consuming nature of this thesis.

ORGANIZATION OF THIS CHAPTER



We begin with **CARBON MONOXIDE DEFINITION** and describe the importance of CO in the world's history. We are aware of CO's ubiquity in a wide field of contexts, from the domestic to the industrial (eg. food and aeronautics), from smokers to occupational activities. Humanity has had to cope with this natural environmental risk since the beginning of time. Then we turn to **CARBON MONOXIDE DISCOVERY** which will lead us directly to the concept of the Haldane Constant to which we have devoted two studies in this thesis.

In the following section, the **HALDANE CONSTANT** is described: the main feature of CO toxicity is to bind metalloproteins as hemoglobin to form carboxyhemoglobin (COHb). Haldane in 1897 proposed a fundamental equation to link various parameters explaining COHb level at equilibrium. He proposed a M constant as the affinity of hemoglobin for CO compared to O₂ being 300 times more important, varying in further literature from 140 to 400. This affinity point of view is essential for the understanding of CO elimination in different O₂ therapy or ventilation mode modalities or CO scavenger antidotes proposed in recent studies. This equation is

still relevant today and is still used in the more complex equations of recent mathematical models which we'll be addressing later.

Following this, we review fundamental **CARBON MONOXIDE TOXICITY MECHANISMS** and the clinical severity impact of CO categorised as light, moderate or severe. The ultimate goal of all studies or treatments investigated in the past has been to attempt to predict and/or avoid delayed neurological syndrome (DNS). Up to this point, this goal has not been achieved.

Thus, after an essential reminder of CO absorption and distribution in the body, we introduce the main topic assessed in this thesis: **CHRONOLOGY OF CARBON MONOXIDE ELIMINATION STUDIES** (or COHb half-life or COHbt_{1/2}). Reviewing historical key-points, we summarize wide data on COHb elimination to understand the historical relevance of different treatments used in cases of CO poisoning, and why COHbt_{1/2} discrepancies exist in studies. The historical context explains the intense debate around effective therapy. So, we report in **CURRENT CARBON MONOXIDE TREATMENT** current recommendations for treating CO intoxication proposed by national or international scientific societies pending more conclusive studies. This review highlights the importance of investigating accurately COHb toxicokinetic and our proposal to use Non-invasive ventilation (NIV).

In subsequent paragraphs, to understand our main hypothesis we approach the emergence of **NON-INVASIVE VENTILATION** and the modes concerned in our protocol. We will define the terminology used to avoid confusion. The devices used will be described in the relevant chapter.

In **AIMS AND HYPOTHESES**, after having reviewed all background specifically relating to this thesis in this chapter, we summarize the relevance of each question addressed here, and synthesize what is currently known and not known in each topic concerned.

The main '*RESEARCH QUESTION*' and four inter-correlated secondary questions are formulated to address each of these hypotheses.

At the end, we provide explanations on **MATERIAL AND METHODS** to investigate each area of research's questions, and to explain the selection of the swine model.

This chapter concludes with a description of the **STRUCTURE OF THE THESIS** and the **SOURCES OF FINANCIAL SUPPORT**.

1. BACKGROUND ON CARBON MONOXIDE

1.1. CARBON MONOXIDE DEFINITION

Carbon monoxide (CO) is a diatomic gaseous molecule previously named carbon oxide (Fig.1).

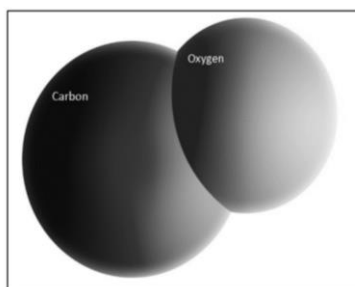


Fig. 1: carbon monoxide molecule

The Earth's earliest prebiotic atmosphere included the trio of gas transmitters carbon monoxide (CO), nitric oxide (NO), and hydrogen sulfide (H₂S), with which all of life has co-evolved [11]. It is estimated that 10% of carbon throughout the cosmos is in the form of CO. Beyond earth, CO is the second-most common diatomic molecule in the interstellar medium, after molecular hydrogen. The nucleus of Halley's comet for example contains about 15% frozen CO [12].

In the body, endogenous CO is produced at physiological rate and is beneficial as a neurotransmitter in the brain and peripheral autonomic nervous system. But it is also a poison when high-enough quantities are inhaled from exogenous source [13, 14].

“Poison is in everything, and nothing is without poison.

The dosage makes it either a poison or a remedy.”

Paracelsus 1493-1541, considered the first pharmacist.

1.1.1. Endogenous carbon monoxide

Humans have been continuously physiologically exposed to small amount of CO endogenously produced in the body from the normal catabolism of hemoglobin (Hb) [15-18] (Fig.2).

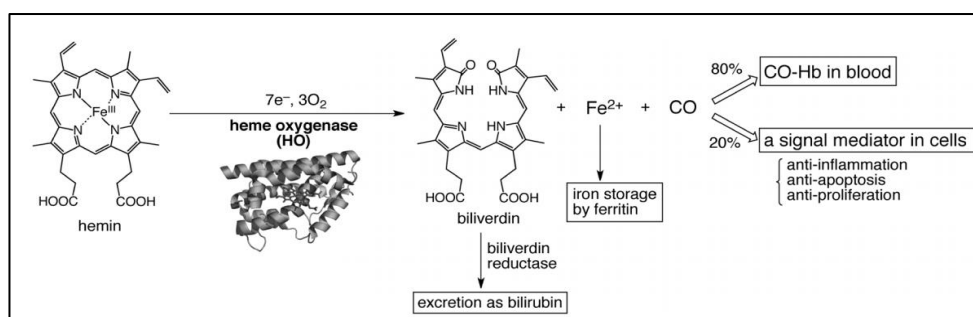


Fig. 2. Endogenous production of CO in mammalian cells and its physiological functions. Free heme is immediately decomposed by an heme oxygenase (HO) enzyme to produce iron, biliverdin, and CO in the cells. Most of the endogenous CO produced will circulate in the blood as COHb [19, 20]. The other endogenous CO might function as a gaseous messenger in the intracellular signalling pathways [21]. In addition, CO has unique protective effects in cells, such as anti-inflammation, anti-apoptosis, and anti-proliferation [21-25].

In a healthy male at rest, the average rate of **endogenous CO** production is close to 0.4ml/h (10ml a day) [16, 26]. In a healthy female at rest during progesterone phase of the menstrual cycle, endogenous CO production is double that of the estrogenic phase [27]. Due to the strong affinity of CO for Hb, this small endogenous CO production results in a physiological basal COHb rate from 0.4 to 0.8% [13]. Endogenous CO regulation and its physiological role existed probably before the discovery of fire - and clearly before industrialisation which greatly intensified multiply exogenous CO poisoning. Hemoglobin has then a very old role, long unrecognized, as a predominant CO scavenging system in vivo, preventing accumulation of excess endogenous CO in tissues to transport it easily to alveolar to be eliminated [28].

Despite its notorious toxicity, CO itself is relatively unreactive compared to molecular oxygen (O_2), nitric oxide (NO), hydrogen sulfide (H_2S), and reactive oxygen species (ROS) such as superoxide ($O_2^{\bullet-}$), hydroxyl radical ($\bullet OH$), hydrogen peroxide (H_2O_2), and singlet O_2 . Although other small molecules show various adverse reactivities towards proteins, lipids, and nucleic acids, the only recognized reaction of CO in the biological system is the binding to low valent metal ions, mostly ferrous heme but also copper and cobalt [28-30].

1.1.2. Exogenous carbon monoxide

Exogenous CO is toxic over a certain threshold. Its production results from the incomplete combustion of carbon-containing molecules such as wood, charcoal, petroleum, tobacco, gases...[1]. Because complete combustion is seldom attained, or if O_2 rate is insufficient, varying concentrations of CO can be expected to be produced in most combustion processes. This is well illustrated by the varying

colours of cooking flames: usually blue, they can become yellow in cases of incomplete combustion, then producing CO.

Exogenous CO can be broadly traced from prehistoric origins and mythological beliefs, with a rudimentary understanding emerging in the Middle Ages. Humans have maintained a complex relationship with CO since first learning to control fire or how to escape volcanic vapour. Early cavemen probably discovered the toxicity of CO after they introduced fire into their habitation for cooking or heating. Over time, humanity has tamed CO, through the metallurgical processes that emerged during the Bronze age, building tribal houses, creating thermal baths, smoking food...[11]. Since the end of the 19th century, with the proliferation of mining, internal combustion engines, the use of gas or coal for heating, the use of 'home lighting' gas containing between 6 and 15% CO (before any combustion, gradually replaced by natural gas), and finally, any incomplete combustion of petroleum, have largely contributed to the accelerating incidence of CO. It is the most common type of chemical poisoning, with an increasing risk of death. This is why scientific literature on CO has long been very prolific.

CO concentration in atmospheric pollution is considered at 0.1 to 0.2 ppm, while cigarette smoke contains 5.000 times more. When smoking, the tobacco smoker is exposed to CO concentrations of around 400–500 ppm and experience a higher COHb concentration than a non-smoker. COHb concentration being usually less than 5% in non-smokers is around 5-7% in smokers for one pack a day, and 7-9% for two or three packs a day. As a general rule, for each pack of cigarettes smoked per day, the COHb increases by approximately 2.5% [4]. Heavy cigar consumers could reach peaks of 20% COHb [26, 31, 32].

CO is transported across the lungs into the bloodstream and the carbon atom of CO forms a particularly strong bond with low valent metal ions such as iron heme

making up hemoglobin (Hb) forming carboxyhemoglobin (COHb); with a high stability and high binding affinity [28, 33]. The affinity of hemoglobin for CO is around 218 times greater than for O₂ [13, 34]. Actually, this concerns all hemoproteins such as hemoglobin (Hb), myoglobin (Mb), neuroglobin (Ngb) and cytochrome c oxidase (CcO) among others, leading to their dysfunction [8, 28]. Formation of these stable hemoproteins-CO complex in vivo is the cause of CO toxicity.

Once exposure to exogenous CO ceases, using almost exclusively the lungs for elimination, the COHb level returns to baseline with a velocity expressed in half-life for COHb (COHbt_{1/2}). For this process, many studies have shown that the COHbt_{1/2} in the body is about 4 h on atmospheric room air [13, 35].

1.2. CARBON MONOXIDE DISCOVERY

Lewin in 1920 listed numerous incidents of CO poisoning which were described in the early Greek and Roman literature [36]. Penney reviewed in 2000 historical descriptions of CO poisoning before and after knowledge of its mechanisms of action and, Hopper in 2021 devoted a very complete review in “A Brief History of CO And Its Therapeutic Origins” [11, 37]. These reviewed a wide range of historical facets related to CO from antiquity to the Renaissance and reveals a link between a CO exposure and personalities such as Aristotle, the Greek physician Galen, the Persian physician Avicenna, Cleopatra, the Byzantine emperor Julian the Apostate, the Spanish alchemist Villanova and scientists of the 17th century [38].

Reports throughout the 17th century begin to investigate CO without identifying, characterizing, or naming it, while rejecting supernatural explanations [38]. Later in 1796, the French chemist de Lassone produced CO by heating zinc oxide with coke, but mistakenly concluded that the gaseous produced was hydrogen, as it burned with a blue flame [36]. At the end of the century of enlightenment, Cruikshank (1800) resolved the CO formula and named the molecule ‘gaseous oxide of carbon’. This discovery marks the beginning of research focused exclusively on CO [11]. In France, Nysten in « *Recherches de physiologie et de chimie pathologiques* » observed symptoms on dogs after CO, compared to CO₂, infused [39]. Leblanc (1842) identified CO as the toxic component of coal fumes, introducing a new period of research in the field of CO toxicology [37]. Chenot (1854) suggested that CO would act as a reducing substance which removed O₂ from the blood to oxidize itself to CO₂ with the liberation of considerable heat [40].

Finally, the identification of the precise mechanism for CO poisoning is widely attributed to Claude Bernard in his memoirs published in 1857. He suggested that CO caused death from anoxia by irreversible combination with Hb [37]. CO linked to Hb seems to disturb gases exchanges between Hb, O₂ and CO₂. Experiments were conducted on dogs and birds. Symptoms seemed to be reversible. He highlighted the ruddy red coloration of blood exposed to CO [41]. He was the first to suggest that pure O₂ at normal barometric pressures could be used as an effective therapeutic aid in the treatment of CO poisoning [41, 42]. Gréhant or Leblanc followed by Linas and Limousin (1868) also studied CO effects on animals during this new industrialization period responsible for an increasing number of CO poisoning (Fig. 3). They are the first to try pure O₂ to treat CO poisoning under normal pressure [43].

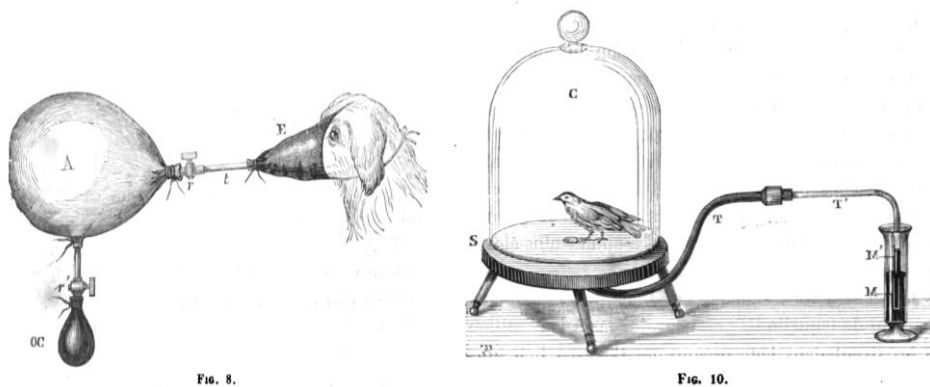


Fig. 3: The beginning of experimental animal studies to understand CO absorption features and toxicity.

Foley (1878) built a hermetic sedan chair with pump and manometer allowing to reach 2.5 atm to treat a wide field of respiratory diseases, but didn't apply it to CO. Bert, in France, developed a pressure chamber (Fig. 4) and suggested a mobile

model to be drawn through streets of Paris by four black horses [44-46]. Nevertheless, CO poisoning wasn't especially identified as a specific indication and Bert (1878) already demonstrated the almost universal cytotoxic effects of O₂ at high pressures on both animal and plant tissues [47].

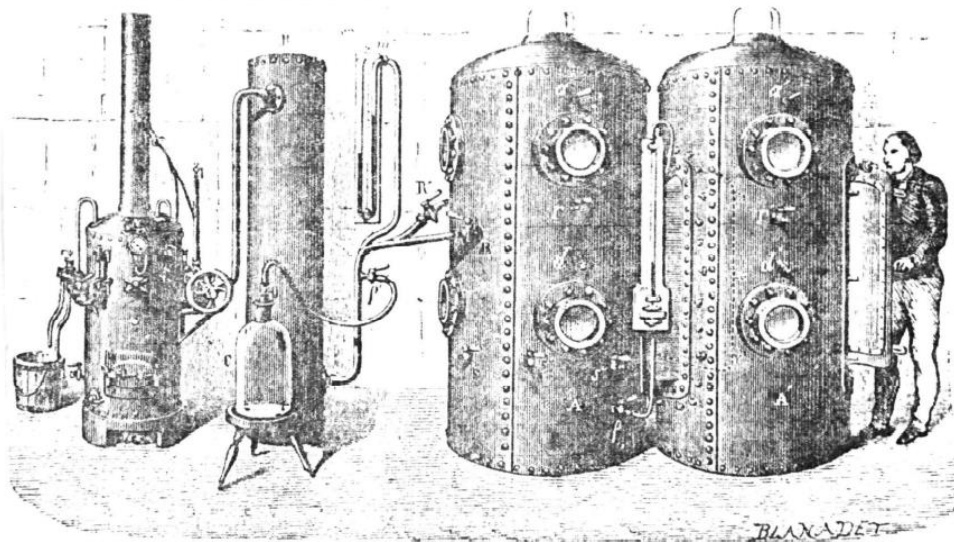


Fig. 4: Bert's pressure chamber constructed in 1869, consists of two chambers connected by an airtight door. Published in *La pression barométrique* [44].

Late 19th century, J.S. Haldane is now considered to be the father of experimental and clinical research into CO poisoning (Fig. 5). Devoting years to this topic, his son J.B.S. Haldane took up the torch and also became a renowned scientist.

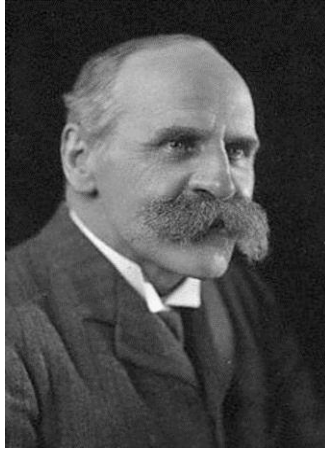


Fig. 5: John Scott Haldane (1860-1936) is a famous physiologist who made many CO discoveries. Clinically involved, he visited many scenes of mining disasters to investigate the origins of the CO toxicity.

J.S. Haldane in 1895 was linking experiments and observations:

- *"CO is not oxidized or otherwise decomposed in the human body...CO passes in by the lungs without there being the smallest loss"*.
- Then, he observed symptoms on a mouse and on himself at COHb until 56% after various CO exposures. He described *'relatively slight hyperpnea, palpitations, and feeling of fulness in the head, together with a tendency to impairment of the senses and loss of power over the limbs'* [48]. He noted that CO causes serious and lasting damage to the central nervous system, which may cause death long after the patient has been restored to pure air [48]. It was the early beginnings of the discovery of DNS. Nevertheless, J.S. Haldane didn't seem to have suffered from any after-effects of these experiences.
- CO primary effect is to displace the O₂ of the blood.

- He confirmed that the combination of CO with Hb is reversible (Fig. 6), and that the disappearance of the symptoms could run parallel with CO elimination when fresh air is again breathed. CO elimination is always much slower than the absorption of the gas [48].

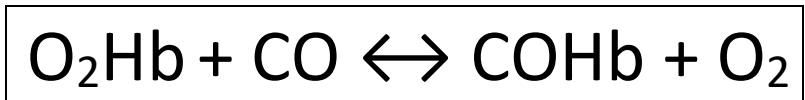


Fig.6: Equation illustrating the reversibility of CO to hemoglobin.

- Haldane recorded $\text{COHbt}_{1/2}$ after COHb at 50% reached during the first hour. Artificial respiration and normobaric O_2 assist in clearing the blood of CO. Nevertheless, the nervous system suffers especially from the deprivation of O_2 and Haldane declared 'treatment is unsatisfactory' [48].
- At a tension of 2 atm of O_2 the poisonous impact of CO on mice is ended [49]. Haldane discovered this potential benefit of HBO but didn't realise the potential application of the treatment for humans. It would be confirmed as a response to CO poisoning treatment only half a century later in 1961 on rodents [50].
- Haldane & Lorrain (1897) concluded that the affinity of Hb for CO was about 300 times greater than that for O_2 [51]. This constant will be named Haldane M constant and is so important that we need to devote the next whole sub-chapter to develop it.

1.3. HALDANE CONSTANT

In this sub-chapter, we discuss an important consideration on CO affinity for hemoglobin and other metalloproteins, which explains CO physiopathology and toxicity. Moreover, this is an important element in the application of our results from swine to humans and the interpretation of a potential COHb rebound after treatment ends that this thesis will investigate. We first consider the **tetrameric structure of hemoglobin** from which the famous **Haldane M constant** derives. Second, we consider the **potential variability of this M**, nevertheless considered as a constant, in case of variability in the experimental conditions. Then we examine the **physiological implication of the hemoglobin tetrameric structure** which is disturbed by the CO presence. And finally, we look at the comparison to **monomeric structure of myoglobin** suspected to be involved in the unknown part CO physiopathology, and in the toxicokinetic controversy that we will discuss later.

1.3.1. Hemoglobin and tetrameric structure considerations

Hemoglobin (Hb) is a tetrameric protein (two α -chains and two β -chains) with four hemes including each a site available to link CO or O₂ molecule. An iron atom is located within each heme, Hb is so named a metalloprotein and either ligand (CO or O₂), once bound, excludes the binding of the other to the same site.

This structure (Fig. 7) confers essential physiological properties. The crude affinity of CO for an isolated ferrous iron is approximately 10⁵ times stronger than O₂; however, when ferrous iron is integrated in the Hb molecule structure this ratio is reduced by a factor of approximately 10³ [52]. Thus, due to the specific quaternary structure of Hb, this value is worth less than 300 for hemoglobin (corresponding to the Haldane Constant or (M) value). From this conformation results a capital

structural reduced CO affinity, allowing an essential endogenous CO physiological elimination [53].

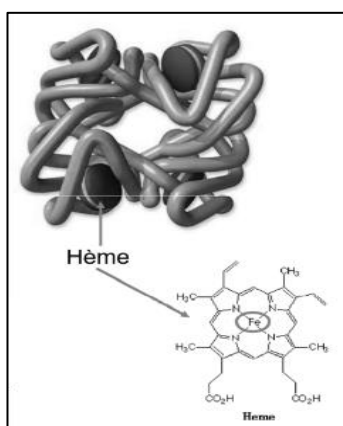


Fig. 7: Hemoglobin molecule's structure composed of four hemoproteins, two α -chains and two β -chains. Each chain contains an iron atom (Fe^{++}) able to bind O_2 or CO. Actually, they are four different affinity constants as the sites are filled (polypeptide chain as m1; m2; m3 and m4).

Comparatively to O_2 and CO_2 , CO has a very low solubility. The equilibrium ratio of bound CO (COHb) to dissolved CO in blood (p_{CO}) would be approximately 1.7×10^6 . In fact, despite a M-value at ± 240 , in vitro, the combination of CO with hemoglobin is not especially rapid but it is the dissociation which is very low. When, for example, blood is shaken in a 100 % CO atmosphere, only 26 % of the Hb is converted to COHb in 5 min; almost complete saturation takes place only in about 20 min [54].

To understand the mechanism of CO toxicity, at the beginning of the 20th century, Haldane experimented to estimate the affinity of Hb for CO comparatively to O_2 with partial pressures of the two gases ($p_{CO}=p_{O_2}$) at 50/50 to assess the proportion of COHb and O_2 Hb formed. He found a proportion around 240, meaning that Hb has 240-fold more affinity for CO than O_2 . As a result, O_2 carrying capacity of blood

will be compromised. Experiments developed to elaborate Haldane's equation where this affinity is named Haldane constant or (M) constant (Fig. 8). In other words, this value gives the partial pressure ratio (pO_2/pCO) needed to achieve equal amounts of COHb and O_2Hb ($\%COHb = \%O_2Hb$).

$$\frac{[HbCO]}{[HbO_2]} = M \frac{(pCO)}{(pO_2)}$$

Fig. 8: Haldane equation where M is the relative affinity ratio of CO to O_2 for blood (≈ 234), (P_{CO}) and (P_{O_2}) are the partial pressures of CO and O_2 , respectively, and [COHb] and [O_2Hb] are expressed as percent of total Hb concentration.

More recent and detailed assessments now allow us to estimate association and dissociation kinetics with constant respectively K_a and K_d . Equilibrium constant (K_{Eq}), the overall affinity for each gas, being K_a/K_d . As shown in the table 1, K_{Eq} is substantially higher for CO ($\approx 750 \mu M^{-1}$) compared to that of O_2 ($\approx 3.2 \mu M^{-1}$). The ratio of the K_{Eq} values ($K_{Eq} CO / K_{Eq} O_2$) is referred to M value respecting the Haldane constant found by Haldane ($750/3.2 \approx 234$ compared to 300) (Table 1).

As shown in the table 1, O_2 combines with Hb about 10 times as fast as does CO but the great affinity of CO for Hb is due to the fact that COHb dissociates ($k_d \approx 0.008 s^{-1}$; $t_{1/2} \approx 87 s$) 2.500-fold more slowly than HbO_2 ($k_d \approx 20 s^{-1}$, $t_{1/2} \approx 0.035 s$) [55, 56]. As a result, exchange of O_2 bound to Hb in blood with alveolar air and tissues is much more efficient than CO exchange. This characteristic, the slow dissociation with CO, allow to link efficiently the endogenous CO product to be scavenged, but contribute to relatively long retention times of CO in blood in case of exposures to CO.

Ligand	k_a ($\mu\text{M}^{-1}\cdot\text{s}^{-1}$)	k_d (s^{-1})	K_{Eq} (μM^{-1})	M
Hemoglobin ^a				
Oxygen	66	20	3.2	1
Carbon monoxide	6	0.008	750	234
Myoglobin ^b				
Oxygen	14	12	1.2	1
Carbon monoxide	0.51	0.019	26.8	23
^a Based on Chakraborty et al. 2004				
^b Based on Gibson et al. 1986				

Table 1: Hemoglobin and Myoglobin binding kinetics and equilibrium constants for oxygen and carbon monoxide. K_a = association rate constant; K_d = dissociation rate constant; K_{Eq} = equilibrium constant; M = Haldane constant ($K_{\text{Eq CO}}/K_{\text{Eq O}_2}$).

The way to physiologically promote CO elimination (decrease COHb) is to decrease P_{CO} by increasing ventilation [20]. As illustrated by the equation (Fig. 8), the large M of about 234 predicts that even small reductions in P_{CO} will result in major improvements in blood O_2 -carrying capacity owing to a large decrease of COHb and equivalent increase in O_2Hb (Fig. 8) [57]. Moreover, according to the law mass action and the equilibrium equation (Fig. 6), $\text{CO} + \text{O}_2\text{Hb} = \text{O}_2 + \text{COHb}$, an increase in p_{O_2} results in a comparable increase in O_2Hb and releases CO ready for exhalation [58]. Oxygen supply (P_{O_2}) is then an important parameter where, for example, a decrease of 12% leads to a 24% increase of the COHb level [59].

In addition, M is then considered as a constant at equilibrium. It is estimated that a decrease of 20% in the default value of M results in a reduction of 13 to 26% from the default COHb value. Thus, varying the M-value over typical ranges reported in the literature (M from 218 to 248) changed the $\text{COHbt}_{1/2}$ assessment, but only slightly (Fig. 9). Nevertheless, as we will see in the next point, could M not be a constant!

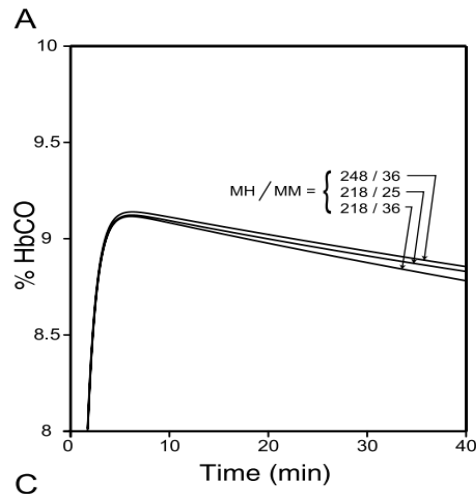


Fig. 9: Varying the M-value over typical ranges reported in the literature changed the slow rate of decay of %COHb, but only slightly. MH (M constant for Hb) and MM (M constant for Myoglobin) [60].

1.3.2. Is (M) really a constant?

By definition, the constant M is considered to be a constant value fixed at ± 240 . Nevertheless, we're compelled to report on various elements of the literature which show that it's probably not quite that simple.

- Historic literature reported M value with a wider range from 140 to 400, which may be due to technical inaccuracy.
- M-value could vary depending on biochemical environment (pH, T° ...) or among different species.
- Haldane's equation is valid at 50/50 CO and O_2 presence and in relation with a fixed (M) value at total saturation (on four binding sites) in which nearly all CO in blood is bound to Hb without taking into account the different linking steps in relation with the tetrameric nature of Hb (m_1 to m_4) [61].

- If 'step by step' from tetrameric nature is considered or CO/O₂ proportion different from 50/50 as is the case with a CO poisoning (Fig.10), M could vary as experimentally reported by several authors from different angles of investigation: (1) T 'Tense' or R 'Relax' allosteric states, (2) M affinity for each binding sites from m₁ to m₄, (3) the COHb% level reached or (4) the binding to β-chains compared to α-chains.

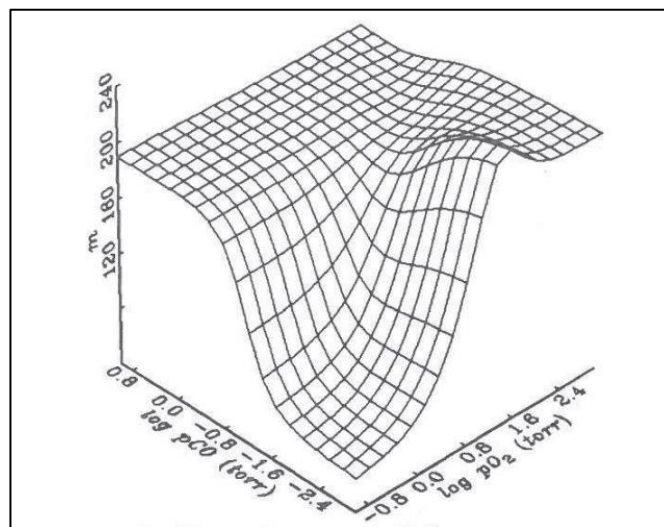


Fig. 10: Partition coefficient (M) surfaces for CO and O₂ binding to Hb as a function of CO and O₂ partial pressures [60, 62].

- (1) Mechanistic interpretation of co-operative O₂ binding to Hb is published from 1970 [63]. Sigmoidal curve (Fig. 12) suggests that Hb could present two allosteric states, T 'Tense' with lesser affinity for ligands (deoxy state), or state R 'Relax' with high affinity for ligands (carboxy state). So, two different values for (M) could correspond to these two states (Fig. 11). Di Cera claimed to have found a (M) lower at 123±34 for T state and higher at 241±19 for R state at 25° [62, 64]. Or, according with mathematical model, (M) could differ between the

two conformations, at ± 185 in T state and higher for binding the 4th ligand, in R-state at ± 225 . Furthermore, transition from T state to R state in the presence of CO alone may be different from that in the presence of O₂ alone [61]. Sirs in 1974 suggested that the value of (M) will decrease with lowered pO₂. For a change of pO₂ of $\pm 50\%$, the variation of (M) would be approximately $\pm 20\%$ [65].

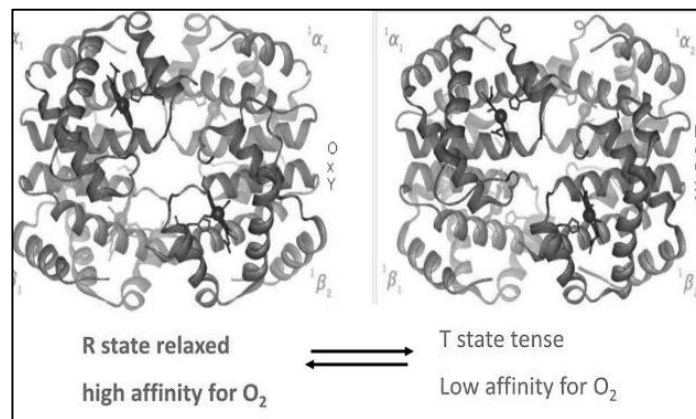


Fig. 11: Two hemoglobin state models could exist. High O₂ affinity as R state relaxed or low affinity for O₂ as T state tensed. Binding of O₂ for Hb is cooperative, binding of first ligand affects the affinity of the remaining sites for ligands.

- (2) Moreover, by analysing each step considering the tetrameric structure with their own association and dissociation velocity constant, it highlights intermediate M values respectively m_1 , m_2 , m_3 and m_4 [66-68]. The ratio for the fully ligated molecule m_4 by CO increased at 262 and m_1 seems to be 176, m_2 at 153 and m_3 , the triply ligated species seems to be negligible, as in the case for O₂ [62]. This supports that a sharp conformational transition occurs at the third stage of ligation [69]. This blockage does not leave enough room for binding O₂ or CO [62].

- (3) In addition, M value could depend on the COHb% level with a M value at 185 if COHb is less than 20% and at 228 from 30 up 80 COHb% [70]. Other study having m_1 mean at 169 for COHb below than 2% compared to a global mean at 236 for (M) [70, 71]. Or, for a wider range from 5 up to 90 COHb% Rodkey and '*in vitro*' data suggest that the value of (M) remains constant [72].
- (4) Finally, β -chains seems to react faster than α -chains [73, 74]. In 1987, Samaja showed that the first step of the dissociation of CO from Hb(CO)⁴ is independent of the subunit, in contrast to the third and fourth steps, where the α -chains dissociate CO twice faster than the β -chains [75].

These M value variations found in relation to historical literature, the consideration for the tetrameric structure or the variability among species, motivated us to previously undertake two systematic reviews on this topic.

1.3.3. Physio-(Patho-)logical implications of the tetrameric structure

Actually, co-operative binding of O₂ to Hb serves an important physiological function [76, 77]. Under conditions of relatively high pO₂ in arterial blood entering the lung (pO₂=100 mmHg), co-operative binding promotes the loading of Hb with O₂ to be easily transported and to be stored. Conversely, at lower pO₂ in venous blood (pO₂≈20 mmHg), co-operative binding promotes unloading of O₂ for utilization; dissociation of a molecule of O₂ decreases the affinity of Hb for O₂, promoting the dissociation of a subsequent molecule of O₂ to be delivered where

is the most useful. Co-operative binding contributes to the sigmoid shape of the O₂Hb dissociation curve (Fig. 12; 'Normal' curve above) [77].

Binding of CO to Hb is also co-operative. Binding of CO increases the associative rate of binding and affinity of both CO and O₂. Douglas and Haldane demonstrated that when the blood is partially saturated with CO, the dissociation curve of the remaining O₂Hb is altered, displaced on the left, in such a way that O₂ dissociates only at a much lower partial pressure than usual [78]. COHb presence shifts the O₂Hb dissociation curve to the left which means that tissue O₂ tensions must fall to much lower levels before O₂Hb can give up its O₂ [13, 79]. The greater COHb% level, the further to the left the O₂Hb curve is shifted (Fig. 12 below) [80]. Thus, in case of CO poisoning, the resultant decrease in the O₂-carrying capacity of the blood and the impaired release of O₂ to the tissues results in a greater tissue O₂ deficiency than would be produced by an equivalent reduction in ambient pO₂, or an equivalent reduction in Hb secondary to anaemia (Fig. 12). A man with COHb at 50% is almost helpless, but a man whose blood contains only 50% of the normal quantity of Hb behaves normally and can often carry on his work. PO₂ and quantity of O₂ carried are the same, but the dissociation of O₂ from the O₂Hb is quite different [55].

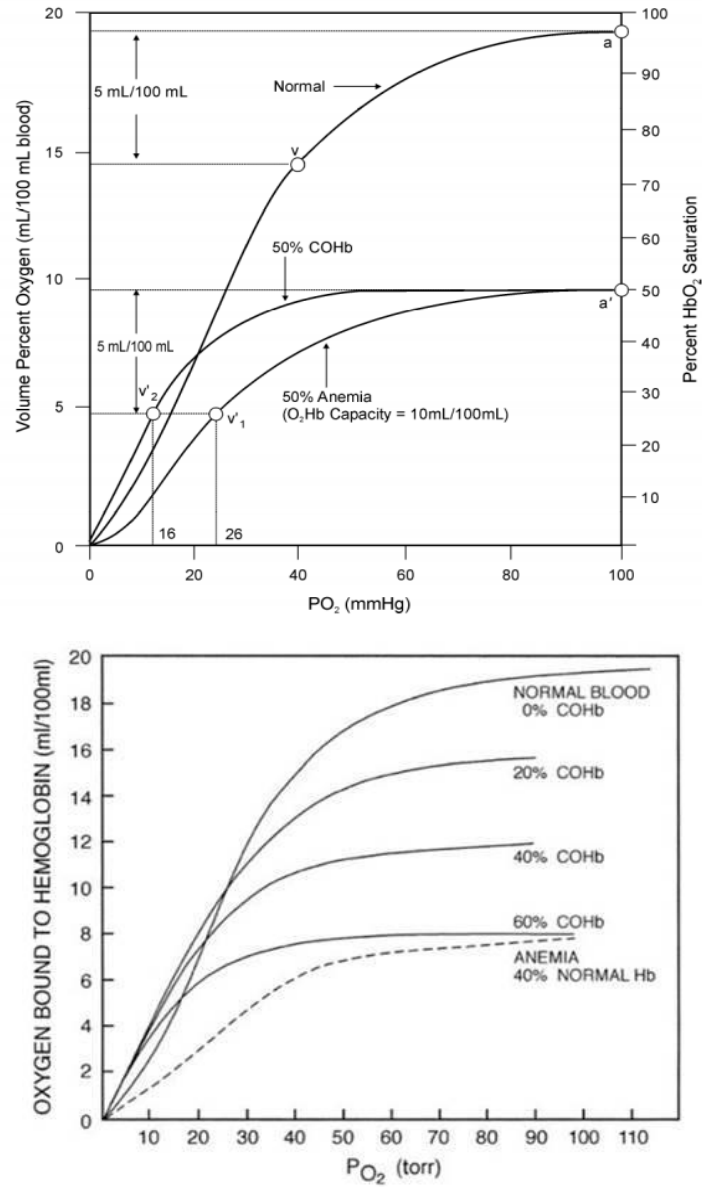


Fig. 12: (Above) O₂Hb dissociation curve for normal human blood compared to blood containing 50% COHb and for blood of an anaemic human. (Below) O₂Hb dissociation curves for normal blood and 20, 40, and 60% COHb. The presence of COHb not only reduces the maximum amount of O₂ that Hb can bind but also changes the shape of the O₂ dissociation curve [81].

1.3.4. Myoglobin and monomeric structure considerations

Myoglobin is also a metalloprotein but with a monomeric nature without co-operative binding (Fig. 13; b). In consequence, the dissociation's curve for O_2 presents only a hyperbolic shape (Fig. 14). Thus, the O_2 Mb dissociation curve is independent of the COMb level [60]. In humans, muscle accounts for $\pm 41\%$ of total body mass and provides an O_2 storage that may serve as a buffer under ischemic conditions [60].

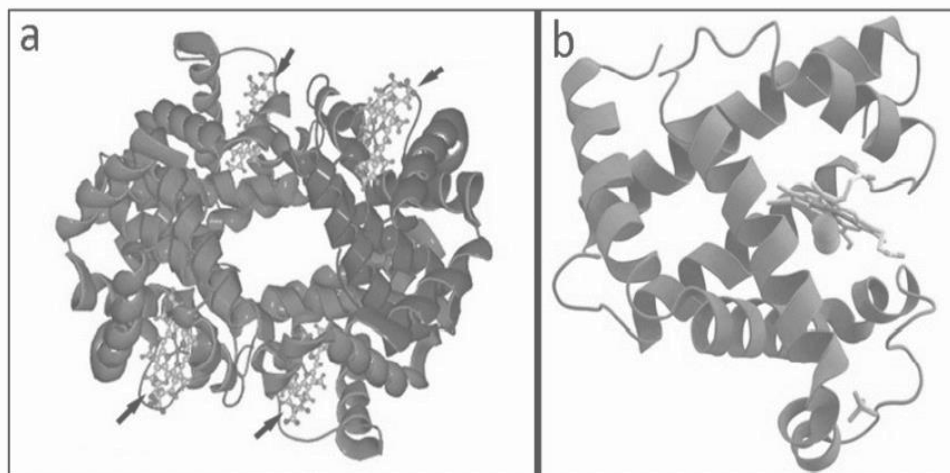


Fig.13: Structure of the tetrameric hemoglobin (a) including the four haem-groups (arrows) compared to the monomeric myoglobin (b).

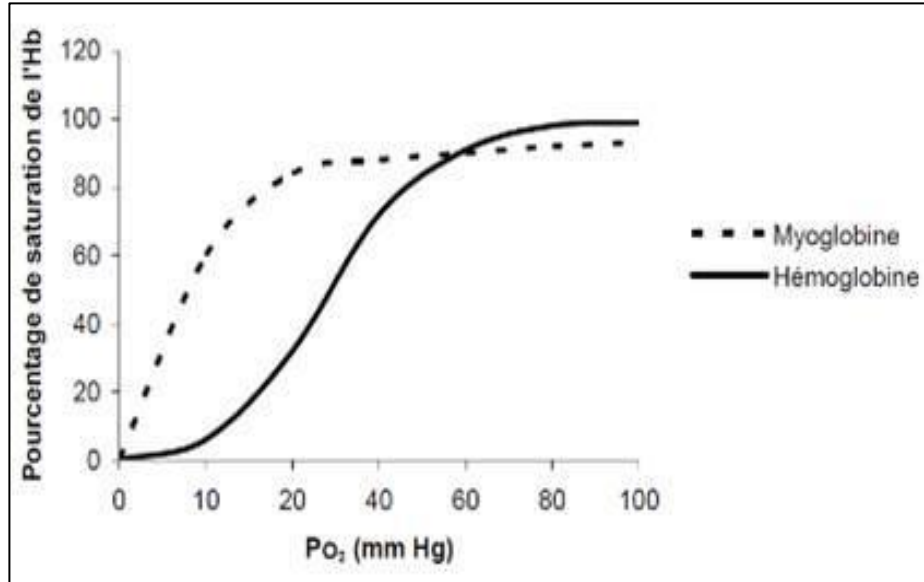


Fig. 14: Tetrameric O₂Hb dissociation sigmoidal curve compared to hyperbolic monomeric O₂Mb one. Curve shape differed due to absence of co-operative binding in monomeric structure. Myoglobin acts as an O₂ reserve which is only released late in case of severe O₂ deprivation.

The binding affinities of CO and O₂ to Mb are lower than that for Hb with a Haldane constant at ≈ 23 (Table 1) and 36 for myocardium, but higher affinity for CO is still in favour of preferential binding of CO over O₂ at similar partial pressures of both gasses [82]. The CO dissociation rate from myoglobin ($k_d \approx 0.019 \text{ s}^{-1}$; $t_{1/2} \approx 36 \text{ s}$) is slower than O₂ ($k_d \approx 12 \text{ s}^{-1}$, $t_{1/2} \approx 0.06 \text{ s}$). As a result, exchange of CO bound to Mb in muscle with blood is much less efficient than O₂ exchange. Moreover, the affinity of Mb for CO ($K_{eq} \approx 27$) results in an equilibrium condition in which dissolved CO concentrations in muscle are very low ($\approx 10^4$ fold) relative to COMb concentrations [83, 84]. These two characteristics (high affinity and slow dissociation) contribute to relatively long retention times of CO in muscle following exposures to CO.

1.4. CARBON MONOXIDE TOXICITY MECHANISM

It has been long understood that '*asphyxia is not immediately terminated when the victim is removed from the gassing chamber...although his body may be surrounded and his lungs filled with fresh air, his brain continues to be asphyxiated*'. Hendersen, 1922 [85].

CO poisoning is classified as a chemical asphyxiant gas, and toxic action results in symptoms that range from headache to unconsciousness and death, depending on the length of exposure, the dose and patient comorbidity.

The clinical severity in CO-poisoned patients does not directly correlate with blood COHb levels, and clinical improvement does not correlate with the rate of clearance of blood COHb [1]. Moreover, as observed in canine studies, the toxicity of a particular concentration of CO is greater when CO is administered by inhalation rather than by transfusion of CO-exposed red blood cells [54]. So, dissolved CO could be the cause of cellular CO toxicity while high concentrations of COHb in the blood should not be intrinsically toxic.

To focus on CO toxicity, we have to understand first (1); how CO is absorbed, secondly (2); the impact of CO on other biological mechanisms, thirdly (3); if CO is transformed in the body, fourthly (4); how CO diffuses to other metalloproteins and if CO is stored in the body, and fifthly (5); the harmful cellular impact of CO. In a following subchapter we will discuss the impact of these three first points on our main topic: the CO elimination.

First (1), exogenous CO is rapidly absorbed through the lungs via the alveolar system and diffuses into the pulmonary capillary blood according to the CO diffusion capacity of the lung (DL_{CO}) and the alveolar-to-blood CO pressure gradient [60]. CO diffuses to the mixed venous blood entering the lungs and is reversibly bonded to Hb in the red blood cells transported via the blood system.

Absorption of CO in blood is described with the Coburn-Forster-Kane (CFK) equation used from 1965 to decide about safety CO limit level in atmosphere, especially in occupational area (Fig. 15) [14]. This CFK model is illustrated by absorption curves and can be used to predict steady-state blood COHb levels increasing in a regular manner that correspond to a given continuous inhalation exposure to CO (Fig. 15) [55]. In addition of the duration of CO exposure, the rate at which the body absorbs CO depends mainly on two factors, the partial pressure of CO in the air breathed as 'the dose', and the volume of pulmonary ventilation. Absorption is, for example, two times faster during moderate exercise (walking) as it is while sitting at rest, and three times as rapid during fairly hard work [86]. Or, the rate at which the P_{CO} increases in different animals is proportional to the respiratory exchange per unit of body weight. In small animals, absorption will be rapid, and the final equilibrium will be reached 20 times faster than for man. Hence, Haldane suggested the use mice or birds to indicate the presence of CO in air of coal mines. While a mouse can be affected within a few minutes, man has an interval 20 times longer [48, 87].

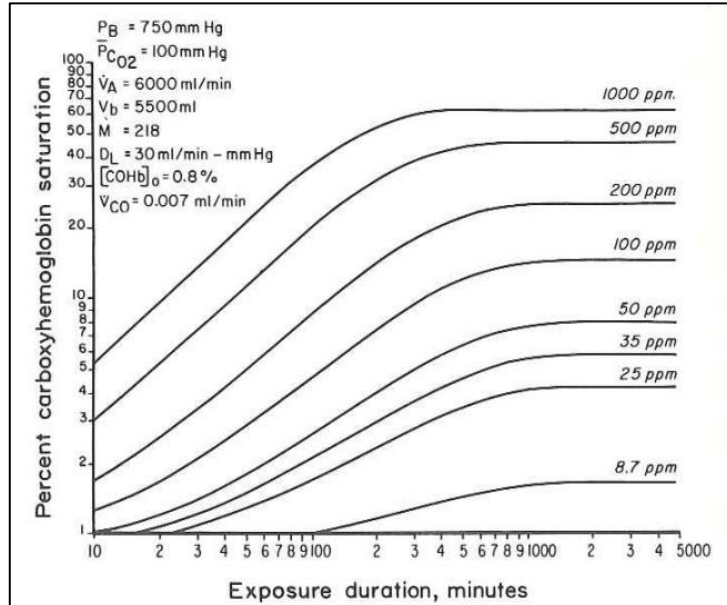


Fig. 15: COHb rate on human in function of exposure duration and CO concentration determined by solving the CFK equation [88]. Rate of increase of blood saturation during exposure of the human subject to CO [26, 88, 89]. Steady state during absorption is not reached in case of <5h CO exposure at 100 to 500 ppm [90].

Moreover, COHb levels measured shortly after multiple exposures were a function of total CO dose rather than concentrations of inhaled CO. If two sets of transients' exposures delivered equivalent total doses at 7.500 ppm in humans (1 min at 7.500 ppm and 5 min at 1.500 ppm), mean increase in COHb% per exposure was equivalent at ± 2.06 [91, 92]. But this does not mean that distribution and CO body storage for these two different procedures are equivalent.

Secondly (2), we saw previously that COHb results a reduction in the O_2 -carrying capacity, the O_2 Hb dissociation curve is a function of the %COHb of the blood and O_2 dissociates at a much lower partial pressure than usual [60].

Thirdly (3), CO is not bio transformed in the body. Absorbed CO is eliminated from the body only by exhalation. There is no evidence that the body can oxidize CO [55]. Some of the very early scientists noted that there seemed to be evidence that an oxidation of CO to CO₂ occurred in the body. Later investigators felt that this view was mistaken, due in all probability to the fact that they were misled by the indirect increased excretion of carbon dioxide (CO₂) observed in the later stages of CO poisoning [80]. Actually, for physiological endogenous CO production in tissues ($\pm 0.2\%/h$) [93], less than 0.1% of inhaled CO is being converted to CO₂ [94, 95]. This oxidation could occur by mitochondrial cytochrome oxidase. Thus, we can conclude that CO in the body is a stable molecule with no significant CO biotransformation.

Fourthly (4), the historical CFK equation cannot be used to address details of CO distribution because it assumes that the body is considered as mono-compartmental with only an intra-vascular CO storage [83]. Forty years later, one of the most successful recent models was developed by Bruce which considers multi-compartmental model including extra vascular compartment as myoglobin [84]. CO distribution in other compartments results in a theoretically bi- or multi-compartmental toxicokinetic model during COHb distribution and elimination. Nevertheless, studies into COHbt_{1/2} fitting with a mono- or bi-compartmental model are still controversial [96, 97]. We will have the opportunity to discuss this controversy later.

Approximatively 80% of the CO is bonded to Hb in circulating erythrocytes blood to form COHb. The remaining CO $\approx 15\%$ is distributed to myoglobin with which it binds and less than 5% will be bound to others compounds stored as neuroglobin and cytochromes [26, 37]. Finally, CO dissolved in blood (p_{co}) is likely negligible (less than 1%). In case of higher exogenous CO exposure, in response to diffusion

gradients, a significant p_{CO} increases in the blood lungs allowing CO to be broadly distributed and stored in the extravascular tissue, especially the ones containing metalloproteins as the heart muscle and brain [83, 84]. Nevertheless, dissolved CO in plasma remains very low, even at high COHb% [54].

The diffusion into muscle tissue is dependent on CO diffusion coefficients (Dm_{CO}). Dm_{CO} depends on multiple physiological factors: the slow diffusible feature from the CO, the diffusion of CO across the capillary wall, the diffusion of CO within tissue, the total surface area for diffusion, and the affinity of CO with Mb [60]. Dm_{CO} would be sufficiently small that blood-tissue equilibration of P_{CO} occurs more slowly than vascular mixing [60]. During exposures, $COMb$ increases more slowly than COHb but, despite M-value at 240 for Hb compared to 23 for Mb, during CO washout on room air, $COMb$ decreases more slowly than COHb [98]. Uptake of CO by muscle tissues could occur within 6 to 30 min, depending on the experimental protocol. Thus, in a short duration/high concentration CO exposure, less CO diffuses from blood into the tissue due to the shorter exposure time.

This CO distribution process is reversible. If P_{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 [98]. However, due to the stronger affinity of CO for Hb, we saw that there is a baseline COHb concentration in the blood from endogenous production [13].

In addition, if exercise can increase CO absorption, it can also increase the rate of transfer of CO from blood to muscle [99]. The effect of exercise is thought to be related, in part, to increased O_2 consumption and lower pO_2 in muscle, which favours binding of CO to myoglobin, and increased muscle blood flow. Hypoxia appears to increase transfer of CO from blood to muscle [98-100].

To date, in practice, no laboratory technique is able to assess COMb. Bruce predicted in mathematical modelling that breathing 100% O₂ during washout causes a counterintuitive sudden rise in COMb levels. Especially in the long-low CO exposure simulation, sharp increases in COMb levels were observed only during hyperoxia (Fig. 16). This suggests that the subject is not able to rapidly exhale all the CO that has been dislodged from Hb by hyperoxia. The CO released is in part dissolved in blood and diffuses into tissues and binds to Mb, resulting in elevated COMb levels in the early part of washout and COMb decrease may lag considerably behind the decrease in COHb during therapy (Fig. 16) [98]. Moreover, this response may exacerbate the impairment of O₂ availability/storage in exercising muscle (e. g., the heart) or in resting muscles in severe CO poisoning. Therefore, victims of CO poisoning may be susceptible to deleterious effects of high COMb for several ≈10 min after %COHb falls. Extension of the model to predict the consequences of therapy using hyperoxia or HBO may result in prolonged elevation of tissue CO levels which might contribute to worse effect or long-term sequelae and will provide additional insights regarding the optimal treatment protocol for CO poisoning [57, 83]. This effect could suggest not to use full O₂ as an initial treatment but encourages the investigation of other treatment modalities, such as hyperventilation.

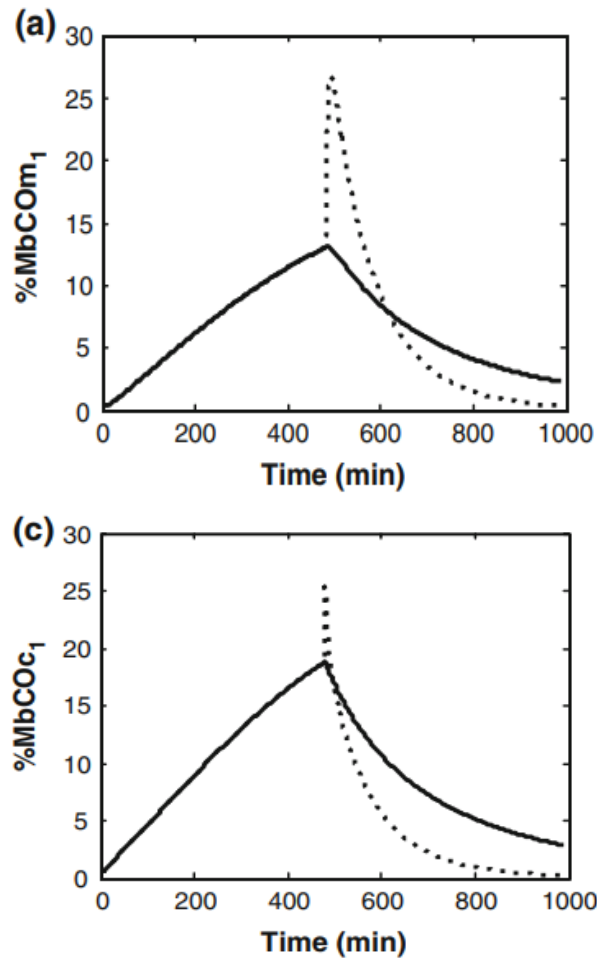


Fig. 16: COMb % after 8h CO exposure at 265ppm from Bruce's mathematical simulation. (a) COMb in the skeletal (a) or (c) myocardial tissue [98]. CO elimination from Mb on air (solid line) compared on hyperoxia (dashed line) [98].

In addition to hemoglobin and myoglobin, CO toxicity is potentiated by the reversibly-binding of CO to other heme-containing proteins as catalase, (hydro-) peroxidase and more importantly mitochondria, where it binds to a metalloprotein including iron or copper cores in cytochrome c oxidase (CcO; complex IV). O₂ is used

in mitochondria to produce energy currency of life from adenosine triphosphate (ATP). This mitochondrial respiration uses electron transport chain through mitochondrial cytochromes. The cytochrome oxidase system is predominant in areas of great metabolic activity and is a target of CO which alters the function of the respiratory electron transport chain [101, 102]; thus, organs with the highest metabolic rate, which contain CO-binding metalloprotein, such as the heart (cardiomyoglobin) and brain (neuroglobin), are affected the most by CO poisoning [103]. CO directly inhibits the enzyme by binding the heme a₃ site of cytochrome c oxidase (CcO), resulting in alteration of the mitochondrial electron transport chain enzymatic activity and the inhibition of respiration and oxidative phosphorylation (Fig. 17).

CO binding to myoglobin, cardiomyoglobin, neuroglobin and cytochromes from mitochondria could be considered as CO storage and may be responsible for the prolongation, or aggravation, of the symptoms after COHb back to the physiological level. It may also explain persistent symptoms after CO poisoning [84, 104].

Cytochrome oxidase affinity (CcO) for O₂ compared to CO is considered as similar, only nine times greater for O₂ under normal conditions, but competitive binding is in favour of CO in instances of hypoxia, as is the case for the COHb decreasing O₂ release [102, 105]. The binding of CO to the cytochrome oxidase system inhibits cellular respiration by displacing O₂ - but how long O₂ remains displaced is unknown [3].

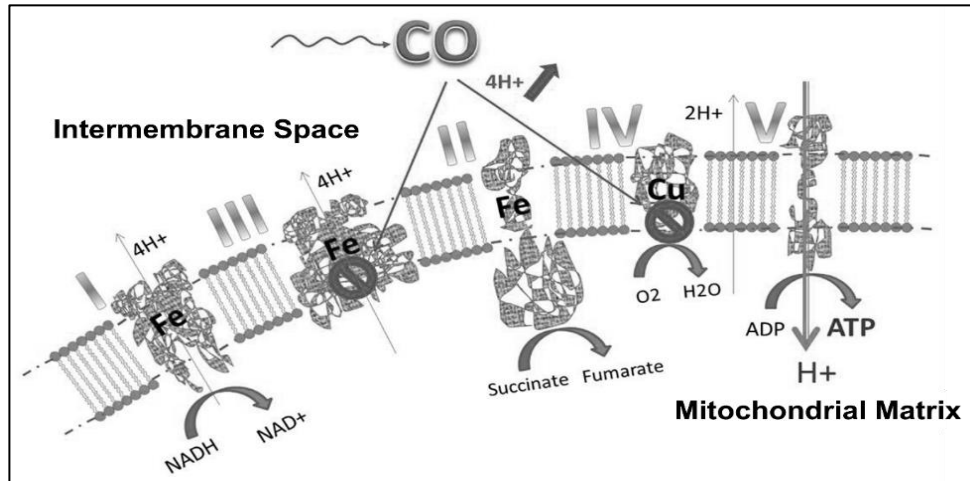


Fig. 17: Undoubtedly CO binds to cytochromes and therefore interferes with oxidative metabolism: inhibition of the reduction of O_2 to H_2O , transfer cessation of H^+ across the intermembrane space, shutting down ATP production through ATP synthase and electrons accumulation entering the electron transport chain through complexes I and III, which can produce free radical superoxide triggering cellular metabolism or death [8, 102, 104, 106]. In addition to inhibition of CcO activity, the activities of complex I and complex II were also significantly decreased with CO exposure [102].

Fifthly, in addition to this loss of ATP production affecting cellular metabolism, this inhibition is believed to potentiate superoxide production by the mitochondria, resulting in oxidative damage to cells and tissues. It has also been suggested that excess CO can displace nitrite monoxide (NO) from heme-containing proteins and activate nitric-oxide synthase enzymes, causing a rapid rise in free NO. The increased NO can react with superoxide to form peroxynitrite, leading to additional nitrosative damage and further NO-dependent inhibition of CcO [102].

Moreover, CO intoxication induces an immune response by activating neutrophils [8] and subsequently inducing the formation of reactive oxygen species (ROS),

which facilitates the development of free radicals [107]. Brain hypoxia from CO poisoning elevates levels of excitatory amino acids, including glutamate, increasing brain nitrite levels, oxidative stress, necrosis, apoptosis, degradation of unsaturated fatty acids and reversible central nervous system demyelination. All these effects contribute to an overall picture of inflammation and injury [108].

For a complete picture, we have also to consider that non-physiological $p\text{CO}_2$ level in blood can indirectly accentuate CO toxicity. Investigators reported inconclusively a certain degree of hyperventilation accompanies CO poisoning [80]. Anyway, if hyperventilation occurred, the decreased $p\text{CO}_2$ in the blood shifted the O_2Hb dissociation curve to the left (Fig. 18), resulting in a slope which decreased O_2Hb dissociation and O_2 delivery to the brain and, moreover induced cerebral vasoconstriction, decreasing cerebral blood flow [109]. In opposite, a hypercapnia could result in opposite effect. This was a great concern before 1960 when 5% CO_2 was added to 95% O_2 inspired gas during treatment of CO poisoning to accelerate ventilation with a view to enhancing $\text{COHb}_{t_{1/2}}$ and, based on the Bohr effect, in an attempt to increase the release of O_2 to the tissues [57].

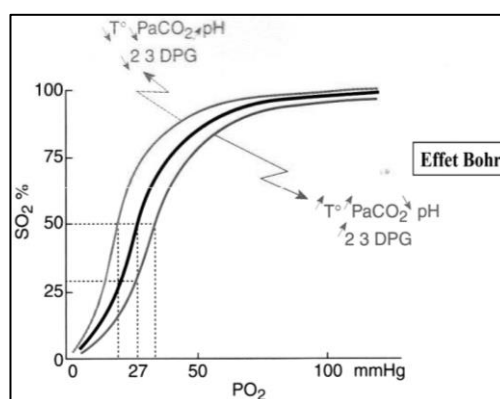


Fig. 18: $p\text{CO}_2$, 2-3 DPG, temperature, and pH could also affect both M and the O_2 dissociation curve [110] as CO [111].

And finally, CO poisoning causes tissue ischemia-reperfusion injury. Free radicals and lipid peroxidation play a role in CO poisoning during reoxygenation after the hypoxic phase [112], and an increase in reactive oxygen species (ROS) was associated with the reoxygenation after CO hypoxia [113, 114]. The oxidative stress may explain the DNS already described in 1880 [37, 115]. Avoiding this DNS is the main goal of CO poisoning treatment, thus studies focused on the possible contribution of ROS, apoptosis and nitric oxide (NO) production as mechanisms of CO cells toxicity are important, but clinically very difficult to assess.

In summary:

- CO acts by preventing the normal carriage of O₂ in the blood allocated to M ratio at 218:1;
- CO impaired release of O₂ to tissue from O₂Hb disturbing the ability of O₂Hb to perform its primary function as supplying the tissues with O₂;
- CO is stored as COHb but also in extravascular compartment as myoglobin (COMb) and neuroglobin (CONgb);
- CO is a direct tissue poison by inhibiting the enzymic oxidative systems and mitochondrial/cellular respiration in the tissues;
- CO absorption and diffusion are modified by ventilation and/or O₂ therapy, moving from different compartments affecting CO clearance from these storage areas and could therefore impact on final toxicity;
- CO facilitates the toxic production of free radicals as excessive O₂ supply could exacerbate [55];
- CO leads to combination of hypoxia/ischemia, effects of NO, and direct cellular toxicity which may initiate a cascade of events leading to delayed brain lipid peroxidation clinically expressed by Delayed Neurologic Syndrome (DNS) [113, 116].

1.5. CLINICAL TOXICOLOGY

The previous sub-chapter helped to understand why the most commonly adverse symptoms reported occurred in the cardiovascular and central nervous systems; however, because CO exposure has the potential to affect nearly all tissues, the clinical presentation of acute CO poisoning includes a wide range of symptoms.

The severity of CO poisoning is typically categorized as mild, moderate, or severe, based on clinical presentation, but no consensus is really defined. Most common symptoms reported of **mild CO poisoning** are headache and dizziness sometimes accompanied by nausea, vomiting, weakness, confusion or blurred vision. Because these symptoms mimic flu-like viral illnesses, mild CO poisoning can easily be misdiagnosed. Symptoms associated with **moderate CO poisoning** may include confusion, syncope, chest pain, dyspnea, weakness, tachycardia, tachypnea, and rhabdomyolysis (Fig. 19).

Toxicity of different concentrations of CO in man has been investigated, though the clinical response to different CO levels exposure may vary between individuals. Symptoms begin more obviously at 20 %COHb while death occurs at 50-80 %COHb [117]. In children a 20 %COHb can lead to unconsciousness [118]. Healthy persons can probably survive higher doses of CO than persons with severe pre-existing diseases.

During exposure to low concentration of CO the symptoms appear gradually, and their severity varies with the duration of exposure. If COHb is raised fairly rapidly, close to $\pm 35\%$ rather considered as a moderate CO poisoning and, maintained at that level for a short period ($\pm 20\text{min}$) the symptoms will be rather slight. If, instead, CO is gradually absorbed over a long period, COHb at 35% represents equilibrium and symptoms will be more severe [55].

At the end, effects of **severe CO poisoning** may be life-threatening, including cardiac arrhythmias, myocardial ischemia, cardiac arrest, hypotension, respiratory arrest, noncardiogenic pulmonary oedema, seizures, and coma (Fig. 19).

A very important point is that studies struggle to highlight a relevant discriminant criterion related to CO poisoning severity to predict complication in the form of delayed neurological syndrome (DNS) [119]. In this context, it remains difficult to demonstrate the superiority of one method of oxygen (O₂) administration over others.

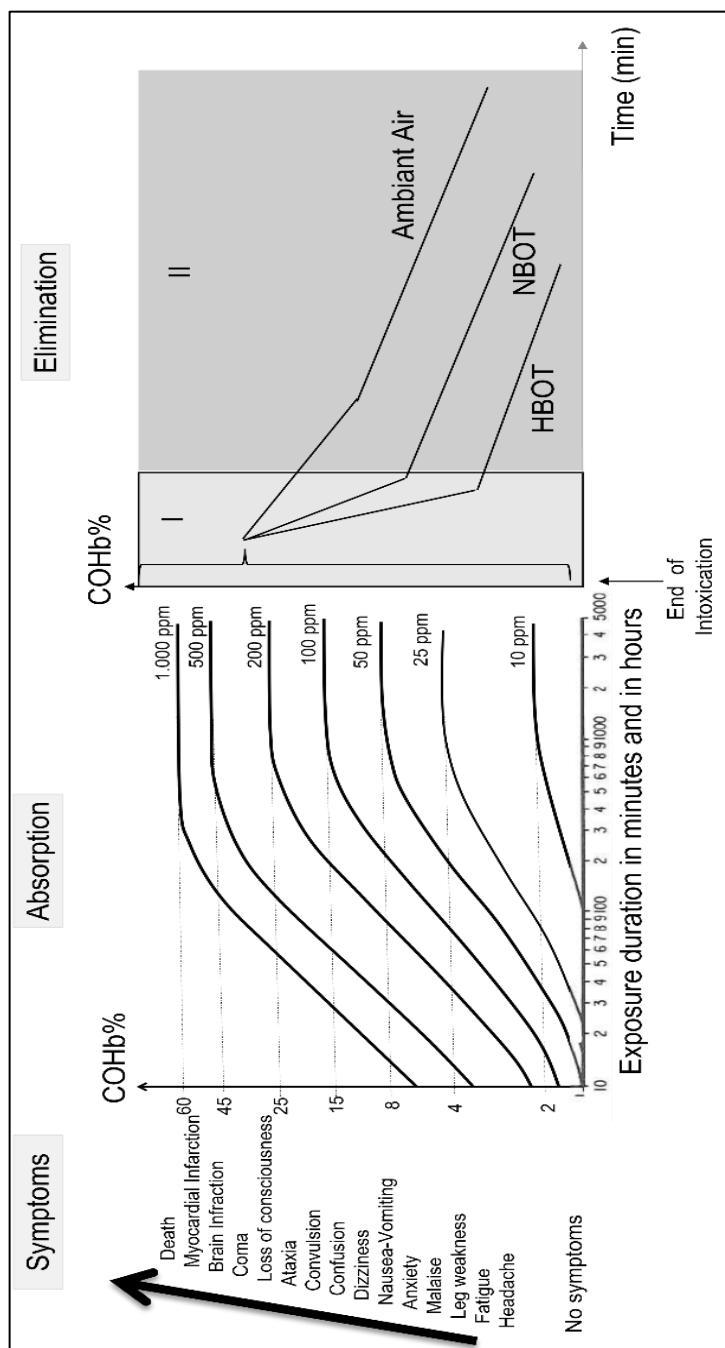


Fig. 19: From left to right: Clinical symptoms on CO poisoning – CO absorption for CO exposure at different levels from CFK equation and biphasic COHb half-life elimination on Ambient Air (AA); Normobaric oxygen therapy (NBOT); Hyperbaric oxygen therapy (HBOT). Adapted from [116].

1.6. CHRONOLOGY OF CARBOXYHEMOGLOBIN ELIMINATION STUDIES

Toxicokinetic (TK) is a term that refers to what the body does to a toxin as a function of dose and time. CO elimination is the cornerstone of this thesis.

The 20th century was the golden age for CO poisoning experiments, including CO elimination. We exhaustively compared our studies with the available global literature. Therefore, this sub-chapter presents the history of the main findings and understanding about CO behaviour and, more specifically, COHb elimination. This allows us to understand the main features, discovered step by step, which led us to construct our own hypotheses.

We divided the presentation of these historic considerations into three groups: (1)*From the first physiologists to current treatment approaches*, (2)*COHbt_{1/2} discrepancies reported in studies*, and (3)*Reported methods for calculating COHbt_{1/2}*.

1.6.1. From first physiologists to current treatment approaches

Before the 19th century (considered the first physiology age), miscellaneous experimental therapies for CO poisoning were put forwards: gargling, drinking lipides, noise reduction, fumigation, inhalation of acetic acid vapor or ammonia, cupping, phlebotomy, transfusion, fresh air, stimulants, emetics, saline solution

infusion, vitamin C, hypodermic injection of whisky, methylene blue injection, rectal infusion of nutrients or digitalis injection [11].

After these speculative treatments there emerged potentially relevant treatments of CO poisoning, to wit: normobaric oxygenation (NBO), Carbogen, hyperbaric oxygen therapy (HBOT), isocapnic hyperventilation, extracorporeal circulation alone (ECMO) or associated with phototherapy, potent CO-binding chelating antidotes, catalysts for CO to CO₂, or techniques optimizing normal ventilation such as continuous positive airway pressure (CPAP) or high-flow nasal cannula (HFCN).

In parallel with their development, controversies have attended each treatment, highlighting either the lack of evidence for their efficacy or their potential side-effects. The current lack of confidence in one treatment over another is explained by the heterogeneity of CO exposures (dose x duration), individual sensitivities, comorbidities, criteria for identifying CO exposure at risk of DNS, protocols administered, delays in initiating treatment and the objectivity of symptoms. Despite 120 years of studies into the subject, there can still be controversy over acute care management, even within the guidelines of scientific societies themselves. It is also in this complex context that mathematical models have been developed from 1965 to attempt to integrate all the variables influencing the absorption and elimination of CO.

We will summarize the history of these relevant CO treatments to better understand the outstanding global issues and the genesis of our thesis' questions.

❖ OXYGENATION

At the end of the 19th century, O₂ and artificial respiration by Schäfer's method begun to be suggested as a treatment [120-122]. The HBO chamber had already

been discovered, but the idea of providing HBO on CO poisoned humans would have to wait for experimental human studies in 1949 and 1950 - and more convincing clinical human studies in 1960 [42, 123, 124]. Weaver in 2000 reviewed COHbt_{1/2} with 100% target NBO from 1985 to 1995 in case series. COHbt_{1/2} was 74+-25min (range 26 to 148 min!). This COHbt_{1/2} was found shorter than in previous clinical reports and became the reference [125]. Interestingly, it's from 1958 that special attention is paid to the immediate administration of O₂ applied with specific masks which must be very carefully fitted and continually supervised, since it is essential that inspired O₂ be at the highest possible concentration [126].

Around 1950, COHbt_{1/2} in humans ($\pm$ 4 hours on AA; FiO₂ 0.21) was confirmed to decrease approximately five-fold while breathing pure O₂ ($\pm$ 45min on O₂; FiO₂ 1.0) corresponding to the five-fold increase in PO₂ [42]. In the triple context of the criticism (1955) of the risk of side-effects of Carbogen treatment which has been in use since 1922 (see below), the success of HBO on COHbt_{1/2} and the emergence of mechanical ventilation to support hypoventilation accompanying coma, authors progressively recommended the exclusive use of pure O₂ or HBO [127-131]. The Undersea and Hyperbaric Medical Society (UHMS) is formed in 1967 and has recommended since then the effectiveness of the HBO in case of CO poisoning implemented from 1962.

Progressively, studies on HBO are trying to demonstrate this efficacy not only on COHbt_{1/2} (Fig. 20) but also on its potentially beneficial cellular action, as Brown et al. (1990) suggested partial dissociation of cytochrome α 3-CO complex with increasing dissolved O₂ on HBO in rats' brains [132]. Following this, larger clinical studies are now beginning to evaluate the efficacy of HBO in avoiding DNS, the ultimate goal of the treatment.

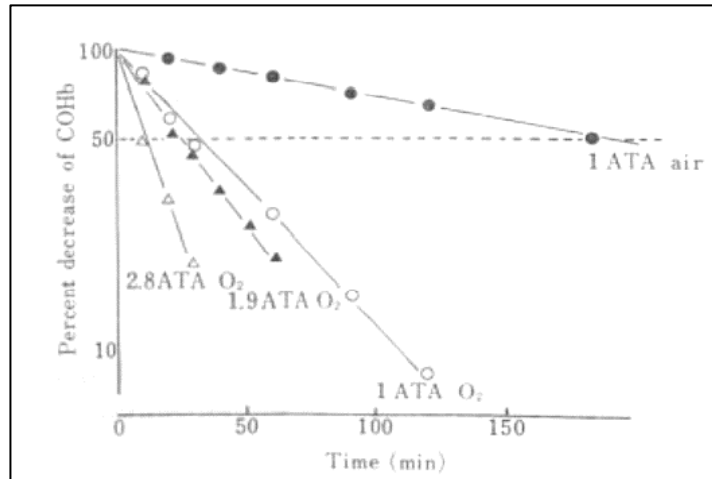


Fig. 20: Dissociation of COHb in the dog. COHbt_{1/2} on dogs (1975): 188min (1atm air); 32min (1 atm O₂); 25min (1.9 atm O₂) and 12 min (2.8 atm O₂) [133].

The six most significant* clinical studies on acute CO poisoning management included later in the meta-analyses are:

(1989*) Raphael compared NBO for 6 hours to HBO at 2.0 atm. Neurologic symptoms at 1 month were assessed. No benefit was confirmed for HBO [5, 134, 135].

(1995) Ducasse compared NBO for 12 hours to HBO at 2.5 atm. EEG impairments at three weeks were assessed with benefits observed in favour of HBO [136].

(1995*) Thom compared NBO until asymptomatic patient to HBO at 2.8 and 2.0 atm. Assessment of DNS prevention during four weeks follow-up were assessed, with benefit demonstrated for HBO [137].

(1996*) Mathieu compared NBO for 12h to HBO at 2.5 atm. Persistent neurologic symptoms at one month were assessed. No benefit demonstrated for HBO [138].

(1999*) Scheinkestel compared NBO for 100 min to HBO at 2.0 atm. Neuropsychologic symptoms at one month were assessed. No benefit confirmed for HBO [139].

(2002*) Weaver published the sole randomized, double-blind, placebo-controlled clinical trial which addressed long-term neurological sequelae after CO poisoning where compared '*normobaric O₂ sham HBO*' to real HBO at 2.0 and 3.0 atm. Cognitive sequelae at six weeks, six months and one year detected by neuropsychological testing were in favour of HBO (25% vs. 46%) [35].

(2004*) Raphael compared NBO for 6 h to HBO at 2.0 atm. Neurologic symptoms at one month were assessed. No benefit was shown for HBO [140].

The first systematic review (2005) of HBO based on these previous studies* concluded that HBO cannot routinely be recommended for CO poisoning. It could be possible that some patients, particularly those with more severe poisoning, may derive benefit from the treatment, but this remains unproven [141]. In 2011, a new meta-analysis with a total of 1.361 participants, confirmed the absence of expected benefit from HBO [135]. In opposition, and without including new determinant clinical studies, Casillas in 2019 proposed a new systematic review and concluded that HBO is effective in moderate to high-risk CO poisoning, being the therapy of choice to avoid sequelae, especially neurologically. Nevertheless, he suggested that NBO can be also considered as a reasonable alternative due to its cost-effectiveness. Surprisingly, he doesn't mention previous systematic reviewing, he avoids evoking Annane studies warning of potential worse outcomes with two HBO sessions, and excluded the Scheinkestel study not in favour of HBO [5, 141-144]. Such Juurlink claims that a future trial of HBO effectiveness is not only justified; it is essential [145].

Moreover, for patients poisoned by CO, the *time* to initiate HBO treatment, as much as the *method* of treatment, is of major importance [139, 146]. Even from the very beginning of HBO treatment of CO poisoning, it was clear that delays between poisoning and treatment markedly reduced its effectiveness [46]. Moreover, there is often significant delay in the delivery of HBO between diagnosis in the field, transportation to an HBO centre, and actual treatment [4, 35, 147-150]. Times to HBO treatment as short as three to six hours, which are all that can be expected given the logistics of patient transport and chamber preparation, seem to continue to show no benefit compared with NBO [6, 131, 144]. In contrast, in experimental studies, HBO is delivered very early.

❖ OXYGEN ADVERSE EFFECTS

For a century, the question of potential oxygen toxicity has regularly reminded us to question the correct dose of oxygen to be administered. In 1945, just before the first human experimental studies on HBO, reluctance to use high pressure O₂ for CO poisoning already considered the toxic nature of O₂ itself at one atm and higher. It was suggested that an optimal combination of exposure time and ambient O₂ partial pressure be selected so that the rate of CO elimination might be materially increased without incurring the risk of O₂ poisoning [3, 42, 151]. During the implementation of HBO for humans, in 1964 and 1974, Ledingham, followed by Agostini, remained O₂ toxicity in the tissues up to 2 or 2.5 atm [152-154]. Observed in clinical trials in 1999, HBO may have even worsened the outcome and was therefore not recommended by the authors [144]. The same conclusions were suggested by Annane's 2011 study, a randomized controlled trial, which compared NBO for six hours to HBO at 2.0 atm. A one-month questionnaire and a physical exam were assessed, with no evidence of superiority of HBO in patients with a

transient loss of consciousness revealed. Moreover, in comatose patients, two HBO sessions were associated with worse outcomes than one HBO session [6]. In experimental human studies (2002), after NBO-treated CO exposure, a cerebral blood flow decrease was observed, completely offsetting the effect of increased blood O₂ content on cerebral O₂ delivery. Nevertheless, they showed that maintaining normocapnia during NBO treatment resulted in significantly higher cerebral O₂ delivery compared with standard NBO [155]. If NBO is indeed an antidote against CO poisoning, the accurate dosage (level x duration x modalities) to generate a maximum beneficial effect without side-effects remains unknown.

❖ HYPERVENTILATION

As early as 1908 it was observed that a considerable increase in pulmonary ventilation was caused by CO₂ administration. Thus, the use of CO₂ was recommended to accelerate COHbt_{1/2} [156] but Henderson and Haggard applied this principle to resuscitation of dogs and humans only from 1920 [157]. They demonstrated that inhalation of Carbogen (O₂ at ±95% mixed with CO₂ from 5% to 10%) increased markedly the COHbt_{1/2} compared with using O₂ alone (Fig. 21) [157]. And they reported dramatic reversals of coma and other neurological symptoms using Carbogen on human [158]. The relative merits of Carbogen, in resuscitation from CO poisoning, would be the subject of debate for 40 years from 1922 to HBO's emergence in 1962 [159]. Nevertheless, due to the inability to regulate the CO₂/O₂ ratio thus associated with a risk of excessive hypercapnia and potentially fatal acidosis, particularly in patients with increased lactate levels and/or impaired respiratory drive, Carbogen began to be shake [159-164]. Moreover, minute ventilation markedly increased above an inspired CO₂ concentration of 4%, but 30% of patients and healthy subjects were unable to tolerate 5% CO₂ [131]. At the end,

the *Medical Research Council Committee for Research on Breathing Apparatus for Protection against Dangerous Fumes and Gases* condemned in 1955 the use of Carbogen in resuscitation [165, 166].

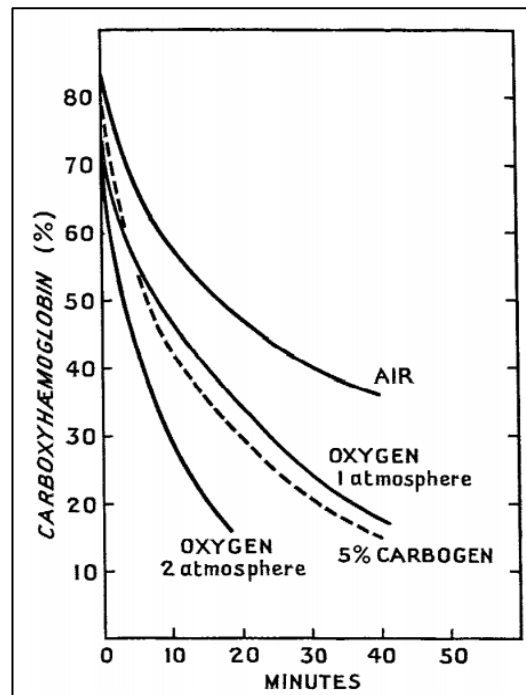


Fig. 21: COHbt_{1/2} in dogs to breathing air, 5% Carbogen or O₂, at 1 and 2 atm [50].

While Carbogen has been completely abandoned for CO poisoning since about 1970 in favour of HBO [8], from 1998 Sommer returned to the concept of hyperventilation for dogs and later for humans. To avoid secondary CO₂-induced adverse effects, he developed the concept of hyperoxic isocapnic hyperventilation. The amount of CO₂ delivered to the patient is no longer used to stimulate hyperventilation but only to maintain isocapnia during a voluntary hyperventilation. In a simple non-rebreathing circuit, a constant O₂ flow at

equivalent minute ventilation amounts is provided. As normal respiration, this fresh gas contributes to a gas exchange where physiological CO_2 produced by the body is eliminated. Then the patient is invited to increase his minute ventilation above his physiological rate, but the additional gas is passively delivered through an inspiratory valve supply by a CO_2 and O_2 mixture. As CO_2 in this additional ventilation is at 6% fixed rate, $\pm$ equal at physiological alveolar CO_2 pressure, there is no additional pCO_2 gradient and thus no CO_2 additional exchange. This hyperpnea, thus allowing to hasten $\text{COHbt}_{1/2}$, without modify to CO_2 gas exchange allowing thus to maintain isocapnia and hastening $\text{COHbt}_{1/2}$ at a rate close to HBO (Fig. 22) [57, 127]. Moreover, normoxic (at 0.21 FiO_2) isocapnic hyperpnoe, could be also possible and would be as effective in eliminating CO as classical NBO, while avoiding the risk of additional oxidative stress from hyperoxia (Fig. 22) [131]. For this purpose, a specific portative device (ClearMate®, Thornhill Research®, Canada), which produces a variable CO_2/O_2 mixture according to the patient's current alveolar ventilation was developed [160]. We contacted Joe Fischer the correspondent/author in Toronto promoting this technique in order to understand the non-use of this technique in clinical practice and why another independent author claims that "the only approved therapy is the ClearMate, which has not gained clinical acceptance" [167]. His response was: "It is true that isocapnic hyperpnoe has not been taken up clinically. It is hard to explain why informed people ignore a simple, inexpensive, safe, highly effective treatment and propose bizarre ones like Zazzeron in his study *Phototherapy and extracorporeal membrane oxygenation facilitate removal of carbon monoxide in rats*" [173].

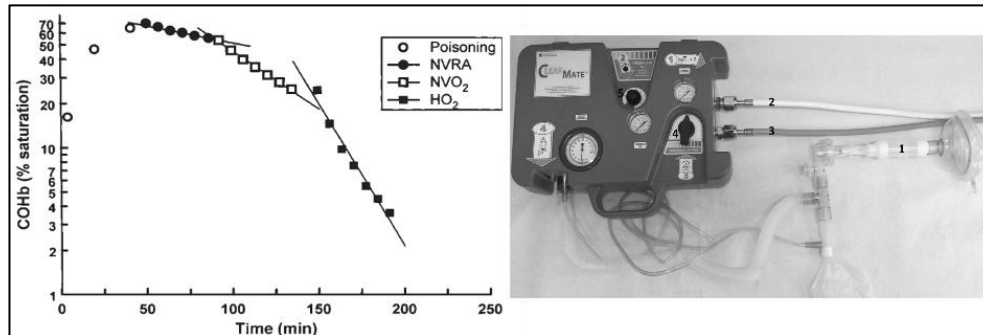


Fig. 22: (Left) COHbt_{1/2} on dog sequentially exposed to CO, ventilated at controlled levels with room air (NVRA) (COHbt_{1/2} at 212min) following 0.95 FiO₂ (NVO₂) (COHbt_{1/2} at 42min), and then, at sixfold ventilation control (hyperpnea) with 0.95 FiO₂ (HO₂) add to supplying approximately 5.5% CO₂ as isocapnic hyperpnea (COHbt_{1/2} at 18min) [57]. (Right) The ClearMate® device and the breathing circuit used. 1. Spirometer flow head; 2. O₂ supply; 3. CO₂ supply; 4. CO₂ supply on/off switch; 5. O₂ flow rate controller [160].

Zavorsky (2012) showed that hyperventilation induced by a mild exercise in addition to NBO is as effective on COHbt_{1/2} as an HBO treatment. Furthermore, moderate exercise on AA would be as effective in removing CO as NBO at rest. Thus they suggested that the combination of mild exercise, hyperventilation and NBO can be considered the "triple therapy" for CO removal in some patients [168]. Since Bruce's mathematical model predicts a rise in carboxycardiomyoglobin at the beginning of NBO, it is probable that exercise, although interesting from a COHb elimination point of view, is not appropriate [83, 84].

❖ ECMO AND/OR PHOTOTHERAPY

The introduction of invasive techniques such as extra-corporeal membrane oxygenation (ECMO) developed in the context of the treatment of respiratory failure from other indications has naturally led to study this coherent technique in

severe CO poisoning. The first case report in 2000 showed the ECMO feasibility as a life-saving procedure to provide cardiovascular stability, in addition to oxygenation, in patients with severe CO poisoning and shock [169]. This was confirmed from 2015 [170].

Moreover, in 2015, on CO-poisoning murine models, Zazzaron studied the photo-dissociation of COHb by direct pulmonary phototherapy after thoracotomy or by oesophageal illuminary therapy to access close to the lungs in a less invasive way. It significantly improves COHbt_{1/2} [171, 172]. The control 100% oxygen COHbt_{1/2} in mice was 6.8 min, and the oesophageal only lowered the COHbt_{1/2} to 5.7 min [171]. Four years later, he combined this phototherapy with ECMO and increased COHbt_{1/2} by threefold compared to ventilation with 100% O₂ alone and was associated with improved rat survival [173]. In 2021, technical modifications of the photo-ECMO system, including the development of a compact, portable device, permitted treatment of patients with CO at the scene of their poisoning or in hospitals that lack HBO facilities [174]. And, in 2022 a triple therapy was studied, when an HBO-photo-ECMO system was in vitro successfully tested to increase COHbt_{1/2} [175].

❖ CPAP and HFNC

Since 2005, Continuous positive airway pressure (CPAP) to treat acute pulmonary oedema is used in emergency departments and, since COVID-19 pandemy, high-flow nasal cannula (HFNC) has become also widespread in emergency rooms. In addition to classical non rebreathing mask (NRM), these alternative methods to provide 100% NBO to patients were assessed in case of CO poisoning [13].

The first case report on severe CO poisoning (COHb at 42%) using CPAP for two days without neuropsychological deficit developed at three days was published in

2010 [176]. This was followed in 2018-2020 by others case reports showing a $\text{COHbt}_{1/2}$ acceleration compared to NRM [177-180]. The discussions are rather poor but authors suggested that CPAP increases the gas exchange area and improves ventilation due to the positive pressure going into the alveoli [13, 180]. Recently, a clinical study on CO poisoning treated on CPAP at 5cmH₂O showed a $\text{COHbt}_{1/2}$ significantly decreased compared to NRM [45min (30–120) vs 105min (70–190)] [181].

In addition, a series of CO poisoning cases treated with HFNC was published with $\text{COHbt}_{1/2}$ observed at 36.8min [182]. Or, a mean $\text{COHbt}_{1/2}$ value in HFNC group at the first hour was found significantly lower than the NRM group: 9.5min $\pm$ 4.7 and 12.0min $\pm$ 5.1, respectively [183].

In contrast, two other recent studies compared $\text{COHbt}_{1/2}$ on HFNC (flow of 60 l.min⁻¹) to create a positive pressure effect to NRM (flow of 15 l.min⁻¹) on humans after mild to moderate CO poisoning. Globally, no significant difference was observed (Fig. 23) [184, 185].

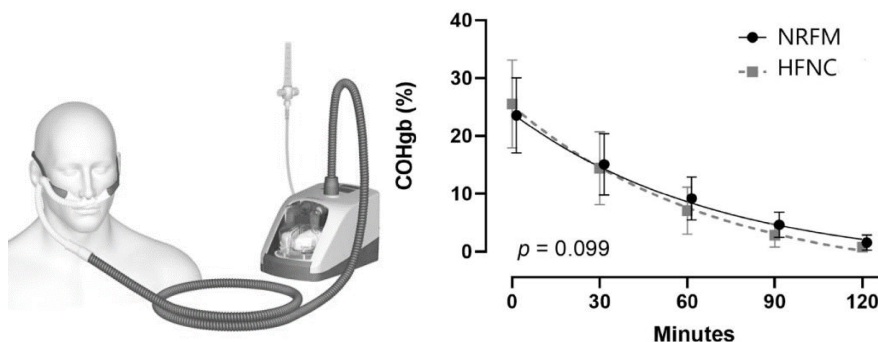


Fig. 23: HFNC as Optiflow® AirvoTM2, Fisher & Paykel© Healthcare, New Zealand; $\text{COHbt}_{1/2}$ on HFNC compared to NRM (NRFM) [184].

❖ PHARMACOLOGICAL CO ELIMINATION BY ANTIDOTE THERAPIES

To date, no antidotes to counteract CO toxicity currently exist, as for example hydroxocobalamin for cyanide poisoning [186]. Nevertheless, research is underway to find an equivalent antidote specific to CO.

Synthetic hemoprotein complex (HemoCD) similar to Hb or Mb has been in development from 2010 (Fig. 24). Its CO binding affinity is ≈ 100 times higher than that usually for Hb, whereas the O_2 binding affinity is moderate [28]. When infused in animals, most of CO-hemoprotein complex is excreted in the urine within one hour [8, 21]. This unique property led to research into the biological function of endogenous CO as a gaseous signal mediator in living systems with investigations into the effects of the total absence of CO in the organism [21, 187].

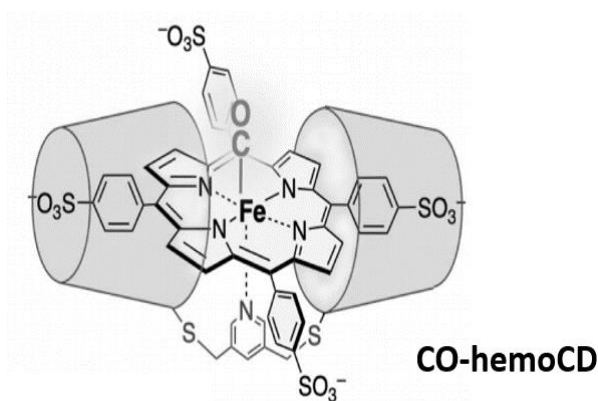


Fig. 24: Supramolecular interaction between Py3CD and FeIITPPS to form 1:1 inclusion complex (hemoCD) [28].

A second chelator, an engineered human neuroglobin (Ngb as Ngb-H64Q-CCC) was developed in 2015. It binds CO about 500 times more strongly than did Hb and about 80.000-fold than Cytochrome C oxydase, near-irreversible, tested in vitro and on mice. This Ngb reduced COHbt_{1/2} to less than one minute and was followed by

rapid renal elimination [188]. This CO scavenger could then reverse the binding of CO to Hb and Cytochrome C oxydase and then CO-dependent inhibition of mitochondrial respiration [102]. Further human studies on Ngb-H64Q-CCC seem to be planned [102].

And, in 2022 a study assessed the scavenger features to remove CO from the red blood cells on murine model by evaluating three readily available hemoproteins: 2,3-diphosphoglycerate stripped human Hb (StHb); N-ethylmaleimide modified Hb (NEMHb); and equine Hb (*Mb*). StHb, NEMHb, and *Mb* improved survival by 100%, 100%, and 60%, respectively [189].

Another manner of improving CO elimination is to foster the catalyse of CO in CO₂. CO is physiologically oxidized to CO₂ by the body, but amounted non-significant to less than 0.1% [20, 94]. This oxidation process was interestingly used from 1924 with the 'Self-rescuer filter' device (Fig. 25) which was able to oxidize atmospheric CO inhaled to "safe" CO₂ as a respiratory protection against CO in mines to escape from toxic atmosphere. Theoretically, the increased blood CO₂ was compensated by hyperventilation due to the escape from the mine [190, 191].

Since 2015, B12r, a Vitamin B12 (hydroxocobalamin with a Cobalt centre) reduced by ascorbic acid (vit C) buffer has been developed. It is able to catalyses a conversion of CO to CO₂ in pre-clinical study in vitro on human blood and on in vivo rodent. This reaction was already studied in vitro in 1970 without suggesting any clinical application for CO poisoning [192]. This antidote seems to be safe for humans and on rat models and shows clinically significant reduction in COHbt_{1/2},

evidence of increased brain oxygenation, and amelioration of myoglia damage [193].

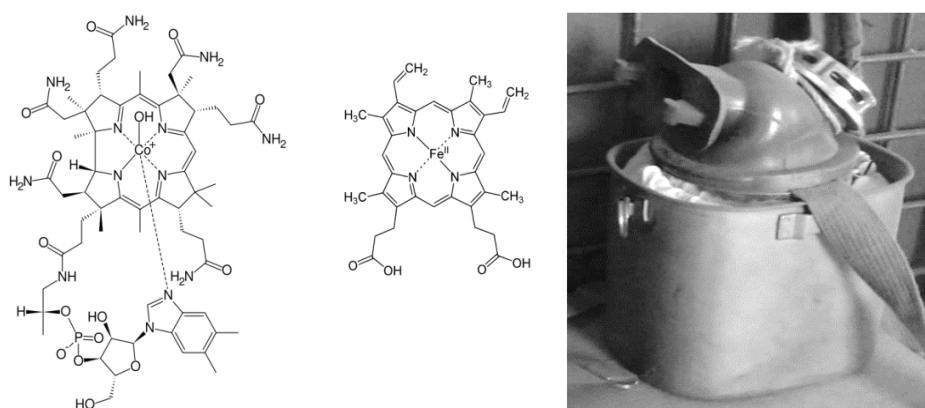


Fig. 25: (Left) Structural similarities between Hydroxycobalamin (Vit B12) and Heme B. (Right) Self-rescuer filter device's oxidative reaction is catalysed with a mixture of copper and manganese oxides. Once in use, the device has a working life of around two hours, time to escape from a mine, but need an atmosphere containing more than 19.5 % O₂ to allow CO oxidation.

In 2023 we are still in search of an effective treatment. Widely different fields like tobacco smoking, domestic and environmental pollution monitoring, all industrial combustion process, the aeronautic and space industries, the food industry - CO is used to colour and conserve meat - are all areas currently sensitive to CO. Where there is the possibility of exogenous CO, all extra hospital front-line care givers are obliged to wear a CO detector. Moreover, endogenous CO is studied to understand the role of neurotransmitters role or as a route to possible treatments for different indications. It is now even possible to administer CO as a pill as a prodrug. Interest in this gas remains very relevant.

1.6.2. COHbt_{1/2} discrepancies reported in studies

The COHbt_{1/2} reported in these studies over the past 125 years are divergent, depending on the subject or model exposed to CO, the characteristics of the exposure or post-exposure, including the treatment modalities (Fig. 26 and 28). After reviewing COHbt_{1/2} found on human or animal investigations from 19th century to date, we report COHbt_{1/2} discrepancies on human data summarized in Fig.26 and table 2. And, in Fig.27, a sheep experimental model is exposed, illustrating the link between COHbt_{1/2} and exposure protocol features. At the end, an additional overview illustrating the origins of these discrepancies is provided in Fig. 28.

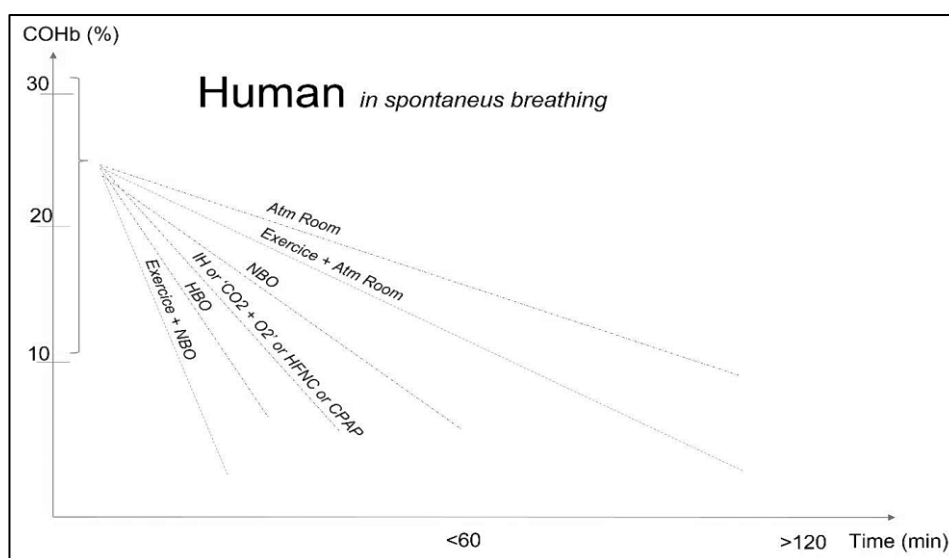


Fig. 26: COHbt_{1/2} discrepancies illustration on Human on different treatment facilities.

Atm Room	NBO	HNC (NBO)	HBO
270 ±60 and 192 ±24 (Deller 1992) 270-180-275 (Sneyd 1993)	12 ± 5,1 (Tomruk 2019) 1h 44 ± 10,5 (Klasner 1998) 57,76 (Myers 1983) FIO_2 60-90% 57 and 194 (Kaiser 2014) 64 (43-114) (Yesiloglu 2020) 74 ± 25 (Weaver 2000) 78 ± 9 (Li 2006) 87 ± 32 (Levasseur 1996) 126 ± 246 (Pierce 1998) FIO_2 63,5+35,5% 80-95-160-170-270-280 (Sneyd 1993) FIO_2 50-60% 80 ±5 (Bal 2020) 105 (70-190) (Turgut 2020) 137 (Burney 1982) 131 ± 133 (Myers 1984) 134 ± 111 (Kim 2020)	9,5 ± 4,7 (Tomruk 2019) 1h 37 ± 9,3 (Ozturan 2019) 41 (31-58) (Yesiloglu 2020) 43 ± 10,6 (Kim 2020) CPAP (NBO) 45 (30-120) (Turgut 2020) 36 ±5 (Bal 2020) NBO + Exercise 23,2 ± 3,5 (Zavarsky 2012)	26,3 ± 3,7 at 1,58 atm (Jay 1997) 27,5 ± 1 at 1,58 atm (Jay 1995) 43 ± 22 at 1,95 atm (Myers 1984) 15,1 [14 -15,6] at 2,5 atm (Pace 1950) 22,3 [19,1-26,5] at 2,5 atm (Pace 1950) 23,2 at 3 atm (Peterson 1970)
150 W to 222 M (Rode 1972) 179 (Peterson 1950) 249 (Pace 1950) 287 ± 56,5 [220,4 to 360] (Zavarsky 2012) 320 [128-409] (Peterson 1970) 236 ±34 and 302 ±39 (cited in Benignius 1994) 330 (Myhre 1974)	36 and 47 (Pace 1950) 52 and 68 (Tesler 2000) 57,11 (Rucker 2002) 71,3 ± 9,9 (Jay1997) 78,4 ± 24 (Takeuchi 2000) 80,3 (Peterson 1970) 90,1 ± 5,22 (Jay 1995)		
252 (Coburn 1965) 248 (206-328) (Bruce 2006) 226 (Tyuma 1981)			
Atm Room + Exercise 101,1 ± 15,2 (Zavarsky 2012)			
	Normocapnic Hyperoxia Hyperventilation (NBO) 31 ± 6 (Takeuchi 2000) 35 ±13 (Sein 2017) 45 ± 8 (Rucker 2002)		
		Clinical study	
		Experimental study	
		Mathematical model	

Table 2: COHbt_{1/2} discrepancies on human experimental, clinical or mathematical studies. COHbt_{1/2} reference is Weaver at 74+-25 min. Treatment modalities were Atmospheric room; Normobaric oxygen therapy; High-flow nasal cannula; Hyperbaric oxygen therapy; Continuous positive airway pressure; Normo-(iso-)capnic hyperoxia hyperventilation; Add exercise.

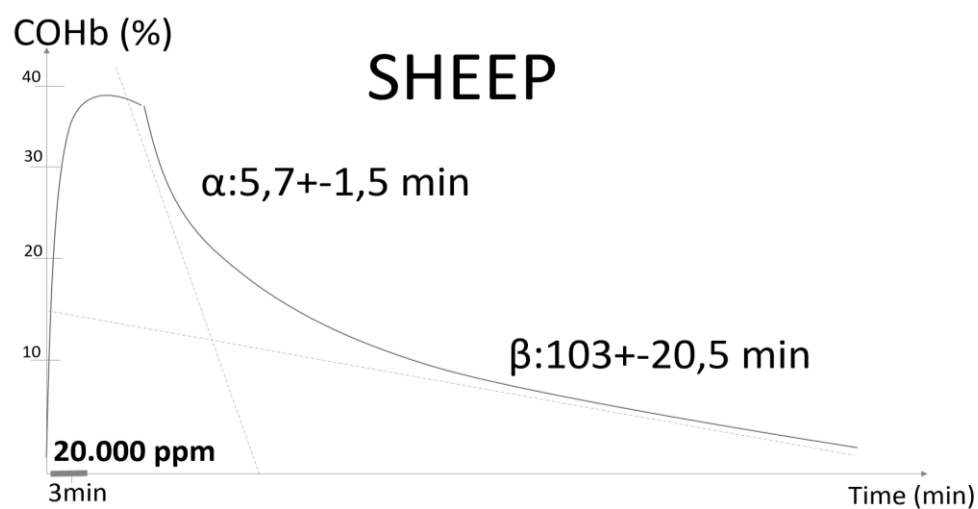
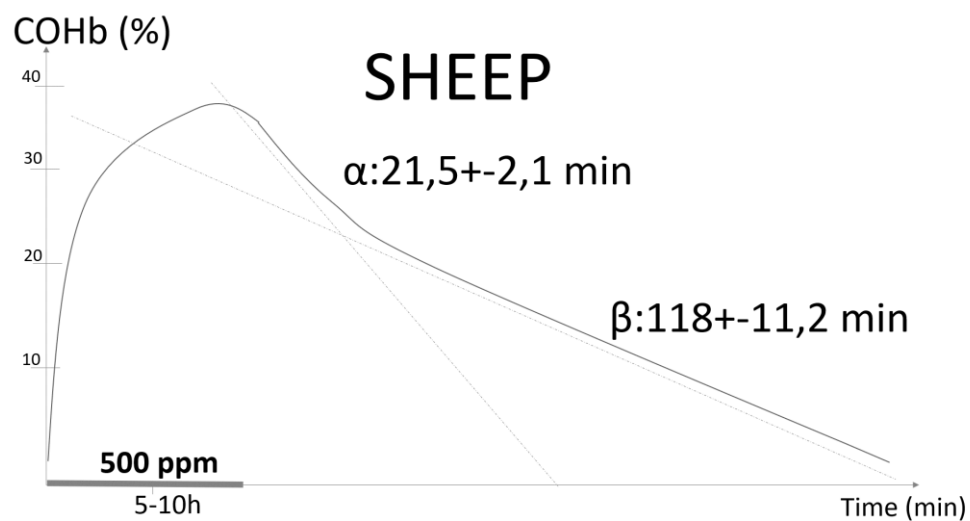


Fig. 27: COHbt_{1/2} comparison on experimental sheep study between low CO level exposure (500ppm) during long exposure (5-10h) and high-level exposure (20.000ppm) during very short exposure (1-3min) (from Shimazu). Peak COHb% were comparable at %COHb 30–40%. Curves observed showed bi-compartmental toxicokinetic (see below) [194].

- COHb% (↗; COHbt_{1/2} ↗)
- Hb mass (↗; COHbt_{1/2} ↗); Anaemia (COHbt_{1/2} ↘)
- CO exposure duration (↗; COHbt_{1/2} ↗)
- CO exposure level (↗; COHbt_{1/2} ↗)
- O₂ Atmospheric pressure (↗; COHbt_{1/2} ↗)
- Alveolar ventilation (Respiratory rate and tidal Volume) (↗; COHbt_{1/2} ↘)
- O₂ concentration or supplied (↗; COHbt_{1/2} ↘)
- Physical activity (↗; COHbt_{1/2} ↘)
- Time at which samples are done (<1h; COHbt_{1/2} ↘); (>1h COHbt_{1/2} ↗)
- Dissociation constant of COHb complex: the (M) constant and its variation,
- pH et body temperature
- Species
- Gender: could be ±6% longer in males compared to females,
- Individual variability
- Pulmonary chronical disease (↗; COHbt_{1/2} ↕)
- Initial %COHb level (↗; COHbt_{1/2} ↕)
- Smoking (↗; COHbt_{1/2} ↕)

Fig. 28: Factors impacting or not COHbt_{1/2} reported in literature. Adapted from [13, 84, 125, 195]. '↕' = no impacted.

All these variability features could explain discrepancies in COHbt_{1/2} observed. They highlight the importance of a tight exposure and post-exposure protocol, control in experimental studies, and the fragility of overall clinical studies.

COHbt_{1/2} is assessed assuming a toxicokinetic model. If the model used is not a good one, the assessment could be incorrect. The type of model observed, mono- or bi-compartmental, gives us more precise indications of CO distribution in the body and of its potential storage in the extravascular tissues. Lack of attention to this point could explain why the studies fail to confirm the superiority of one treatment over the other. Historical studies on different CO topics sometimes briefly identified the toxicokinetic model of the COHbt_{1/2} curve observed as mono- or bi-compartmental, but without focusing on the importance that this determination

can bring to the understanding of the physiopathology of CO and treatment implication.

To better understand the origins of the current controversy on mono- or bi-compartmental issue, here are, in chronological order, the key studies evoking the toxicokinetic behaviour of CO elimination or the existence of CO binding to other quantitatively significant metalloproteins such as myoglobin - which could at the end suggest extravascular storage.

(1929, Sayers) COHb elimination is observed to be in **two phases falling**, fairly quickly, from COHb at 30% to 20% in less than two hours, followed by seven to eight hours to backed at COHb 5% [196].

(1934, Theorell) **Myoglobin** is similar to Hb in its combination to CO, but M is only at 13.8 for horse Mb, 27 for dog and 20 or 40 for human [20, 197-200].

(1941) CO binds to **myoglobin** as shown by Pagniez and confirmed in 1958 by Rossi-Fanelli and by Coburn in 1971;1973 [100, 199-201].

(Roughton, 1945) Shows that the amount of CO expired during 15-45 minutes after the end of a CO exposure lasting ≈ 4 min is significantly less than the amount lost from the blood, implying that **significant blood-to-tissue flux of CO persisted after CO exposure** period [202].

(1948, Schwerma) **Biphasic** elimination is observed on dog NBO-treated (Fig. 29) [203].

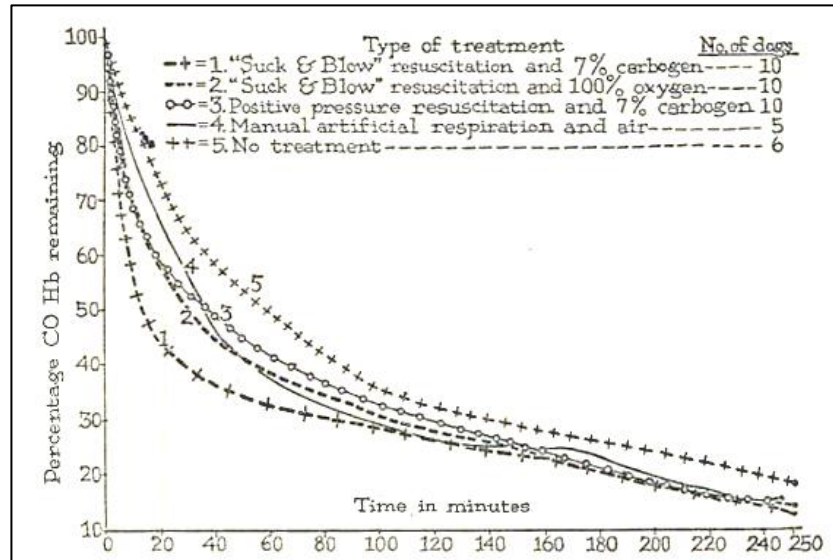


Fig. 29: %COHb elimination from the blood on dog AA-; NBO-; or Carbogen-treated.

(1965, Coburn) Coburn–Forster–Kane equation (CFKE) described physiological variables that define pulmonary CO uptake and excretion, and %COHb in man (Fig.15). They assumed a CO washout following **first-order kinetics** [204].

(1970, Coburn) Reviewing on CO body store and CO binding in **extravascular tissues using myoglobin** as model. Distribution of CO between blood and tissues is approximately constant from %COHb 0 to 50%. Otherwise, myocardial %COMb is three times greater than in skeletal muscle [20].

(1972, Godin) Model improvement to discuss the course of CO uptake and release in humans AA-treated after low CO exposure. Model underestimated the early fall of COHb level following exposure; equilibration with **extra-vascular pigments was evoked** [205].

(1974, Myhre) **Biphasic excretion** pattern occurred at an altitude of 1630 meters on humans AA-treated. However, he noted that $\text{COHbt}_{1/2}$ was much longer (5.5 h) [206].

(1994, Benignus) Experimental studies on 15 humans who inhaled the same amount of CO over a 4- to 6-min period and monitored for 4.5 h on AA [207]. In 2006, Bruce used these data to show the washout curves were **not mono exponential** [204].

(1975, Sasaki) **Mono-compartmental** elimination is observed in human in vitro blood and in canine AA-; NBO-; HBO-treated (Fig. 30) [133].

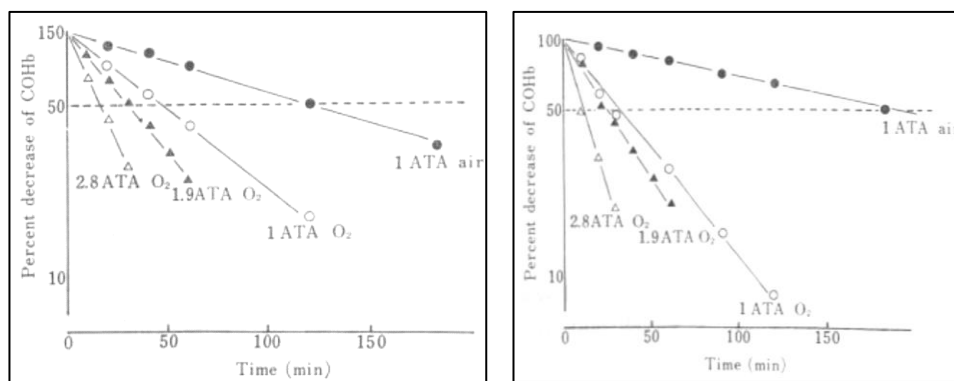


Fig. 30: %COHb decrease in vitro on human blood (left) and in vivo in canines (right). A semi-logarithmically linear tendency was observed.

(1975, Wagner) **Biphasic** (multi-compartmental elimination) $\text{COHbt}_{1/2}$ is observed on canines AA-treated [208].

(1996, Wazawa) **Biphasic** (multi-compartmental elimination) $\text{COHbt}_{1/2}$ is observed on rabbits AA-treated [209].

(2001, Kreck) speculated briefly that CO follows first orders kinetics with **mono-compartmental** elimination on sheep isocapnic ventilation AA-treated [210].

The three following recent studies are at the heart of the actual controversy. Nevertheless, in our opinion it seems that the results of these studies aren't really in opposition but that the controversy stems from a methodological issue.

(2000, Weaver) **On retrospective clinical study on human NBO-treated.**

COHbt_{1/2} has been reviewed on 100% (FiO₂ 1.0) target NBO from 1985 to 1995 in his clinical centre. COHbt_{1/2} was 74+-25min (range 26 to 148 min), shorter than in previous clinical reports and became the reference [125]. Weaver observed **mono-compartmental** COHbt_{1/2} TK model on a clinical study and contradicted Shimazu results on experimental studies [211].

(2000, Shimazu) **On experimental prospective study on animal AA-treated.**

COHbt_{1/2} were compatible with a **bi-compartment model** reported on sheep poisoned at %COHb 30 to 35% and treated three hours on AA after short-term (few min) or long-term (5 to 10 hours) exposure [97].

(2003-2006-2008, Bruce and Bruce) **On mathematical model using prospective experimental data on human AA-treated.** New mathematical model, more comprehensive than CFKE, is combined with data from Benignus' previous experimental studies on human with short CO exposure at 5min and high level at 6000ppm [207]. This study was selected because COHbt_{1/2} assessment treated with AA were as long as 4h [207]. It fitted with **multi-compartmental model** and the authors' highlighted difficulty linked to retrospective analyses of toxicokinetic [83, 204, 212]. Purpose is to respond to the need of a better understanding of the factors that influence CO washout to optimize treatment strategies and especially COMb which is not available in clinical practice [212]. Bruce claims that Weaver did not have sufficient data to distinguish a linear from an exponential decrease in COHb [204].

In these three studies there is no investigation on toxicokinetic model with a prospective experimental procedure on animal NBO-treated as it was done in 1948 and 1975 on dog with opposite results [133, 203].

Thus, experimental studies in the 21st century seem to confirm that the decline in blood %COHb following CO exposure exhibits at least two kinetic phases [84, 194]. The fast phase is thought to reflect a combination of exhalation of CO along with slower distribution of blood CO to tissues that continue after cessation of exposure [84]. Because P_{CO} in the blood remains high for a long-time during washout on room air, CO could continue to flow from blood to the muscle compartment for ± 250 min after termination of the exposure. The flow of CO into the muscle compartment causes the rate of fall of COHb during the early washout phase to be greater than that achieved by washout through the lungs and ventilation alone (phase- α); however, later CO flows from the muscle compartment back to the blood and $COHbt_{1/2}$ is less than the rate at which ventilation removes CO from the blood (phase- β) [204]. The $COHbt_{1/2}$ for the slow phase- α is approximately 100–300 min. $COHbt_{1/2}$ for both phases appear to increase with exposure intensity and/or duration; a contributing factor to this effect may be the amount of CO accumulated in extravascular stores.

Moreover, if the observations are in favour of a multi-compartmental model, it needs to compare studies of CO uptake and elimination under different treatments with more precision - by comparing, for example, the different elimination phases, or considering residual CO storage even when COHb has returned to normal. In addition, using the appropriate model could allow extrapolation of initial COHb peaks from the curve obtained with blood samples collected during a victim's first care. The initial peak could be used by also considering the estimation of the duration of CO exposure as an objective criterion in order to decide on a therapy.

According to a bi- or multi-compartmental model, the time at which COHb samples are obtained can influence calculated COHbt_{1/2} as we are now able to develop in the following sub-chapter [84].

1.6.3 Reported methods for calculating COHbt_{1/2}

Literature reports a wide range of values for COHbt_{1/2} (table 2) [59, 84]. Ideally, calculated COHbt_{1/2} should closely estimate the actual time required for COHb to fall from its peak value to 50% of the peak value; however, in clinical studies, the accuracy of these calculations would depend on the post-exposure times at which COHb samples were obtained.

To address COHbt_{1/2} estimation, three different methods are used.

- A first method used to determine COHbt_{1/2} is designated **the “true” half time (t₅₀)** for washout of CO, calculated from minute-to-minute %COHb, but often it did not accurately reflect COHbt_{1/2} at later times when COHb was below one-half of its peak value. The first half time calculated by this method, is designated t₀₋₅₀ and the second half time is designated t₅₀₋₇₅ [204].

For example, from Begninus' experimental study, the mean true COHb half time (t₀₋₅₀) for the 15 subjects was 248.5 ± 39.6 min (range 206 – 328 min), whereas for the (t₅₀₋₇₅) was 302.5 ± 50.3 min (range 251– 406 min). The mean difference between the two half times was 54.0 ± 12.1 min [204, 207].

- The second method called **the two-points method**, estimated the COHbt_{1/2} at two different time points and therefore privileged in clinical studies that do not have many samples available. If c1 and c2 are the %COHb values at times t1 and t2 during the CO washout phase, then COHbt_{1/2} based on this two-points method is given by:
$$\text{COHbt}_{1/2} = \ln(2) \times (t_2 - t_1) / [\ln(c_1) - \ln(c_2)] \quad [204].$$

This two-points method assumes that COHb follows a mono-exponential decay during washout.

- The third method is **the linear regression method**, that involves fitting a straight line via linear regression to a plot of the natural logarithm (ln) of %COHb vs. time during washout. This is the most accurate method, requiring more sampling, which is preferred in experimental studies. From the slope of the line, COHbt_{1/2} is calculated as $\text{COHbt}_{1/2} = \ln(2)/(\text{magnitude of slope})$. If COHb follows a mono-exponential decay, then the plot of ln(COHb) vs. time will be a straight line and COHbt_{1/2} can be properly interpreted as a half time. In contrast, if plots are curvilinear, it's possible to determine linear fits to both early and late portions of the washout curves. Thus each COHbt_{1/2} value represents an “apparent” half time given only the data used to fit each line [204].

Bruce in 2006 tried in part to resolve the mono- or bi-compartmental controversy between human observational clinical studies from Weaver [125] and experimental animal studies from Shimazu [97]. He took over data from experimental study of 12 humans from Benignus [207] extracting variable %COHb at various times, applying the two-points method to mimic a clinical context [84]. Actually, it's this method which is used in observational clinical studies when only two or three samples are available to estimate COHbt_{1/2} depending on the time of sampling after exposure considered. COHb levels were available at 5, 19, 35, 67, 131, and 259 min postexposure on the 12 subjects and were compiled and compared between them and to COHbt_{1/2} obtained with the t₀₋₅₀ values from first method (Fig.31 A) [204].

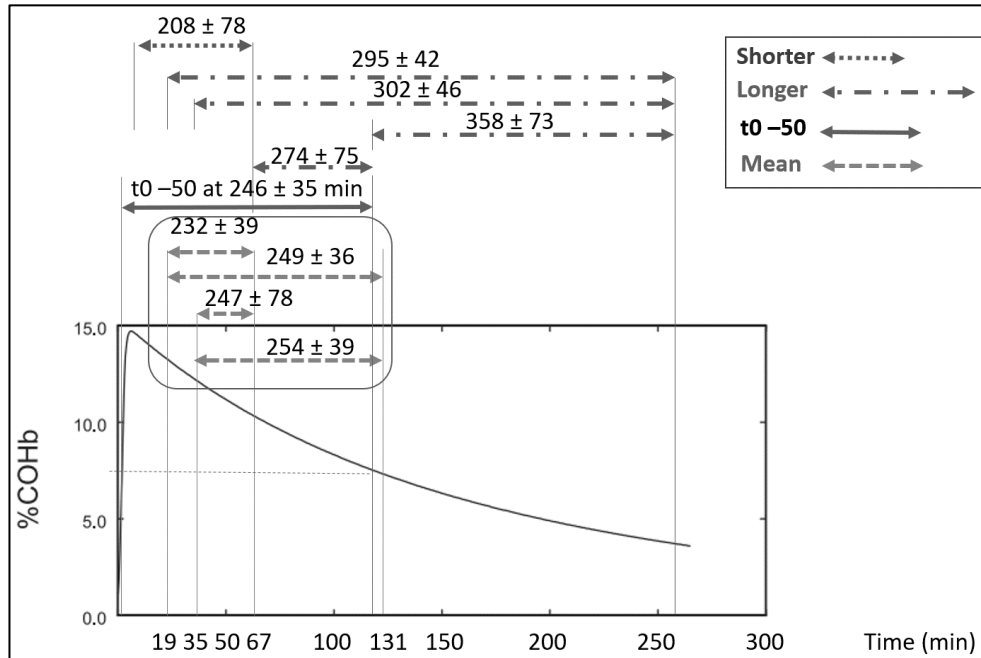


Fig. 31 A: Different means (SD) COHbt_{1/2} results on AA which could be obtained using the **two-points method** depending on the postexposure blood sampling time considered. Only 'Mean' COHbt_{1/2} values were similar to the value using the '**true method**' t₀-50 [207]. In contrast, the COHbt_{1/2} calculated at earlier time points was shorter whereas the COHbt_{1/2} were considerably longer when determined from blood COHb levels obtained with more spaced time [84].

In the following figure (Fig. 31 B), COHbt_{1/2} obtained from the same data but with the linear regression method are exposed to highlight the origins of the discrepancies.

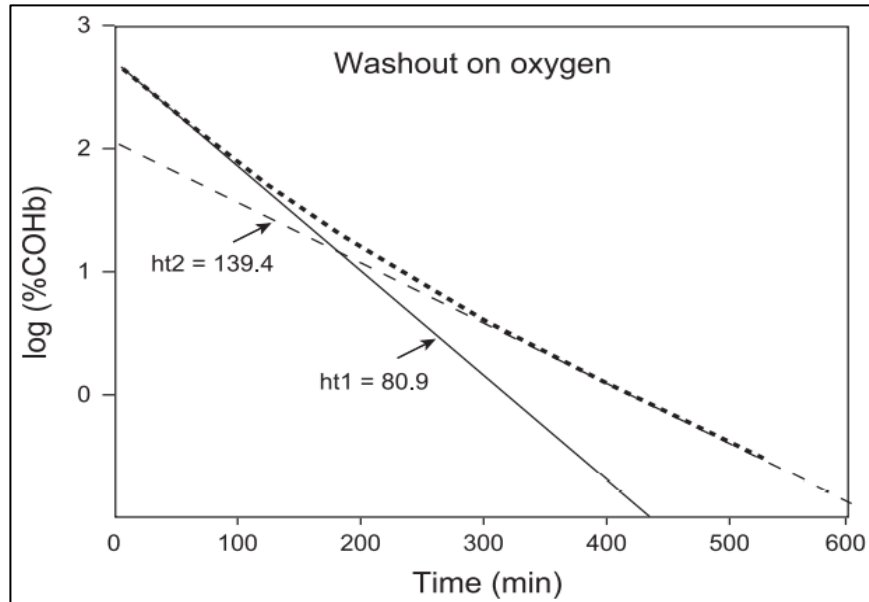


Fig. 31 B: CO washout from the same data assessed with the **third method** fitting with a bi-compartmental model with two COHbt_{1/2}. A short COHbt_{1/2} at 80.9 min is followed by a longer COHbt_{1/2} at 139.4 min (from Benignus' data on experimental human study) [84, 207].

We conclude from these considerations that blood sample times are essential to assess accurately COHbt_{1/2} in clinical studies. In addition, starting treatment time or real FiO₂ delivered estimated by well-adjusted treatment devices is also to consider [213].

COHbt_{1/2} may be also affected by the duration and dose of CO exposure in experimental or mathematical modelling. This could explain the discrepancy with retrospective clinical CO poisoning which does not have these data at disposal [97, 194]. Nevertheless, age, gender, smoking, smoke inhalation, loss of consciousness, initial COHb, or a metabolic acidosis does not seem to impact the COHbt_{1/2} in retrospective clinical study [96, 125].

In fact, there is no way today to assess muscle $\text{COMb}_{t1/2}$ in clinical practice. But, if this was possible, $\text{COHb}_{t1/2}$ discrepancy observed could also probably be correlated to CO amount uptake by muscle (COMb) as shown by Bruce's model (Fig. 32) [84].

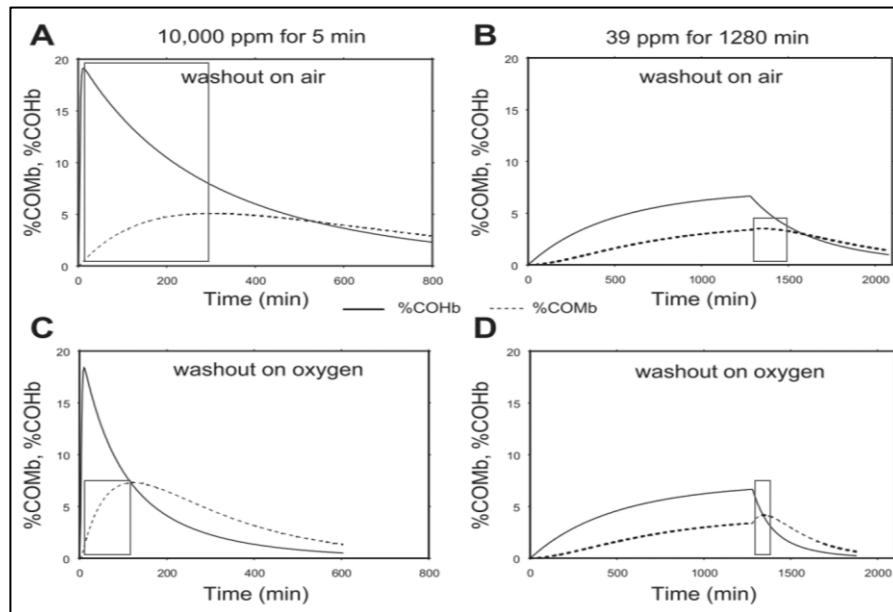


Fig. 32: Multi-compartmental model of factors affecting CO washout included CO diffusion from blood to muscle. Model from Bruce is illustrated after 2 different CO exposure concentrations and durations. (A) On washout on air, CO continued largely to diffuse to the muscle compartment for ± 300 min after CO exposure at short and high concentration (10.000ppm for 5min). (B) A much smaller but longer CO exposure rate results in a smaller CO diffusion from blood to muscle during washout on air (39ppm for 1280 min). (C) On washout on O_2 , CO uptake by muscle was much greater after 10.000 ppm exposure for 5min than (D) after the 39ppm exposure for 1280 min [84]. Thus, COMb levels would increase progressively during CO exposure and continue to increase, at an even faster rate, during the initial part of hyperoxic therapy. This response during therapy was most significant following short exposures to high CO levels. This behaviour occurs because arterial P_{CO} continues to be greater than muscle P_{CO} early in hyperoxic therapy, and this pressure difference drives CO from blood into muscle tissues [83].

1.7. CURRENT CARBON MONOXIDE TREATMENT

1.7.1. Importance of prevention

Despite more than 150 years of studies into CO, effective treatment to avoid DNS are still controversial; despite treatment, up to 40 % CO poisoning patients suffer from DNS, permanent or transient neurocognitive and/or affective disorders [214, 215]. This highlights the importance of prevention. Thus, prevention concern must remain the first approach, as identified by Haldane in 1895, considering little animals as the first effective CO living detector used until 1987 when they were replaced by electronic monitors [216]. Mice and birds, have a lower affinity (M) of Hb for CO, but are, however, well known to be more sensitive to CO as demonstrated by their use in coal mines. This paradox was discussed by Haldane. *How is it possible to be more sensitive to a toxic substance with a lower affinity to it?* [217]. The explanation given was that these animals had a high basal metabolic rate, causing symptoms of poisoning before gas levels became critical for adults and workers [218]. In 1874, Gréhan had already proposed keeping birds in apartments when gas was used for heating [219].

More recently, in 2012, Mutluoglu reported a clinical demonstration of the relationship between CO poisoning and the species. A mother, a 3-year-old child and a bird were accidentally exposed to the same CO atmosphere. First, the domestic animal blacked out, then the child was symptomatic, and lastly the mother. The author linked this chronological event to the fact that children have less blood volume and increased minute ventilation per unit of body mass compared with adults [220]. Another similar story was described in 2013. '*Sudden death of bird alerted a family to danger*' [216].

Considerable protection or detection devices have now been developed and are available on the market for occupational, industrial or domestic use. All extra hospital front-line care givers are obliged to wear a CO detector.

U.S. National Health Promotion and Disease Prevention Initiative “Healthy People 2010”, has listed the CO exposure surveillance as one of the major environmental health priority areas [160]. If residential CO alarms are as effective as smoke alarms, or even 75% as effective, the cost-benefit ratio for their universal application could be overwhelmingly favourable [2, 221].

When prevention fails, the target of any treatment proposed for CO acute exposure is to reduce mortality, normalise COHb levels, alleviate symptoms, reduce the incidence of delayed neuropsychological sequelae (DNS), and reduce cardiovascular morbidity, with minimal adverse effects of treatment [222].

1.7.2. Carbon Monoxide Poisoning Therapies

Ideally, a therapy could be portable to be provided immediately in the field by emergency medical services to limit treatment delays or in the emergency department, being targeted, rapidly effective, and safe even in severely-ill patients without access or unable to tolerate HBO [8, 223].

Current therapy for CO poisoning is 100% NBO or HBO (2.5–3 atm) [7, 222]. NBO and HBO remove CO at a faster rate from the blood by increasing the partial pressure of O₂, which improves the COHbt_{1/2} [125, 222]. NBO reduces the COHbt_{1/2} from 320 min in room air to 74 min [7, 89, 125, 224]. HBO can reduce COHbt_{1/2} to 20 min [35, 42]; however, in actual clinical practice observed, the half-life may be higher, up to 42 min [225]. Almost all patients receive NBO upon rescue or arrival at the emergency department [8]. Nevertheless, no systematic review, RCTs, or

cohort studies indicated the optimal duration of NBO 100% O₂ treatment after mild CO poisoning [222]. In practice, it is recommended to continue for at least six to 12 hours or until COHb levels fall below 5% considered as a safety level or even ≤3% [5, 7, 8, 125, 149, 222, 226, 227]. After this, if patients feel well, repeating the COHb level seems not to be necessary [7].

Increased pulmonary ventilation and a maximum safe rise of inspired p_{O2} combine to hasten the dissociation of COHb and excretion of CO [10]. Carbogen used from 1922 to ± 1960 to increase ventilation was considered as less secure compared to HBO since the 60s. Technological progress with more accurate CO₂ monitoring and specific CO₂ delivery device with isocapnic hyperventilation allowed clinicians to reconsider the hyperventilation approach from the beginning of the 21st century [131], but in practice, this therapy has not gained clinical acceptance [167] and studies on this topic are focused on COHbt_{1/2} more than on DNS. Waiting for more studies, HBO which has the capacity to avoid DNS for some authors, remains at the forefront.

Historical arguments for applying HBO were that the increased tension of O₂ speeds the COHbt_{1/2} and the elimination from myoglobin, particularly of the heart muscle, but also enough O₂ is physically dissolved to correct the hypoxia immediately, despite much of the Hb being useless as an O₂ transport because of combination with CO [228]. We have seen that the effect on myoglobin could be now questioned in mathematical models [84]. HBO could have demonstrated a reversal effect on inflammation such as inhibition of lipid peroxidation, impairment of leukocyte-endothelium adhesion, improvement of mitochondrial function and resolution of cerebral oedema induced by CO poisoning [147, 229-231]. Theoretically, HBO seems to be the ideal treatment for CO poisoning but proof of real clinical impact remains very controversial [145]. Its use is therefore to be balanced against the potential risks of induced O₂-toxicity [6].

1.7.3. Current Carbon Monoxide Therapy Recommendations

Despite the demonstration of an efficiency of HBO in counteracting some biological steps responsible for the toxicity of CO, clearing CO from the blood and some beneficial effects demonstrated in animals [19, 132, 232], in practice, clinical studies struggle to demonstrate a real clinical efficacy in avoiding DNS [5, 131]. Thus, treating CO-poisoned patients with HBO therapy remains controversial [5, 6, 35, 233] and there are different policies in different countries. Although HBO therapy is not systematically recommended for CO-poisoned patients in the United Kingdom, it is the treatment for CO poisoning that is widely used in Taiwan or in U.S. [7, 13, 149]. Even when the diagnosis is correctly established, appropriate therapy is widely debated. Hyperbaric societies and acute medicine societies do not share the same level of recommendations/conclusions even in the same country. Thus, HBO is the standard therapy, and the efficacy of HBO stays unclear [234].

For example, according to the “Clinical Guidance for Carbon Monoxide (CO) Poisoning”, as published by the **U.S.** CDC (The Centers for Disease Control and Prevention) or the **U.K.** Department of Health and the NHS England, HBO therapy shall *“be considered when the patient has a COHb level of more than 25–30%, there is evidence of cardiac involvement, severe acidosis, transient or prolonged unconsciousness, neurological impairment, abnormal neuropsychiatric testing, or the patient is ≥36 years in age. HBO is also administered at lower COHb (20%) in case of neurological signs or symptoms other than headache, myocardial ischaemia/arrhythmia diagnosed by ECG, or pregnancy”* [7, 235, 236]. More nuanced, the American College of Emergency Physicians (2008) ACEP clinical policy addressed critical questions about the role of HBO therapy and concluded [224] that although HBO is a therapeutic option for CO poisoning patients, its use cannot be mandated.

The Undersea and Hyperbaric Medical Society (UHMS) was formed in 1967. It is an international non-profit association serving some 2,000 physicians, scientists, associates and nurses from more than 30 countries in the field of hyperbaric medicine. It organizes an annual scientific meeting in U.S. Scientific societies such as the UHMS and the European Committee for Hyperbaric Medicine (ECHM) have published guidelines on the use of HBO in patients with CO poisoning.

While UHMS recommends the use of *HBO therapy for all cases of acute symptomatic CO poisoning* [224, 237], ECHM recommendations include only: *unconsciousness at or before admission, clinical neurological, cardiac, respiratory or psychological signs or symptoms and pregnancy* [238]. Recent practice recommendations by Hampson or Weaver, U.S. experts in the hyperbaric medicine field, however, do recommend HBO for all cases of serious acute CO poisoning [224].

In **Europe**, the criteria used for patient selection of CO poisoning for HBO therapy are still not universally agreed. Additionally, HBO therapy protocols used at European hyperbaric centres vary significantly, suggesting a need for more education regarding the published guidelines [147]. The dilemma is the fact that HBO schemes are applied vary widely across Europe, which imposes limitations on future meta-analyses and their validity [7, 149, 239].

In **Germany**, a national guideline is currently under development by the DIVI (German Interdisciplinary Association for Intensive Care and Emergency Medicine) and has concluded that '*a common opinion nowadays seems to be that routine HBO treatment cannot be recommended in general, but may be beneficial in patients with severe intoxication*' [240, 241].

At the end, a meta-analysis of seven randomized control trials (see above), with a total of 1,361 participants [135], did not reveal an overall benefit from HBO. No

further larger prospective studies have been published since then [239]. Furthermore, at the time, no clinical variables seemed to identify poisoned patients for whom HBO was most likely to provide benefit. This point of view was confirmed by the ACEP in 2017 as *“Despite the existing literature on this topic, there are few well-designed clinical trials, and the results of these trials are not conclusive in regard to the efficacy of HBO in preventing neurologic sequelae. An adequately powered multicentre randomized controlled trial with well-defined inclusion criteria, standardized treatment protocols, minimal delay in administration of HBO therapy, and adequate retention for long-term follow-up is needed to definitively answer the question. Ideally, further research should include groups long thought to be at greater risk for neurologic sequelae, such as children and fetuses”* [9, 242]. Of the question *“In ED patients diagnosed with acute CO poisoning, does HBO therapy as compared with NBO therapy improve long-term neurocognitive outcomes?”* ACEP concluded a “Level B recommendation” reflecting moderate clinical certainty. No controlled randomized multicentre study with defined exclusion and inclusion criteria, defined treatment algorithms, and an adequate follow-up protocol has been conducted so far [9, 239].

In summary, we saw that evidence for the benefit of HBO in adults with regard to neurological sequelae subsequent to CO poisoning is inconclusive, and conclusions are careful and nuanced [239]. The meta-analyses are inconclusive [5], but HBO defenders cite Weaver's study as the most relevant [142, 224], while others dissect it to find other weaknesses which qualify its conclusions [5, 141, 145]. HBO fundamental studies are promising, but clinical studies assessing the effectiveness in avoiding DNS, the ultimate goal, seems to fail after 60 years of clinical practice. At the end, HBO societies recommend HBO while other Scientific's societies recommend prudence.

2. BACKGROUND ON NON-INVASIVE VENTILATION

2.1. NON-INVASIVE VENTILATION

2.1.1. Development of the technology

The emergence of invasive mechanical ventilation (MV) in its modern sense occurred during the devastating polio epidemics of the 1950s (Fig. 33). Demonstration was made that lives could be saved, acutely with invasive MV and apneic patients supported it virtually indefinitely with tracheostomy and positive-pressure ventilation [243].



Fig. 33: Iron lung based on negative-pressure ventilation used in the treatment of polio victims during Denmark's polio epidemic in 1952, before MV era [244].

By the early 1970s every U.S. acute-care hospital had an Intensive Care Unit (ICU), and a respiratory therapy department. Progressively, specialists in invasive MV were aware of the complications of invasive MV and artificial airways, and subsequently of ventilator-induced lung injury, the refusal of some patients to be

intubated led to renewed interest in less aggressive, potentially less injurious ventilatory support as Non-invasive positive pressure ventilation (NIV as NiPPV) [243].

Although it was first tried as early as the 1950s, and was subsequently used in a few centres of special expertise, long-term support of ventilation via NIV as NiPPV only became widespread starting in the 1980s. Otherwise, Non-invasive Continuous positive airway pressure (CPAP), delivered via nasal mask to patients with obstructive sleep apnea, had been introduced in 1981 [243, 245]. In 1989, Meduri reported the successful application of NiPPV via facial mask in some chronic obstructive pulmonary disease (COPD) or congestive heart failure patients, and the avoidance of intubation in most of them [246].

The increased use of NIV in the ICU and in other acute care settings was facilitated by the introduction of improved bi-level ventilators and the availability of improved patient masks improving comfort and resolving air leaks. The years since 1995 have brought an avalanche of clinical investigations and other publications on the use of NIV in acute respiratory failure (ARF) [243].

3. AIMS AND HYPOTHESES

After having overviewed the main concepts concerning CO physiopathology, we can conclude that after 120 years of CO research, great uncertainties remain regarding the modalities of emergency CO poisoning management. The field of CO investigation being extremely vast, we will reduce it for the purposes of this thesis to emergency medicine assessing the five following interrelated CO toxicokinetic topics. Our aim is to investigate a new therapeutic approach and to provide relevant elements to consider in future study protocols in early care management.

Considering the lack of evidence of the efficacy of HBO, the lack of availability of HBO modalities everywhere, the potential toxicity of O₂, the effect of ventilation on COHb clearance, the optimization of NiPPV or CPAP ventilation for other indications as COPD or APE, the availability of NiPPV or CPAP devices in first care departments, we propose to investigate these techniques in cases of acute CO poisoning **(TOPIC1)**.

While the %COHb peak itself or related symptoms don't seem to be directly correlated with CO severity poisoning, COHbt_{1/2} toxicokinetic could never be accurately assessed on a case-by-case basis in acute care, to be tested as an objective potential indirect criterion of CO severity poisoning. It could, in fact, be an indirect sign of the level and the duration of CO exposure, and therefore of the CO storage still present in the extravascular system. COHbt_{1/2} could be selected as an inclusion and comparison criteria in future studies evaluating the different treatments and HBO in particular allowing researchers to compare more homogeneous CO poisoning severity levels. Therefore, the first step is to remove

the controversy over the mono- or bi-compartmental $\text{COHbt}_{1/2}$ TK model to be applied **(TOPIC 2)**, in order to assess the most accurate $\text{COHbt}_{1/2}$ possible.

A new rise in %COHb after a return to normal ($\text{COHb} < 5\%$) following cessation of treatment could be an indirect sign of a CO storage which could be responsible for a late CO release **(TOPIC 3)**. On the other hand, the adequate duration of NBO treatment remains unclear in practice. A rebound could provide arguments to prolong the duration of treatment.

We need a good understanding of the CO toxicokinetic and thus a review of the CO affinity compared to O_2 for different metalloproteins in humans and in the animal world. In order to confirm our swine model as adequate, to interpretate our results and to discuss extrapolation to human, we had to consider the value of M for both swine and humans, as well as all the variables that could alter it during our experimental protocol **(TOPIC 4)**. These values reported in the literature are too heterogeneous.

And finally, the prognosis of a cardiac arrest on CO poisoning (CA-COP) remains dramatic whatever the management. We would like to investigate the COHb toxicokinetic on CA-COP to improve the management strategy in this context **(TOPIC 5)**.

In this next section, on these five topics, we will first summarize and discuss what is currently known and unknown and, why it is relevant to investigate these fields. Following this, the objective of each research question will be stated, the

hypotheses will be formulated, and the method chosen to answer them proposed. The main theme, Topic 1, will be explored across swine experimental studies which form the core of the PhD, Topics 2 and 3 explore other data from this experimental procedure, Topic 4 on M affinity reviews are needed to compare our data for a global understanding, and, finally, Topic 5 is a case report based on COHb toxicokinetic, including part of the reflections of this thesis.

3.1. What is the COHbt_{1/2} on NIV (NiPPV or CPAP) compared to NRM on NBO? (Topic 1)

What is known?

- ✓ The aim of any CO care treatment is to reduce mortality and cardiovascular morbidity, normalise COHb levels, normalise CO storage from myoglobin or cytochromes, alleviate symptoms, and at the end reduce the incidence of DNS, with minimal adverse effects of treatment [222].
- ✓ Literature struggles to show the clinical efficiency of HBO concerning reduction of DNS or mortality [145].
- ✓ Hyperoxygenation, and therefore HBO as the reference, could even be deleterious by promoting the production of free radicals and/or by increasing free CO at the beginning of O₂ therapy [6].
- ✓ Even if symptoms correlate poorly with blood COHb levels, COHb up to 25% is considered as a risk factor for DNS [7, 148]. COHb is an easily available and an objective biomarker to confirm COHb poisoning. COHb elimination is the first step to clearing exogenous CO. FiO₂ and ventilation were both key features to improving COHbt_{1/2} [131, 222].
- ✓ As mathematical modelling suggests that during washout on NBO close to 100% O₂ or HBO, COMb could increase sharply because the subject is not able to rapidly exhale all the CO that has been dislodged from Hb by hyperoxia. This response may exacerbate the impairment of O₂ availability in exercising muscle, in heart muscles or in resting muscles in severe CO poisoning [83, 84]. On the one hand, all means of increasing ventilation improve COHbt_{1/2} but also could avoid new free CO uptake in extravascular tissue. Carbogen is disused because

of hypercapnia risk and its induced hyperventilation seems to be too uncomfortable for the patient [131]. Isocapnic hyperventilation seems to avoid the risk of hypercapnia and be effective in clearing COHb, but has not gained clinical acceptance [167].

- ✓ NRM allows NBO at 10 to 15 l.min⁻¹ (60%–80% FiO₂ depending on the seal quality of the NRM against the face) [222] while CPAPb allows NBO at 25 to 30 l.min⁻¹ with an optimal seal quality of the mask and promoting alveolar recruitment. Nevertheless, CPAPb provide only 60%–80% FiO₂ due to the open system specific valve feature depending of inspiratory flow level. So, the clinician hopes to improve oxygenation at normal inspiratory flow. NRM and CPAP have no real impact on ventilation compared to NiPPV. NiPPV in a close system provides up to 90% FiO₂ and promotes ventilation.
- ✓ Other alternative treatments were investigated as CO specific antidotes with very high CO affinity, such as hydroxocobalamin for cyanide intoxication [21, 186, 188] or Extracorporeal Membrane Oxygenation [169]. Currently these therapies have no effective clinical application.

What is not known?

- ✓ Controversy is still debated on HBO efficiency for CO poisoning in 2023 [145]. In 2022, Juurlink summed up his overview of HBO efficiency like this: *«Don't carry out new investigations on CO poisoning and HBO constitute nothing less than a monumental failure of the field of hyperbaric medicine [145].*
- ✓ The potential O₂ toxicity remains a concern. On O₂ therapy, the concept of oxidative damage results from the production of ROS at rates which exceed the ability to be detoxified. Reintroduction of O₂ to ischemic tissue may trigger a series of harmful reactions leading to an over-production of ROS and,

consequently, inflammation and oxidative damage rather than the restoration to normal functions. HBO could be associated with increased oxidative stress in several studies, mainly linked to excessive duration of exposure along with high concentrations and pressures [249]. A randomized controlled trial named 'HBO for acute domestic CO poisoning' suggested '*This study did not confirm that HBO improves recovery from pure CO poisoning. In addition, in comatose patients, repeating HBO resulted in worse outcomes compared to one session. The apparent worsening of patients treated with inadequate HBO in the current trial may be reflective of O₂ toxicity in the absence of the benefit offered by HBO at higher pressures*' [143].

In contrast, Weaver (2000) discussed these conclusions arguing that beneficial molecular effects are not reached at 2.0 atm (Annane's protocol) but at 2.8 or 3 atm, and so failed to prove efficiency. Nevertheless, Sluijter (1963) has already encountered O₂ toxicity at 3 atm pressure after coal gas CO poisoning [250]. The presence of CO thus could predispose to O₂ poisoning and there seems to be little point in exposing patients to higher pressures of O₂ than necessary [164].

The mechanism for CO poisoning includes lipid peroxidation, decreased O₂ carrying capacity, and inhibition of Cytochrome c oxidase or Complex IV. Complex IV inhibition with CO alone does not result in significant ROS production. Nevertheless, treatment with HBO could result in improved cellular respiration but higher H₂O₂ production. It remains unclear if the increased production of ROS (H₂O₂) in HBO treatment is detrimental [251]. ROS generated in the brain after CO hypoxia seems to originate primarily from mitochondria. These ROS may contribute to CO-mediated neuronal damage during reoxygenation after severe CO poisoning [114]. If HBO could be beneficial on COHbt_{1/2}, on initial cellular respiration, or uncertainly on DNS outcomes, we don't know the real impact of O₂ toxicity which could produce ROS.

- ✓ Few studies assessed CPAP or nasal high flow O₂ therapy impact on COHbt_{1/2} [181, 185].
- ✓ None of studies published to date estimate COHbt_{1/2} on NiPPV after acute CO exposure.

Why is it relevant?

Historically, the benefit of HBO was assessed only on COHbt_{1/2} and 60 years later meta-analyses don't provide evidence for HBO's clinical efficiency in cases of moderate CO poisoning [135, 222]. No other new treatment has yet emerged and it is recommended to reconsider the historical place, or the wise dosage of O₂ therapy. It is relevant to investigate new HBO therapy, potentially improving ventilation and limiting hyperoxygenation on CO poisoning as it could be assumed by NIV.

Avoiding the use of hyperoxia therapy could avoid the potential initial rapid increase in COMb suggested in mathematical modelling. Applying hyperventilation on room air or on initial limited O₂ therapy leaves time for CO dissolved in blood to be cleared by lungs, and not to be available for new binding on Mb, Ngb or cytochromes. The aim is to promote the release of CO from the COHb by a well-dosed supply of O₂ to avoid iatrogenic toxicity, while at the same time promoting the capacity of the lungs to eliminate free CO in order to avoid recapture by extravascular tissues. It is to be remembered that COHb is less toxic in itself compared to P_{CO} [55].

To date, there is no biomarker or objective sign to assess the severity of an intoxication considered intermediate. And only COHb exists to evaluate the evolution of CO elimination.

We hypothesize that NiPPV or CPAP could be assessed for the first time as a first care alternative for acute CO poisoning. NiPPV and CPAP are now well-mastered in the context of emergency care, and devices are easily available. In case of positive result, application could be rapid and easy. Experimental pilot studies are needed to identify COHbt_{1/2} behaviour in these techniques. This will motivate additional clinical studies. Finally, early CO clearance with NIV does not delay or exclude any other treatment, including subsequent treatment with HBO if evidence for it could be consolidated in the future.

Research Question 1

Question: What is the COHbt_{1/2} on NIV (NiPPV or CPAP) compared to NRM on NBO?

Aim: To identify if NIV (NiPPV or CPAP) could be an effective first care treatment after CO exposure considering only the COHb elimination.

Secondary aim: To identify if we found any adverse effect on NiPPV or CPAP applied after acute CO exposure on a pilot study.

Hypothesis: As NiPPV or CPAP improved ventilation and oxygenation in other acute respiratory disease, providing a positive pressure and better sealing to face improving FiO₂, we expected a COHbt_{1/2} improvement compared to NRM.

Method: As it is not possible to carry out a controlled clinical study comparing NiPPV, CPAP and NRM on CO poisoned humans for technical and ethical reasons, we propose a swine experimental model to control CO exposure characteristics.

Topics 2 and 3

We wanted to introduce topics 2 and 3 by reminding interested parties that COHb itself is less toxic than free CO which is responsible for the extravascular CO storage and thus for mitochondrial toxicity and/or neurological injuries. As it is impossible to evaluate pigs neuropsychologically, we wanted to estimate the CO bound to extravascular tissues under our different treatments, independently of the efficacy on COHbt_{1/2}. One way to estimate this extravascular reserve would be to measure the COMb which is currently impossible in clinical practice.

So, our experimental protocol will include many muscles biopsies during CO poisoning and COHb elimination phases. Nevertheless, there is no analytical technique that allows us to assess accurately COMb in a muscle biopsy at thesis beginning. Therefore, we would like to freeze and store the biopsies and hope that a technique will develop in the coming years. Thus, in the meantime, we want to investigate in Topics 2 and 3 two indirect methods which could estimate this COMb or extravascular reserve which could be accessible in clinical practice, and being experimentally evaluable.

3.2. Does COHb toxicokinetic fit with a mono- or a bi-compartmental model? (Topic 2)

What is known?

- ✓ Located in the extravascular tissues, myoglobin (Mb), neuroglobin (Ngb) and other heme-containing protein as cytochrome oxidase, are able to link CO and account for approximately 10 to 15 % of the total body CO [20, 252].
- ✓ In humans, muscle accounts for $\pm 40\%$ of total body mass suggesting that muscle could be a significant storage site for CO [60, 253, 254].
- ✓ Experimental studies on sheep AA-treated suggested bi-compartmental COHbt_{1/2} elimination [97]. In contrast, Weaver in retrospective human clinical study NBO-treated suggested a mono-compartmental model [96, 125]. Nevertheless, Weaver agrees that *'It is possible that an early rapid fall in COHb may have been missed'* [97, 125]. In his mathematical model, Bruce demonstrated also a bi-compartmental model of experimental human assessment, using real data treated on AA or extrapolated on NBO [84].

What is not known?

- ✓ If literature struggles to show the clinical efficiency of HBO concerning reduction in DNS, one of the keys could be the inadequacy of the inclusion criteria currently used in studies to evaluate HBO and the real CO poisoning severity [9, 145].
- ✓ CO poisoning severity could be related to a CO storage non-assessable in clinical practice.

- ✓ CO storage could be correlated to CO exposure level and duration difficult to estimate in the clinical context.
- ✓ Correlation between CO exposure level and duration and, $\text{COHbt}_{1/2}$ investigable in clinical practice is almost never studied.
- ✓ So, $\text{COHbt}_{1/2}$ has never been specifically explored as a CO poisoning severity criterion.
- ✓ It still debates on toxicokinetic model to assess the $\text{COHbt}_{1/2}$ with mono- or bi-compartmental models and, $\text{COHbt}_{1/2}$ discrepancies are observed in experimental or clinical study [96, 97, 125].
- ✓ Only two studies of animal experimental CO exposure NBO-treated in 1948 and 1975 assessed the appropriate model to use for $\text{COHbt}_{1/2}$ determination (mono- or bi-compartmental) with opposite conclusions [133, 203]. No assessment has been done since on experimental animal CO exposure based on accurate real data, NBO-treated, to be compared to mathematical model [84].

Why is it relevant?

There are no biomarker or signs to assess CO storage, and thus the COMb , in acute clinical practice. To study the most effective treatment for CO poisoning, it could be practicable to find a way of estimating this CO storage. $\text{COHbt}_{1/2}$ toxicokinetic could give usable information during the first hours of acute care management on CO body storage. Ignoring the correct toxicokinetic model being mono- or bi-compartmental is a source of error in the estimation of the $\text{COHbt}_{1/2}$.

The first step before any attempt to assess $\text{COHbt}_{1/2}$ is to clarify the discussion between experimental studies that favour the bi-compartmental and clinical studies that favour the mono-compartmental.

It is only after having determined the toxicokinetic model to consider, that future studies will be able to be based on an accurate COHbt_{1/2} assessment with a view to demonstrating its usefulness as a discriminating criterion for determining the severity of CO poisoning. This would improve the establishment of patient cohorts for studies seeking to compare different treatments.

Moreover, the bi-compartmental model is compatible with extravascular muscle CO storage and thus, in clinical practice, a longer COHbt_{1/2} could be favourable to extending treatment in order to promote CO release from extravascular CO storage.

And finally, the bi-compartmental model could be compatible with what mathematical models have argued: a potential adverse effect due to early free CO peak (P_{CO}) coming from significant CO storage in early phase during COHb washout on NBO, which underlines the need for O₂ dosing.

We need an experimental model with many and extremely accurate COHb determinations, sampled directly after exposure to assess the accurate toxicokinetic model, being mono- or bi-compartmental. And, if possible, we need COMb biopsies to correlate COHb to COMb levels.

Research Question 2

Question: Does COHb toxicokinetic fit with a mono- or a bi-compartmental model?

Aim: To make statements around the current controversy between experimental and clinical studies.

Secondary aims: The bi-compartmental model is an argument in favour of CO storage. If this model is confirmed, it will allow us to compare COHbt_{1/2} to the first and second toxicokinetic phase in treatment efficiency studies. Moreover, the proportion from one phase to the other could reflect CO storage and severity of exposure. In addition, to highlight clinical practice on NBO duration currently widely estimated from 6 to 12h or longer.

Hypothesis: COHb toxicokinetic fits with a bi-compartmental model; in clinical practice we must consider this model, assessing COHbt_{1/2} to improve NBO duration. Treatments compared in our protocol could have different efficiency depending on the phase- α or - β considered.

Method: Use our data from a swine experimental study (COHb elimination) to assess the toxicokinetic modalities with high accuracy and statistical analyses. We will take muscle biopsies during the intoxication and detoxification phases to compare intravascular (COHb) and extravascular (COMb) CO elimination.

3.3. Is there a %COHb rebound after COHb backed at 5% and NBO cessation? (Topic 3)

What is known?

- ✓ Based on physiological studies, the benefits of NBO 100% by NRM in the emergency care is universally accepted [222]. Despite agreed potential HBO superiority in avoiding DNS, NBO can be considered a reasonable alternative due to its cost-effectiveness [142].
- ✓ CO binds to metalloproteins including myoglobin to form COMb. The extravascular reserve of CO is estimated at 15%. Mathematical models estimate that the release of CO from COMb is delayed compared to COHb and that CO released from COHb by NBO would increase the dissolved vascular CO (p_{CO}) that could be redistributed to extravascular tissues as myoglobin.

What is not known?

- ✓ No systematic review, RCTs, or cohort studies have indicated the optimal duration of treatment after CO poisoning and thus the duration of in-hospital monitoring [222]. Thus, there is still debate about the ideal duration of normobaric 100% O₂ after mild CO poisoning. In practice, however, it is recommended to be continued for at least 6 to 12 hours or until COHb levels fall below 5% or even $\leq 3\%$ [5, 8, 125, 149, 222, 226, 227]. After this, if patient feels well, repeating the COHb level does not seem to be necessary [7].
- ✓ Nevertheless, COMb is not clinically or analytically evaluable.

- ✓ No data are available on COHb rebound after oxygen treatment when COHb backed to infra toxic level at 5%. If this rise in COHb exists, we do not know if the level of rise, or if the clinical impact, are significant.

Why is it relevant?

If this rise in COHb exists, then it is a second way, after a COHbt_{1/2} toxicokinetic bi-compartmental demonstration, to have an argument for CO storage and potentially have an access to its estimation.

In doubt, it could be easy to systematically prolong the treatment duration several hours after COHb backed to 5% to clear potential CO storage, but in the overcrowded emergency room context, this is probably inappropriate and not cost-effective if no P_{CO} or COHb rebound occurs, or if rebound is not significant. Six hours at 15 l.min⁻¹ oxygen therapy corresponds to 5.400 oxygen litres and postpones discharge up to 6 hours, corresponding generally to a one-day admission fee.

Moreover, a significant rebound estimated case-by-case could reflect a CO poisoning severity and help in the decision for a recourse to other therapies such as HBO.

Research Question 3

Question: Is there a %COHb rebound after COHb backed at 5% and NBO cessation?

Aim: To present an argument in favour of delayed CO release from extravascular CO storage as COMb.

Secondary aim: To argue in favour of the multi-compartmental toxicokinetic model. To emphasise the importance of establishing pertinent duration after CO exposure. To be more cost-effective regarding the in-hospital monitoring duration.

Hypothesis: There is a COHb rebound after NBO stopped up to 5% in favour of multicompartmental toxicokinetic model. This rebound is expected moderate because the extra vascular CO storage itself is estimated at 15%.

Method: Ongoing our experimental study beyond %COHb backed to 5% to assess the COHbt_{1/2} evolution without any treatment with accurate blood samples. Compare curves with statistical analyses.

3.4. Which are the accurate M values for human and animals? (Topic 4)

What is known?

- ✓ CO has a high affinity for all metalloproteins and particularly for hemoglobin (Hb), with which it forms, reversibly, the carboxyhemoglobin complex (COHb). The Haldane constant, also called the M constant, represents the relative affinity of Hb for CO versus O₂ as the equilibrium constant for the reaction of CO + O₂Hb, resulting in COHb + O₂ (Fig. 34) [78, 255]. M was estimated at ±240 [256].

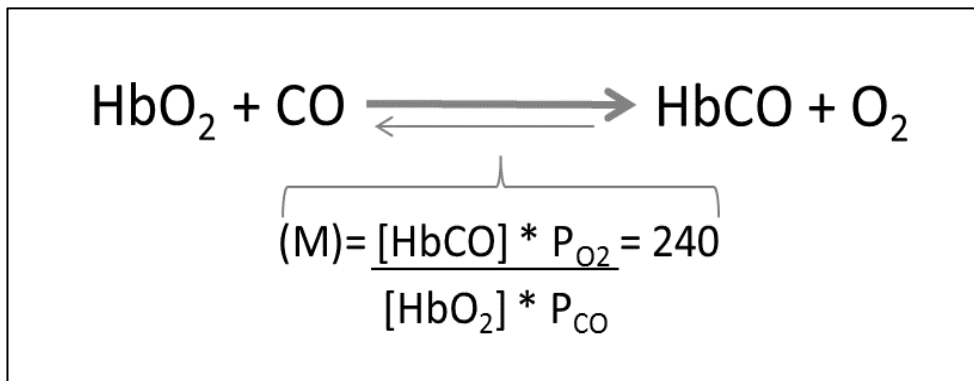


Fig. 34: The equation included M constant at equilibrium in blood where [COHb] = [HbCO] = carboxyhemoglobin rate; [HbO₂] = [O₂Hb] = oxyhemoglobin rate; p_{CO} = partial pressure in CO dissolved; and pO₂ = partial pressure in oxygen dissolved.

- ✓ Thereafter, historically, other estimations of M for humans, ranging from 140 to more than 400, were published [256, 257]. In recent decades, researchers considered human M values ranging from 210 to 245, but in most instances without justifying their choice [258]. From 1969, experimental studies found,

at 37°C and any pH, a M_{37} value of 218 ± 8 for adult Hb and 178 ± 10 for foetal Hb; these appear to be the most extensive and detailed studies but needed to be confirmed [259, 260]. Moreover, there is M variability between species and experimental conditions.

What is not known?

- ✓ In humans, accurate M value seems to present discrepancies and the influence on M of factors like pH, temperature, 2,3-DPG concentration in red cells, atmospheric pressure are hotly debated.
- ✓ M for swine is scarcely studied.
- ✓ M for other animals differs widely compared to humans but were studied in very different physiological conditions, making it difficult, ultimately, to compare one species to another.
- ✓ M is a value at equilibrium but could vary at different COHb level and/or during CO absorption or elimination.

Why is it relevant?

Fifty years after the reference of Rodkey's study on M, our main objective was to determine whether more recent literature offered any new data related to M estimation, in order to confirm or improve the accuracy of the M human value and, what is more noteworthy, under physiological conditions. Over 20 years, no new evaluations of M in animals have been published.

It is important to investigate this gap in the current knowledge to interpret our results on our experimental study. Some experimental or physiological factors, such as temperature, pH conditions and type of Hb (adult or foetal) may influence the M

value and explain, at least in part, discrepancies from previous studies. Given that COHb rates vary directly in relation to M, using a widely variable M value may introduce a margin for error in determining COHb.

The elaboration of the experimental research protocol requires to establish the swine M value in order to estimate the time needed for each experiment on each swine.

Moreover, our toxicokinetic model being a swine model, we have to identify and compare the M value for swine and human blood with a view to discussing extrapolation for humans.

We would like to provide evidence for the existence of a species-specific Hb that would approximate to the human M-value sufficiently to propose it as a replacement for human blood in future studies.

In addition, although HBO could reduce DNS resulting from CO poisoning, we saw that substantive sequelae could remain. As new and effective approaches are urgently needed to treat CO poisoning, we want to investigate ventilation while others are investigating newly engineered molecules with very high affinity to CO as scavenger antidotes. Understanding affinity constants or dissociation of CO for Hb could allow scientists to compare treatment mechanisms. And this in-depth understanding could allow us to interpret COHb rebound origin from Topic 3, if it occurs.

Research Question 4

Questions: What are the accurate M values for human and animals? What are the impacts of species and various physiological conditions on M?

Aim: To estimate the time required for CO intoxication and elimination to develop the study protocol. To extrapolate results from our experimental swine study to humans.

Secondary aim: To confirm or improve the M value accuracy to avoid error in determining COHb from Haldane's equation. To find the best animal blood model fitting with humans for M value.

Hypothesis: M value for humans is similar to mammals. M value differs according to physiological conditions, explaining discrepancies in previous studies. M value in the animal world was widely investigated.

Method: Process to a systematic review on Human M and another systematic review on M for species in the animal world.

3.5. What is the COHbt_{1/2} during CPR on CO-induced CA? (Topic 5)

What is known?

- ✓ A review conducted in 2001 concluded that there was an extremely poor survival prognosis for cardiac arrest (CA) following CO poisoning, with or without HBO [261].
- ✓ Cardiac arrest includes changes in the patient's alveolar ventilation, alterations in gas exchange efficiency, or variability in cardiac output during the time of the COHb measurements. All of these factors adversely influence COHbt_{1/2} [97].

What is not known?

- ✓ There are currently no specific recommendations that deal specifically with CO-induced CA [262-264].
- ✓ No studies mentioned COHbt_{1/2} during CPR. Intuitively, it is expected that CO elimination during CO-induced CA resuscitation is counteracted by poor ventilation and circulation conditions. But, this has not been objectively demonstrated.
- ✓ Recommendations have reported that, in certain cases, some patients with CO poisoning could benefit from more aggressive or prolonged treatment, but no objective criteria are exposed [265].

What is it relevant?

COHbt_{1/2} is affected by ventilation, oxygen therapy, and cardiac output, all of which are conditions severely impaired by CA [125, 168]. Assessed COHbt_{1/2} could modify management of CO-induced CA providing objective data to decide the duration of resuscitation which may be extended up to the conventional recommendation of 30 min [266]. From our point of view, resuscitation success with COHb level up to 10-20% seems, typically, to be utopian.

The chances of conducting a quality prospective study specifically on CO-induced CA to objectively determine the intrinsic factors influencing the resuscitation success are extremely low. It is therefore essential to explore CO toxicokinetic to understand the CO-induced CA specificity.

Research Question 5

Question: What is the COHbt_{1/2} during CPR on CO-induced CA?

Aim: Assist in decision making during CPR on CO-induced CA management. If COHbt_{1/2} allows to reach infra-toxic COHb level in a time close to realistic CPR time, CPR could be ongoing beyond standard recommendation CPR time.

Hypothesis: COHbt_{1/2} being perturbed by alveolar ventilation, alterations in gas exchange efficiency, or variability in cardiac output, COHbt_{1/2} during CO-induced CA is expected to be largely prolonged compared to COHbt_{1/2} on NBO and spontaneous cardiac rhythm, estimated in literature at 74min.

Method: During the thesis process, we will try to investigate human COHbt_{1/2} during prolonged CPR in clinical cases of CA following unintentional CO exposure.

4. MATERIALS AND METHODS

4.1. SWINE AS A MODEL

Studies on animals have investigated toxicokinetic and adverse effects of CO exposure over a much wide range of exposure concentration or duration and have evaluated outcome measures that are not possible technically or ethically to assess in humans.

Species choice for investigation depends on the outcomes assessed, the animals and laboratory availability, the local cultural and ethical point of view in the historical moment in which the study is carried out.

In the first part of the 20th century, dogs, who were supposed to have a similar physiology and (M) value as humans, were used as the models for severe experimental intoxication, probably due to the availability of this species in animal pounds. Later, between the 1960s and 1980s, researchers more frequently used sheep to analyse the kinetics of CO association and elimination after severe CO poisoning - firstly for ethical reasons and, secondly, sheep lungs appeared to be more closely related to human and thus became a suitable model for studying the cardiorespiratory physiologic effects of CO [267]. Thirdly, in terms of our thesis focused on COHb elimination, investigators also had to pay attention to CO absorption features to be sure that the intoxication and treatment phases could take place in the same working day. Sheep and swine Hb is supposed to have a lower affinity (M) for CO (≈ 137) than most of the other 'big' species like dogs (≈ 215) or humans (≈ 209). Therefore, sheep or swine, could be used successively in repeated experimental poisoning; similar studies in dogs would require more time, not compatible with laboratory schedules [168, 268, 269]. Animals like sheep were

also chosen because of the close resemblance of the pathophysiological changes seen in human respiratory distress, as previously described [270, 271].

Moreover, CO saturation is reached earlier in animals with high motor activity like dogs, or small animals like rats or mice, and $\text{COHbt}_{1/2}$ faster compared to sheep or swine. Authors like Killick in 1940 or Klimisch in 1975 demonstrated that a slower rate of saturation of blood with CO is observed in big laboratory animals [55, 272, 273]. The equilibrium of saturation is thus reached twice as rapidly in hamsters (~4h) compared to swine (~8h) [55]. Large laboratory animals, such as swine, which have a lower respiratory minute volume per unit of body weight, have slower rate of CO blood saturation. Klimisch noted that preliminary experiments with domestic swine showed no difference in M-value, "*in vitro*" evaluated, compared with that of miniature swine [272].

Moreover, the respiration rate of small mammals like mice is around 150-200 per min, and its O_2 consumption per unit of its body weight is about 12 times greater than in humans [274]. And, during the elimination phase, $\text{COHbt}_{1/2}$ value is mainly influenced by the ratio of blood volume to ventilation [84]. Small animals have greater hypoxia tolerance in relation to large ones. This was explained by the lower affinity of their Hb for O_2 which facilitated O_2 unloading at the tissues [275].

In addition, myoglobin content in skeletal muscle varies from one species to another. For example, wet mass in mg.gr^{-1} is 8 in rabbit, 1.9 in mice and 2.1 in human [276]. We know that CO links also myoglobin, another metalloprotein, with a lower affinity than for Hb in human, evaluated at 39 [277].

Thus, we rapidly excluded using small animals to facilitate extrapolation to humans and with regard to the necessity of attaching an adapted human face mask. And, among larger animals, looking to select animals of similar weight and physiology to humans, we turned to the Experimental Surgery and Transplantation (CHEX)

laboratory in Brussels which has an excellent experience of the anesthesia and surgery processes with swine required for our experimental design. The swine chosen for our study had an average human weight of 77 kg [72–88]. Nevertheless, it is the first time that swine of human weight have been used experimentally in a CO poisoning model. The veterinary literature, however, provides us some data studied in the context of industrial food to preserve the red color of meat through voluntary exposure to CO or through the analysis of swine involuntarily intoxicated in their breeding.

4.2. NIV TERMINOLOGY USED

During their evolution, ventilatory supports have been called different things by different researchers, clinicians, and manufacturers, which has led to confusion on the part of both clinicians and investigators [278]. To some extent this diversity of terminology persists.

Non-invasive positive-pressure ventilation (abbreviated NPPV or NiPPV) was formerly used to distinguish it from Non-invasive negative-pressure ventilation with iron lungs, although considering the rarity of the negative today, the simpler term NIV is more convenient. Moreover, one should not confuse BIPAP (a proprietary product name using a capital 'I') used for biphasic mode and BiPAP used for bilevel mode which is the only concern for us in this thesis. Use of the term BIPAP as a generic term for NIV should be discouraged [243]. So, the terms BiPAP, NiPPV, NIV are to be considered in this thesis as a single entity providing Non-invasive ventilation as a real inspiratory aid. To summarize, the ventilator detects the onset of the patient's inspiratory effort with a sensitivity set by the practitioner, which then triggers the administration of inspiratory pressure support (PS) to the level also set by the practitioner. On exhalation, the pressure level is maintained at a lower but staying positive level also set by the practitioner. There are therefore two levels of positive pressure following each other during the respiratory cycle (Fig. 35).

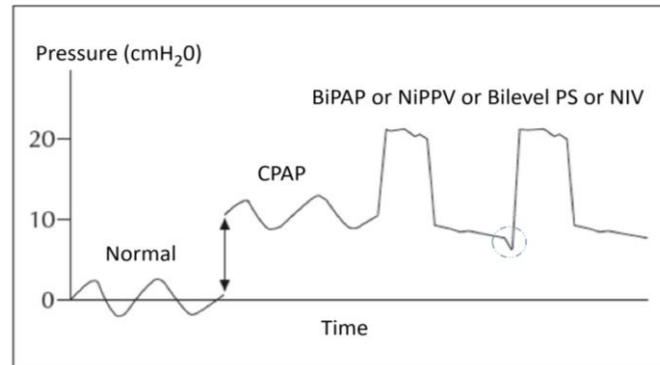


Fig. 35: Succession of breathing cycle in normal condition, under CPAP or under NiPPV. The circled depression in NiPPV mode corresponds to the beginning of the patient's inspiratory effort which will trigger the pressure support (PS).

Moreover, in experimental studies, applying a mask with Non-invasive pressure support on animals requires partially sedation. This sedation could partially collapse the animal's airways, so an intubation tube could be used even if breathing stays spontaneous. Non-invasive ventilation being, by definition, applied without an invasive tube, the term PSV as 'pressure support ventilation' and not NiPPV is then used, but the pressure effect is considered to be the same.

Continuous Positive Airway Pressure (CPAP) has not been considered as NIV since it does not have a ventilation mode. It only provides a fixed and constant positive pressure during inspiration and expiration. CPAP is frequently erroneously translated in French by 'PEP' as 'Pression Expiratoire Positive' instead of 'PPC' as 'Pression Positive Continue'.

NiPPV mode delivers PS triggered by patient him or herself when he/she inspirates, and can result in some patient-respirator asynchrony. The simpler CPAP does not use a trigger and maintains positive pressure fixed independently of the respiratory phase or inspiratory effort.

4.3. NIV DEVICES AND MODALITIES

Ventilators rapidly became more capable and more sophisticated, with a plethora of new modes and other features. Their uses were guided by blood gas analysis and other new ways of physiologic monitoring. Using the most suitable devices and/or available in emergency medicine, we started NIV in the 2000s in our emergency department, using first the BiPAP ST/D30 (Respironics©) and Legendair (Covidien©(AIROX)) following by new generation Elisée 250 (Resmed©) (Fig. 36) to delivery NiPPV in case of hypercapnic ARF.

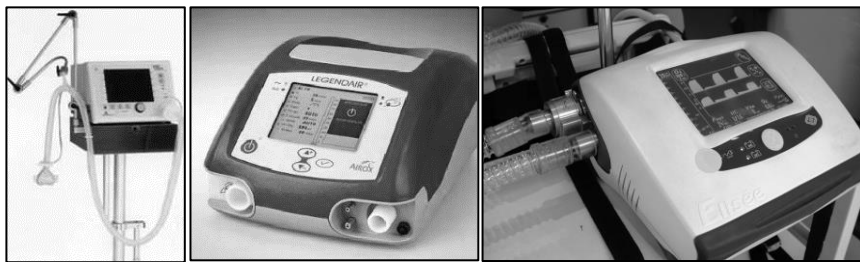


Fig. 36: NiPPV devices delivering Bilevel Airway Pressure as BiPAP ST/D30 (Respironics©); Legendair (Covidien©(AIROX)) as PSV-Leg following by new generation Elisée 250 (Resmed©) (from left to right).

Dr Georges Boussignac developed from 1991 a new CPAP concept named CPAP Boussignac (Vygon©, France), very useful in the context of emergency care or pre-hospital care. The Boussignac CPAP (CPAPb) system consists in applying on a classical naso-buccal mask, a virtual Boussignac valve (Fig. 37). Oxygen flowing through the CPAPb is split into a small cylinder with four narrow, high-velocity streams which are deflected to converge at the centre of the chamber (Fig. 37; Left), creating turbulence, resulting in a positive pressure, providing resistance as a 'virtual valve' that the patient breathes against during expiration. In contrast to our

turbine ventilator, the performance of the CPAPb system is an open system without a real physical valve, depending only on the delivered oxygen, unreliant on electricity, which makes it a perfect design for extra-hospital use.

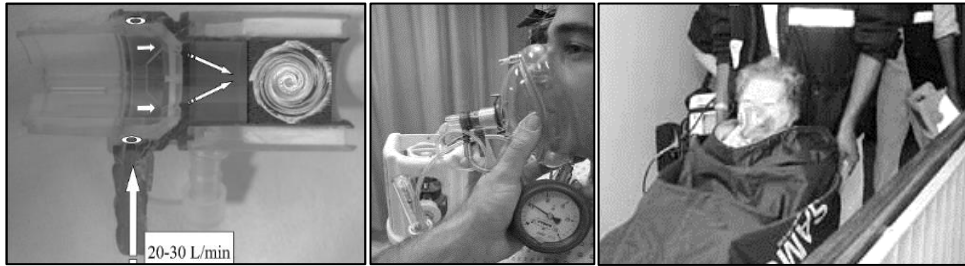


Fig. 37: CPAP Boussignac (CPAPb). CPAPb device longitudinal cutting including the virtual valve; the pressure level guiding by oxygen flow, independent of electricity; design adapted for extra hospital use (from left to right).

The level of CPAP generated is determined by the O_2 flow rate which is adjustable by the practitioner. A flow of 15 l.min^{-1} yields a CPAP at $5 \text{ cmH}_2\text{O}$ and a maximum of 30 l.min^{-1} delivery with an adapted debimeter close to $10 \text{ cmH}_2\text{O}$. To provide target pressure in APE at $7.5 \text{ cmH}_2\text{O}$, oxygen flow has to be up to 25 l.min^{-1} while the maximum oxygen flow that can be delivered by normobaric high flow mask (NRM) is up to 15 l.min^{-1} . Oxygen delivered by NRM being recommended as CO poisoning treatment, a few clinicians began to use CPAPb from 2010 [176]. Nevertheless, FiO_2 delivered is only around 55% [279, 280], not as expected at $15\text{-}20 \text{ l.min}^{-1}$ due to the fact that CPAPb is an open system through the virtual valve allowing the patient to compensate a high inspiratory flow rate during ARF taking overflow in atmospheric air resulting in FiO_2 decreasing.

Then Dr Boussignac developed a VyLife (Vygon©, France), based on a similar concept than CPAPb which is an NiPPV ventilator (also unreliant on electricity) to generate pressure and easily portable for extra-hospital use (Fig. 38).



Fig. 38: Vylife Boussignac for NiPPV (PSV-Vy), electricless, generates the two pressure levels from the pressure of the oxygen bottle.

Acute cardiopulmonary oedema (APE) should be treated in addition to necessary cardiological interventions with continuous positive airway pressure (CPAP) or NiPPV. And acute respiratory failure (ARF) due to chronic obstructive pulmonary disease (COPD) has to be treated with NiPPV. It is apparent that NIV is used by more and more clinicians and is now available in most, if not all, acute-care institutions. Nevertheless, NiPPV was never, and CPAP rarely, investigated in cases of CO poisoning.

4.4. METHODS

In a first phase, we conceptualized the facial interfaces to be adapted to the pig so that they would mimic a human interface as closely as possible. A sculpture moulded in plaster and latex from a first pig used to test the relevance of our procedures was used to build and test different interfaces (Fig. 39).

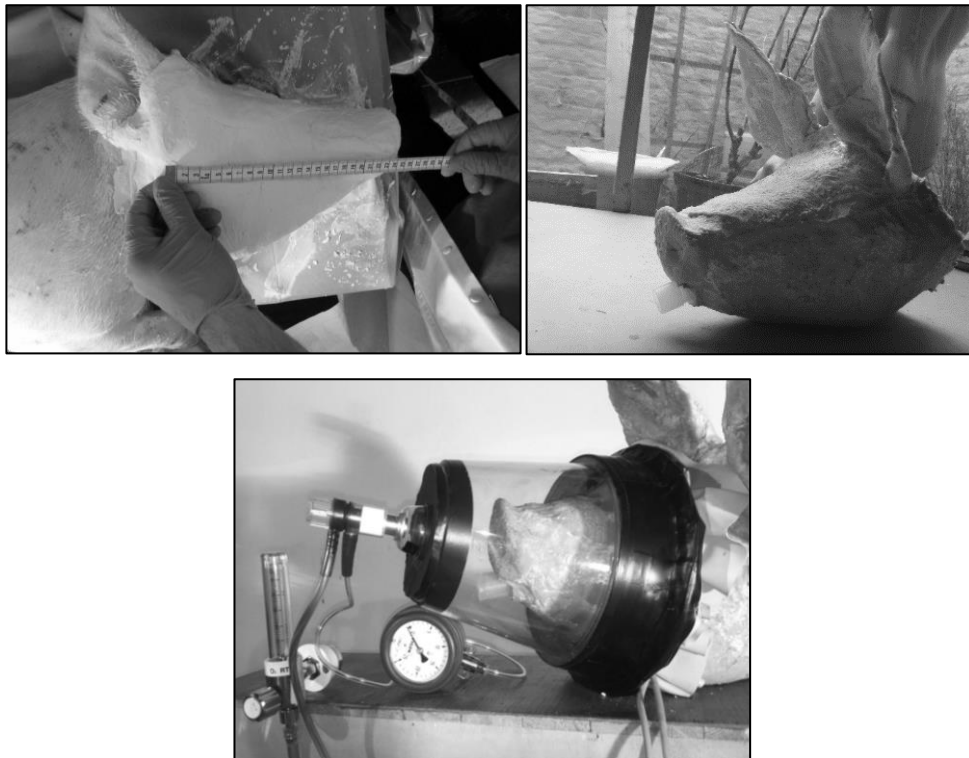


Fig. 39: Different hermetic masks manufacturing trials were tested to satisfy the intoxication phase and the treatment phase. The interface below, for example, was abandoned on account of its excessive dead space.

Intubation is performed 15 to 20 min after the swine have been pre-medicated with intramuscular injections of Tiletamine/Zolazepam (Zoletil® 100, Virbac©) ($6\text{mg}\cdot\text{kg}^{-1}$) and Xylazin (Rompun® 2%, Bayer©) ($2\text{mg}\cdot\text{kg}^{-1}$). Intubation is required to minimized dead space, to avoid obstructive apneic episodes during sedation and to record minute ventilation and end-tidal CO_2 levels during the experiment (Fig. 40).



Fig. 40: Intubation is performed on the animals using a cuffed endotracheal tube (7.5 to 8.0) attached prolonged with a pneumotachograph (end part).

The pigs were sedated with a continuous intravenous administration of Propofol (1.5 to 2.3 mg.kg^{-1}) in order to maintain spontaneous breathing throughout the study. The Propofol infusion rate is adjusted to obtain a balance between sedation and awakening, and P_{CO_2} values are maintained between 35 and 40 mmHg . Propofol is also titrated to control hemodynamic changes, spontaneous motor activity and palpebral reflexes, hypoventilation, and responses to noxious stimuli (Fig. 41).

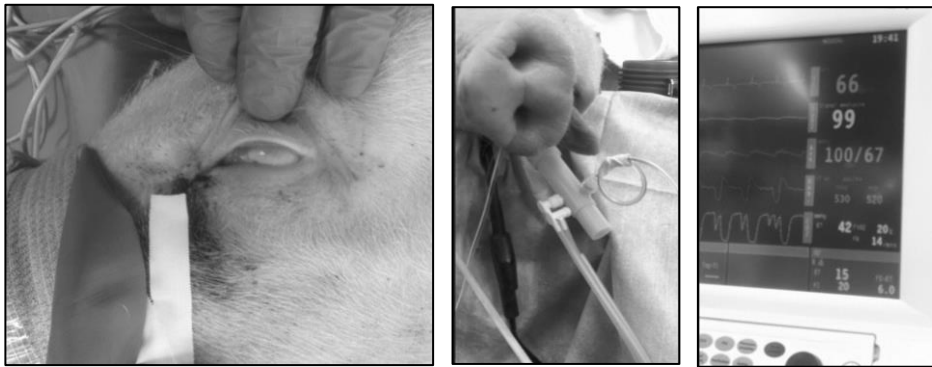


Fig. 41: Palpebral reflexes tested. For each experiment, minute ventilation (ml.min^{-1}), invasive blood pressure (mmHg), FiO_2 (%), heart rate (min^{-1}), SpO_2 (%), end-tidal CO_2 (mmHg), and temperature ($^{\circ}\text{C}$) were measured continuously using a pneumotachograph and a Datex-Ohmeda® S/5™ light monitor (Datex-Ohmeda®, Finland). Monitor was continuously recorded during all procedures.

Catheters are inserted into the carotid or femoral artery to continuously measure blood pressure and to determine %COHb levels. This procedure lasted around 1h30min (Fig. 42). Afterwards, the animals are placed in the lateral decubitus position for the whole duration of the study (Fig. 43).

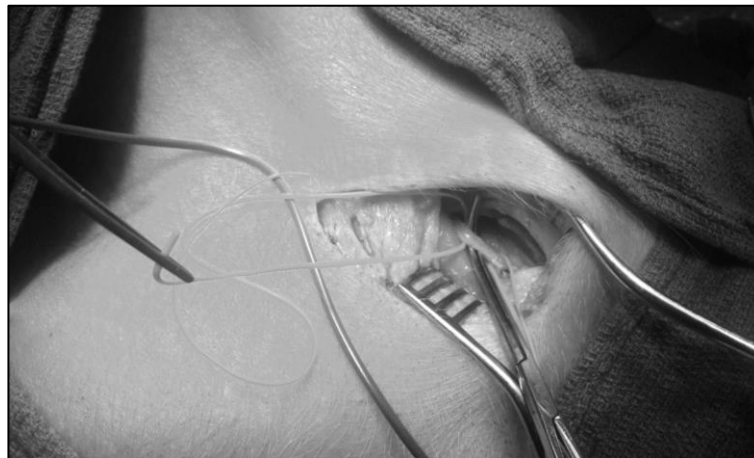


Fig. 42: Anatomical marker and surgical procedure to catheterize the carotid.

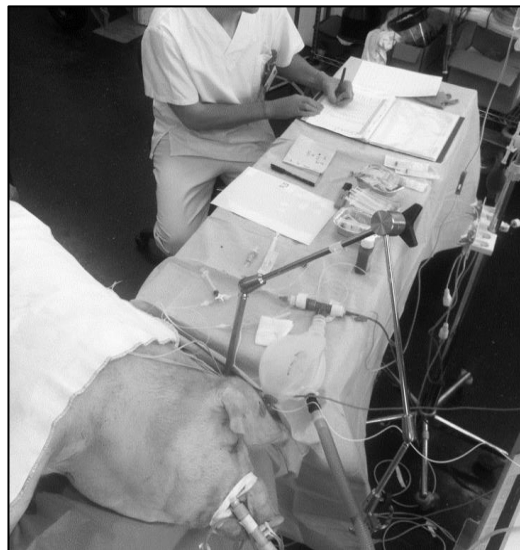


Fig. 43: Table warming is adjusted to maintain a target temperature of 37.5 °C. The principal investigator is assisted by the laboratory technician, while an acute care nurse is present at all times during the entire procedure. He notes all parameter variations every 6 to 10 minutes, and takes blood samples at precise times. The CO concentration in the ambient air was continuously tested (PAC7000®, Dräger®, Germany).

Each day, one protocol is applied to a single pig, using a randomized treatment that is assigned after the intoxication phase. In order to limit the number of animals required and due to economic constraints, the same animal could be exposed to a new intoxication phase and new randomized treatment using the same protocol the following day. A minimum time of 12h is established between experiments, and we determined COHb levels before each experiment so as to exclude significant residual exposure. We measured lactate levels so as to exclude end-organ damage. Overall, four experiments were conducted with ambient air (AA), eight with NRM, five with CPAPb, five with PSV-Leg (NiPPV-Leg), and three with PSV-Vy (NiPPV-Vy). Target levels of intoxication are obtained by administering a combination of 940 ppm (0.094%) CO gas, 20% O₂, and 79.906% N₂ (Pulmo1®, Air Liquide Medical systems©) (Fig. 44; right). This is administrated via a secured system using the endotracheal tube during spontaneous breathing. All exhaust gases from the respiratory circuit are scavenged and released outside the building (Fig. 46).

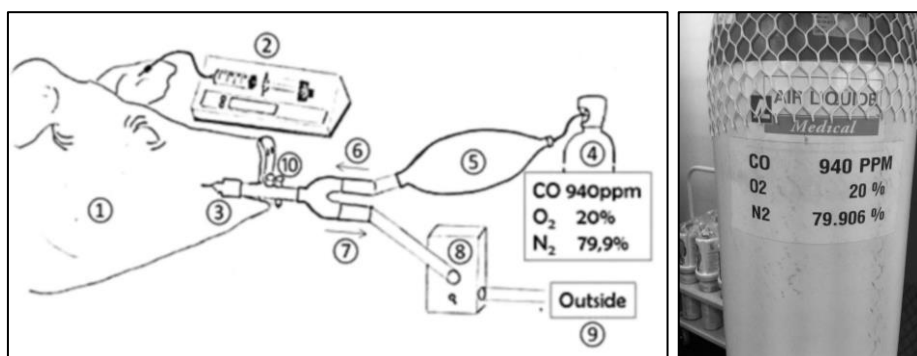


Fig. 44: CO intoxication phase. 1: Pig. 2: Anaesthesia with Propofol 2%. 3: Spontaneous breathing through endotracheal tube. 4: Gas source containing CO 940 ppm. 5: Balloon with gas reserve for inhalation. 6/7: One-direction valve. 8: Exhaled gas scavenger. 9: Gas evacuation outside the room (Fig. 46). 10: Pneumotachograph and FiO₂ measurement. After the intoxication phase, the endotracheal tube was kept in the same position and was covered by an adapted facial mask connected to the respiratory device.



Fig. 45: (Above) Spontaneous breathing through endotracheal tube; One-direction valve; Pneumotachograph and FiO_2 measurement. (Below) Balloon with gas reserve for inhalation with a quantitative CO detector inside to double-check the 940 ppm delivered (PAC7000®, Dräger®, Germany).



Fig. 46: Exhaled gas scavenger; Gas evacuation outside the room.

For the treatment phase, we simplified the treatment interface by adapting material used in clinical practice as NRM (Fig. 47), CPAP Boussignac (Fig. 48); NiPPV-Vy (Fig. 49); NiPPV-Leg (Fig. 50).

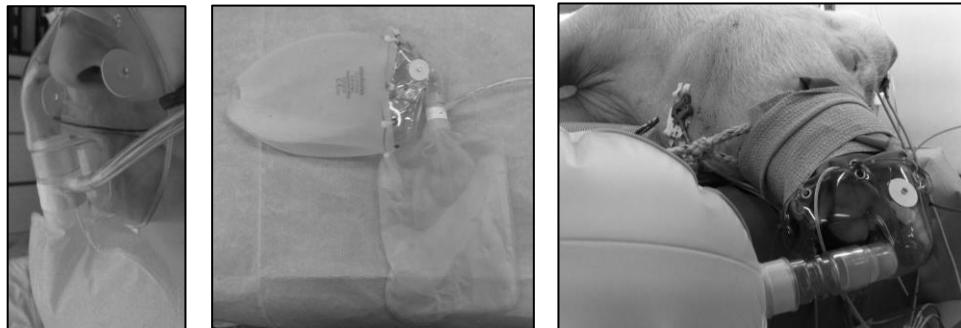


Fig. 47: Non-breathing mask (NRM) on man; NRM construction for protocol; NRM applied during a NBO procedure with NRM. The mask was adapted using an elastic strip to cover their labial commissure and limit air leak but some classic leaks around the mask have been reproduced.

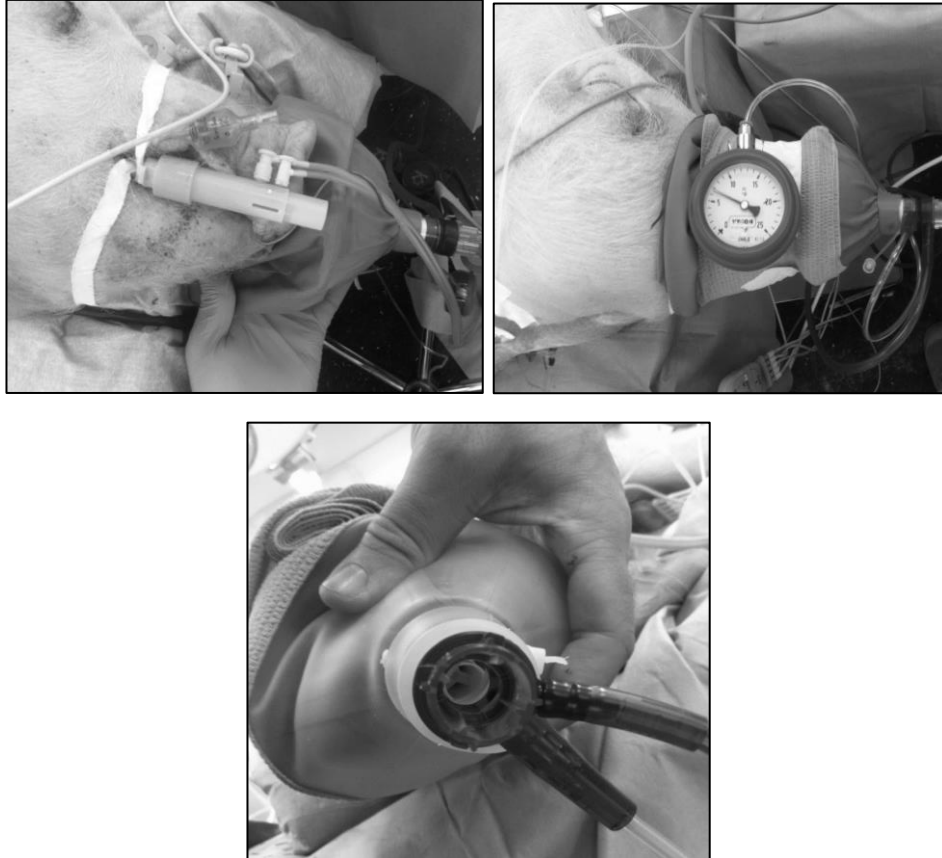


Fig. 48: CPAP Boussignac. (Above on Left) On the front: Endotracheal tube prolonged by the pneumotachograph. On the back: Interface mask for CPAP placed on the picture just to understand how the protocol is designed. (Above on Right) Effective procedure. To reproduce non-invasive procedure, we did not connect the device directly to the endotracheal tube. (Below) The CPAPb system provides continuous positive pressure by injecting high-speed gas into a cylinder through angled side channels. This open system is fed by pure O_2 at $25\text{--}30\text{ l}\cdot\text{min}^{-1}$ and delivers FiO_2 values expected from 70% to 100%, depending on changes in respiratory rate and tidal volume. The pressure created from the flow of gas is targeted at $7.0\text{--}7.5\text{ cmH}_2O$.

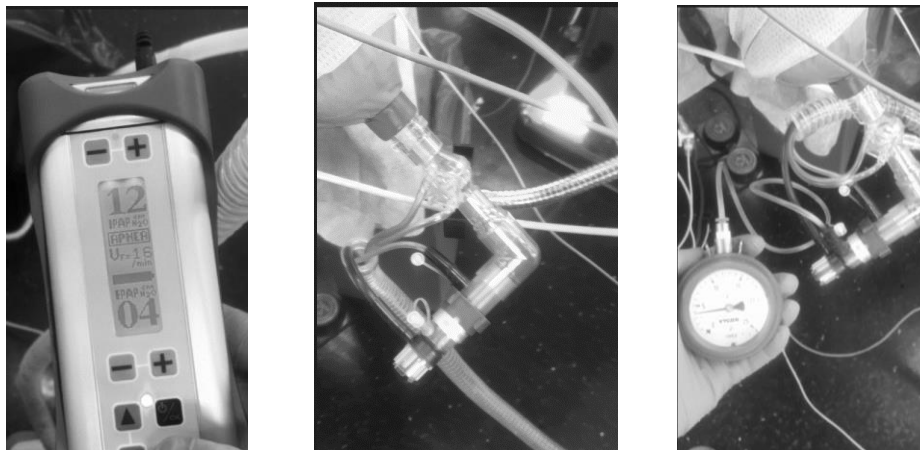


Fig. 49: NiPPV-Vy is an open system. Positive airway pressure is set at + 12 cmH₂O for the inspiratory phase and + 4 cmH₂O for the expiratory phase, with pure oxygen, FiO₂ at 100%. The device measures the pressures delivered, but a double check is performed with a round manual manometer, the end of which is placed in the internal space of the interface.

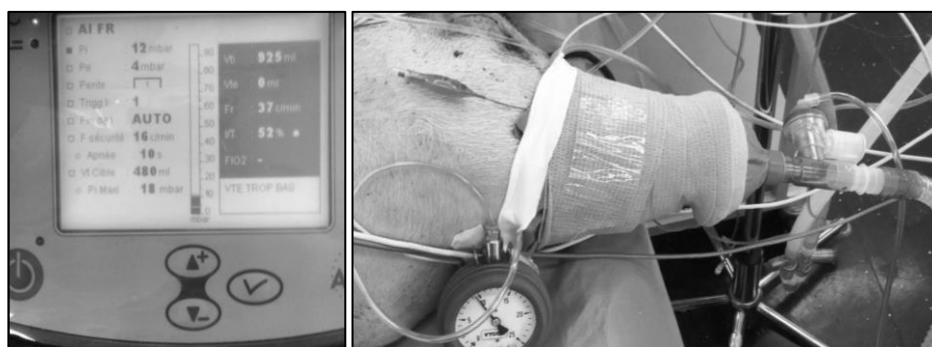


Fig. 50: NiPPV-Leg is a close system. Positive airway pressure is set at + 12 cmH₂O for the inspiratory phase and + 4 cmH₂O for the expiratory phase, with pure oxygen, FiO₂ at 100%. The device measures the pressures delivered, but a double check is performed with a round manual manometer, the end of which is placed in the internal space of the interface.

For the control group AA-treated during phase III or for the phase IV, we left only the endotracheal tube to avoid airway collapse and the pneumotachograph measuring atmospheric room air (Fig. 51) was continuously monitored to be FiO_2 at 21% and with no CO atmospheric presence.



Fig. 51: AA-treated during phase III or for phase IV.

A complete experimental protocol is composed of four consecutive phases (Fig. 52): (phase I): the intoxication phase with determination of baseline COHb blood level followed by iterative sampling every 10 min until COHb levels reached 30% ($\pm 2\%$). (phase II): a 'no intervention phase' with disconnection of the CO circuit. During that period, the animals were placed under ambient air (AA) for 10 min. This corresponded to the virtual delay between the extraction of the victim from the toxic environment and the arrival of the first responders in the context of human exposure. This delay was required for the installation of the respiratory device. (phase III): the treatment phase, with blood samples collected every six minutes until COHb returned to 5% (Fig. 53). (phase IV): the post-treatment phase without any intervention for 60 min at AA in order to document the decrease in COHb or rule out a rebound.

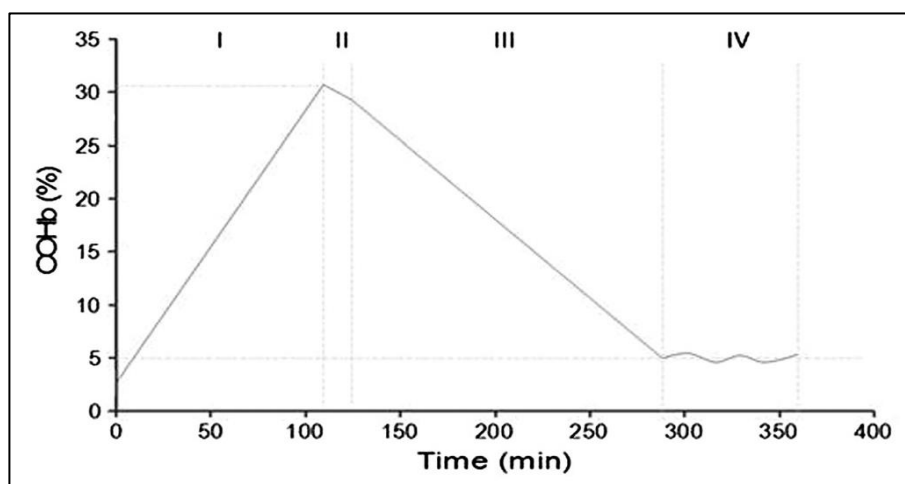


Fig. 52: Experimental protocol. Phase I: intoxication; Phase II: no treatment (atmospheric room air); Phase III: treatment phase with different oxygenation/ventilation devices; Phase IV: no treatment (atmospheric room air) to assess COHb levels and verify the absence of a rebound.



Fig. 53: Blood samples are collected every 10 minutes during the intoxication phase I until COHb reached 30%. And, blood samples are collected every 6 min during treatment phase III and during potential rebound phase IV. All blood samples were analysed for pH, paCO_2 (mmHg), paO_2 (mmHg), lactate (mmol.l^{-1}), and COHb using the blood gas analyser ABL90Flex® (Radiometer®, Radiometer Medical Aps, Denmark) available on site.

In parallel with the COHb samples, thigh muscle biopsies are taken at precisely the same time (Fig. 54).

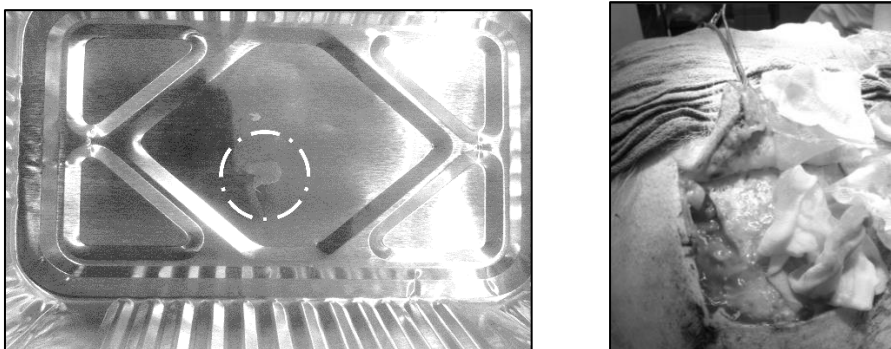


Fig. 54: The biopsy samples, weighing just a few grams, are immediately placed in an aluminium container, sprayed with liquid nitrogen and stored in a suitable freezer.

Illustration in fig.55 summarizes the questions raised by our first four topics and how our experimental protocol will try to answer them. Firstly, during phase I absorption, we considered 30% COHb a significant level of intoxication because this level is supposed to induce clinically significant symptoms in human CO poisoning and require HBO therapy if recommendations are followed. After the sedation of the pigs to avoid any motor activity and under CO exposure of 940 ppm, referring to the CKF equation cited previously, we expected to reach 30% COHb in 100 min. Nevertheless, M-value for swine being 130 and not ± 240 as for humans we anticipated a safety margin at 150 min. For the elimination phase III, the usual $\text{COHbt}_{1/2}$ expected for human with M-value at ± 240 on AA is 320 min and 74 min with NBO coupled to a non-rebreather mask (NRM) at 15 l.min^{-1} . In addition, $\text{COHbt}_{1/2}$ with our new treatments tested are hypothetically expected less than NRM. In accordance with a theoretical complete clearance ($\pm 97\%$) of COHb expected at five-fold $\text{COHbt}_{1/2}$ ($5 \times 74 \text{ min}$), the time required for a complete

procedure is estimated at 100 min for installation, 150 min for CO absorption (phase I) and 370 min for total O₂ treatment time, so in total 620 min or ±10 hours. Nevertheless, our target is not a complete COHb elimination but a COHb at 5% and M-value for swine being not at ±240 but 130 we expected a shorter elimination time and a total procedure time of around seven hours compatible with the laboratory's working hours. The first pig will verify this procedural hypothesis as well as others. For the four experimental days dedicated to the control group on AA, procedure time could take between 15 and 20 hours, so we negotiated a wider range of opening hours with the laboratory technicians.

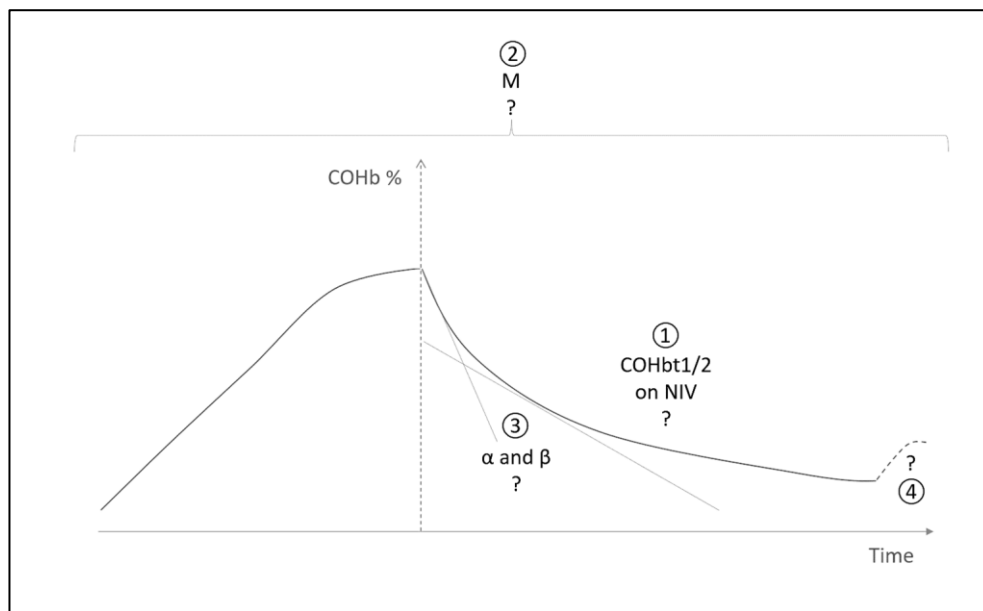


Fig. 55: Experimental protocol. Phase I to IV as explained in fig. 52 above. ① Research question 1: COHbt_{1/2} assessment on different treatments; ② Research question 4: M-value systematic reviewing is necessary to establish the protocol design and to discuss extrapolation results to human; ③ Research question 2: Mono- or bi-compartmental COHb elimination profile assessment; ④ Research question 3: COHb rebound observed after treatment stopped.

To investigate the $\text{COHbt}_{1/2}$ on NIV (NiPPV or CPAP) compared to NRM on NBO (Research question 1), we considered firstly a non-compartmental assessment to avoid ambiguity regarding the controversy regarding mono- vs bi-compartmental models and to compare our data with clinical studies suggesting the mono-compartmental $\text{COHbt}_{1/2}$ toxicokinetic model. First, $\text{COHbt}_{1/2}$ were then assessed and compared using non-compartmental analysis and the areas under the curve (AUCs) $\ln(\text{COHb}) \times \text{time (h)}$ calculated and compared between treatments completed the analyses. Results were expressed as medians [range], and continuous outcomes were compared using the Kruskal-Wallis test and Dunn's test for multiple comparisons using GraphPad Prism 4® software. Secondly, to challenge the research question 2, we tested bi-compartmental elimination plotting $\text{COHb}\%$ on a log scale vs. time on a linear scale, using the linear regression method with least square method. To decide on the best model, models were compared using the Bayesian information criterion (BIC). This allowed us to observe if COHb elimination profile fit best with a mono- or bi-compartmental model. This will also enable us to compare the efficiency of different treatments on each of these $\text{COHbt}_{1/2}$ phases (α - and β -phase) if bi-compartmental elimination is demonstrated.

To investigate the research question 3 on the occurrence of a COHb rebound, data were also collected during the last phase IV on AA. When any therapy was stopped because COHb level target had reached 5%, blood was sampled every 6 min for 60 min. Analysis was focused on potential COHb rebound during phase IV. In the case of no COHb decay, $\text{COHbt}_{1/2}$ would be impossible to assess. Thus, we tested if data corresponded to a quadratic model used to assess the importance of a potential rebound. To interpret the significance of a potential rebound, we discussed previously the M-value in K_{Eq} , K_a and K_d . For the rebound assessment, swine that were immediately on AA after CO exposure without NBO treatment (the control group; $n=4$) were not considered.

4.5. STRUCTURE OF THE THESIS

The following five Chapters will address our research questions (Chapters 2 to 6). Each of these Chapters were published in a peer-reviewed journal. The final Chapter 7 will discuss the implications of the findings and draw conclusions.

✓ Research question 1

CHAPTER 2: Effect of Pressure Support Ventilation on Carboxyhemoglobin Toxicokinetic after Acute Carbon Monoxide Intoxication: a Swine Model

Journal of Medical Toxicology, 2018; 14 (2):128-133

✓ Research questions 2 and 3

CHAPTER 3: Carboxyhemoglobin Half-life Toxicokinetic Profile During and after Normobaric Oxygen Therapy: On a Swine Model

Submitted in Am J Emerg Med; Jun 02 2023

✓ Research question 4

CHAPTER 4: Relative Affinity M Constant of Adult and Fetal Hemoglobin for Carbon Monoxide in Humans: a Systematic Review and Meta-Analysis

Human Physiology, 2020, Vol.46, N°2, pp.191-199

CHAPTER 5: Report on the Relative Affinity Constant (M) of Hemoglobin for Carbon Monoxide in the Animal World: A Comparative Review with a Meta-Analysis Based on a Systematic Review

Journal of Evolutionary Biochemistry and Physiology, 2019, Vol. 55, No. 5, pp. 348—364

✓ **Research question 5**

CHAPTER 6: Unexpected Carboxyhemoglobin Half-life during Cardiopulmonary Resuscitation: a Case Report

International Journal of Emergency Medicine. 2023 Mar 21;16(1):22.

✓ **Discussion and conclusions**

CHAPTER 7: This chapter will draw links between each of these five chapters and will make general conclusions. The clinical implications of these pieces of research will be discussed for health care providers. The strengths and limitations of the studies will be considered, as avenues for further research.

4.6. SOURCES OF FINANCIAL SUPPORT

Belgian Health Care Knowledge Centre (KCE) and the 'Société Française de Médecine d'Urgence' (SFMU) were asked to participate in the study and unfortunately our application did not result in financial support compatible with the costs of the study.

Our principal study benefited from the help of the 'Saint-Luc Fondation' up to 3.500 euros.

The experimental protocol also included the collection of muscle biopsies to evaluate the actual kinetics of COMb and CO storage at each stage of COHb elimination. After soliciting many research laboratories throughout Europe, no one had mastered the technique of measuring CO in muscle. The only positive response came from a laboratory in Strasbourg which required 35.000 euros, far exceeding the budget allocated to the study. We froze all the biopsies for five years, but unfortunately, the technique did not develop in the meantime.

The statistical work was done by the biostatisticians in their academic time.

Considerable assistance on a voluntary basis was provided by the CUSL emergency department nursing team who used their own time to perform all experimental procedures.

The company Vygon provided us with the equipment to ensure some phases of the protocol.

The help of the library unit of the Catholic university of Louvain, UCL, Brussels, Belgium was invaluable for researching and locating a considerable quantity of old or elusive articles.

The Experimental Surgery and Transplantation unit (CHEX, Catholic university of Louvain, UCL, Brussels, Belgium) supported our project until the end and found creative solutions to reduce the human and material costs of our protocol.

The Radiometer company has provided us free of charge with the technical means to set up a delocalized laboratory in order to carry out over 1600 blood tests.

The rest of the financing was provided by personal funds.

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CHAPTER 2

EFFECT OF PRESSURE SUPPORT VENTILATION ON CARBOXYHEMOGLOBIN TOXICOKINETIC AFTER ACUTE CARBON MONOXIDE INTOXICATION: A SWINE MODEL

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2.1. ABSTRACT

Presented at 37th ISICEM Congress, 2017, March 2017, Brussels, Belgium.

Effect of Pressure Support Ventilation on Carboxyhemoglobin Toxicokinetic after Acute Carbon Monoxide Intoxication: a Swine Model

Introduction: In an experimental study on carbon monoxide (CO) exposure in swine, we aimed to compare the influence of oxygen therapy using a non-rebreathing mask (NRM) to continuous positive airway pressure (CPAP) and two pressure support ventilation (PSV) devices on the decrease of the terminal elimination half-life of carboxyhemoglobin (COHbt_{1/2}). This was the primary outcome.

Methods: Eight spontaneously breathing pigs were sedated by propofol and exposed to 940 ppm CO several times (n = 25) to obtain COHb levels of 30%. CPAPb (high flow open system, CPAP Boussignac® [7.5 cmH₂O]), PSV-Vy (open system, Vylife Boussignac®), and PSV-Leg (closed system, Legendair® [inspiratory/expiratory airway pressure 12/4 cmH₂O]) devices were used in a randomized order and compared to NRM (O₂ at 15 l.min⁻¹) and atmospheric air (AA). The primary outcome was COHbt_{1/2}. Multiple comparisons were performed using Dunn's tests.

Results: Median FiO₂ and minute ventilation were significantly higher in the PSV-Leg group than the NRM group (p < 0.05). Median COHbt_{1/2} was 251, 85, 82, 93, and 58 min for AA, NRM, CPAPb, PSV-Vy, and PSV-Leg, respectively. All the interventions were superior to AA in terms of CO elimination (p < 0.001), but there was no statistically significant difference between CPAP or PSV and NRM. There was only a trend between PSV-Leg and NRM (p = 0.18). The median AUCs for ln (COHb) × time (h) were 170, 79, 83, 100, and 64 for AA, NRM, CPAPb, PSV-Vy, and PSV-Leg respectively, with a statistically significant difference only between AA and PSV-Leg (p = 0.002).

Conclusions: In conclusion, in our study on CO intoxication in swine, the use of the closed PSV-Leg system led to the shortest COHbt_{1/2}. These results suggest that PSV-Leg can be more efficient than NRM in eliminating CO and support the design of a clinical study to assess this hypothesis.



Effect of Pressure Support Ventilation on carboxyhemoglobin toxicokinetic after acute carbon monoxide intoxication: a swine model

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Introduction

Fraction of inspired oxygen ($FiO_2\%$) and minute ventilation influence half-life of carboxyhemoglobin ($COHb\ T_{1/2}$) after acute carbon monoxide (CO) poisoning but the effects of continuous positive airway pressure (CPAP) or pressure support ventilation (PSV) were never been studied.

Methods

We compared O_2 therapy by non rebreathing mask (NRM) to CPAP using high flow O_2 and to two procedures of PSV. Eight spontaneously breathing pigs sedated by Propofol were exposed several times, separated by washout period, to CO until $COHb$ level of 30%. CPAP (CPAPBoussignac[®], 7.5 cmH_2O), PSV-Vy (Vylife Boussignac[®]), PSV-Leg (Legendair[®]) [Inspiratory/Expiratory PAP 12/4 cmH_2O] were used in random order and compared to NRM [O_2 at 15l/min] and atmospheric air (AA). $COHb\ T_{1/2}$ was assessed by non-compartmental analysis. Multiple comparisons were performed by Dunn's tests. Results are expressed as median [range].

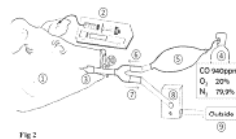
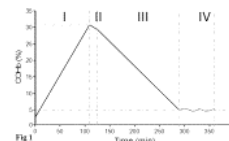


Fig 1: Experimental procedure: Phase I: intoxication; Phase II: no treatment (atmospheric room air); Phase III: elimination; Phase IV: no treatment to assess $COHb$ behavior post treatment.
Fig 2: Procedure of phase I.
① Pigs (72-88kg) ② Anesthetized with Propofol 2%
③ Spontaneous breathing through endotracheal tube
④ Gas source containing $CO\ 940ppm$
⑤ Gas reserve in balloon inhaled by pigs ⑥ One direction valve
⑦ Scavenger taking out exhaled gas
⑧ Gas were draw up out of the room through window.

Results

	NRM (n=8)	CPAP (n=5)	PSV - Leg (n=5)	PSV - Vy (n=3)
TRT time (min)	168 [102-228]	168 [90-210]	114 [102-120]	222 [210-228]
$COHb\ T_{1/2}$ (min)	85 [46-116]	82 [40-94]	58 [52-79]	93 [92-113]
FiO_2 (%)	72 [59-83]	54 [49-73]	92 [76-93]	51 [51-53]
pCO_2 (mmHg)	43.3 [39.2-52.5]	47.8 [46.5-48.3]	44.6 [39.4-48.6]	40.6 [37.1-46.7]
pO_2 (mmHg)	369.4 [230.1-418.2]	281.0 [234.0-441.2]	475.0 [323.4-478.6]	286.2 [247.1-300.5]
MV (min^{-1})	6539 [5587-10312]	7418 [6788-8460]	9726 [9377-11404]	8096 [6695-8602]

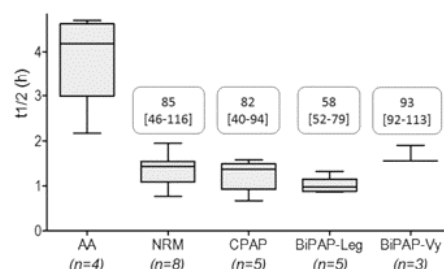


Fig 3: $COHb\ T_{1/2}$ (minutes); median [Range]

Conclusion

In our swine model of CO poisoning, CPAP and PSV-Vy led to similar $COHb\ T_{1/2}$ than NRM (85 min). With PSV-Leg, $COHb\ T_{1/2}$ was lower (58 min) but without significant difference. A lack of statistical power may explain these results. Further studies are required to explore the comparative efficacy of PSV in CO poisoning.

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Effect of Pressure Support Ventilation on Carboxyhemoglobin Toxicokinetic after Acute Carbon Monoxide Intoxication: a Swine Model

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Abstract

Introduction In an experimental study on carbon monoxide (CO) exposure in swine, we aimed to compare the influence of oxygen therapy using a non-rebreathing mask (NRM) to continuous positive airway pressure (CPAP) and two pressure support ventilation (PSV) devices on the decrease of the terminal elimination half-life of carboxyhemoglobin (COHb $t_{1/2}$). This was the primary outcome.

Methods Eight spontaneously breathing pigs were sedated by propofol and exposed to 940 ppm CO several times ($n = 25$) to obtain COHb levels of 30%. CPAPb (high flow open system, CPAP Boussignac® [7.5 cmH₂O]), PSV-Vy (open system, Vylife Boussignac®), and PSV-Leg (closed system, Legendair® [inspiratory/expiratory airway pressure 12/4 cmH₂O]) devices were used in a randomized order and compared to NRM (O₂ at 15 l min⁻¹) and atmospheric air (AA). The primary outcome was COHb $t_{1/2}$. Multiple comparisons were performed using Dunn's tests.

Results Median FiO₂ and minute ventilation were significantly higher in the PSV-Leg group than the NRM group ($p < 0.05$). Median COHb $t_{1/2}$ was 251, 85, 82, 93, and 58 min for AA, NRM, CPAPb, PSV-Vy, and PSV-Leg, respectively. All the interventions were superior to AA in terms of CO elimination ($p < 0.001$), but there was no statistically significant difference between CPAP or PSV and NRM. There was only a trend between PSV-Leg and NRM ($p = 0.18$). The median AUCs for ln (COHb) × time (h) were 170, 79, 83, 100, and 64 for AA, NRM, CPAPb, PSV-Vy, and PSV-Leg respectively, with a statistically significant difference only between AA and PSV-Leg ($p = 0.002$).

Conclusion In conclusion, in our study on CO intoxication in swine, the use of the closed PSV-Leg system led to the shortest COHb $t_{1/2}$. These results suggest that PSV-Leg can be more efficient than NRM in eliminating CO and support the design of a clinical study to assess this hypothesis.

Keywords Carbon monoxide · Swine · Positive pressure ventilation · Continuous positive airway pressure (CPAP) · Toxicokinetic · Carboxyhemoglobin (COHb) · Oxygen therapy

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2.2. INTRODUCTION

Carbon monoxide (CO) is one of the most frequent causes of toxic deaths in the world [1]. The main objective of oxygen therapy for CO poisoning is to increase the CO elimination rate, i.e., to decrease the elimination half-life of carboxyhemoglobin ($\text{COHbt}_{1/2}$). The final objective of this therapeutic approach is to prevent delayed neurological sequelae.

The mode of oxygen administration strongly influences its efficacy: $\text{COHbt}_{1/2}$ ranges from 320 min at atmospheric room air (AA) to 71 min with normobaric oxygen therapy coupled to a non-rebreather mask (NRM) at $15 \text{ l}\cdot\text{min}^{-1}$ and to 21 min with hyperbaric oxygen (HBO) therapy at a pressure of 1.5 to 3.0 atm absolute (ATA) [2, 3].

Nevertheless, the clinical benefit of HBO therapy for the prevention of neurological sequelae is still under debate. HBO therapy could even expose the victims to deleterious effects [4–6]. While oxygen seems to be the natural antidote for CO poisoning, there is some concern regarding the role of massive oxygen therapy on free-radical production, leading to further cellular damage [4, 6]. Moreover, HBO therapy is not uniformly available in all areas at all times, which leads to significant delays in initiating therapy [7]. This led us to investigate other convenient ways to improve CO elimination. Continuous positive airway pressure (CPAP) with high flow oxygen device and pressure support ventilation (PSV) may positively influence fraction of inspired oxygen (FiO_2) and minute ventilation and may be candidates for CO intoxication treatment [8]. Our hypothesis was that these ventilation techniques, which are more convenient and more widely available than HBO, could be more efficient in eliminating CO than NRM. However, as these techniques had not been previously evaluated in this context, a preliminary assessment was required in order to ensure that our hypothesis was plausible and could be safely

tested in a clinical setting, find the most promising ventilation support, and estimate the number of patients required for a clinical study. We therefore performed a pilot experimental study on swine in which we compared the effect of CPAP and two PSV devices to NRM and atmospheric air (as a control without treatment) on $\text{COHbt}_{1/2}$.

2.3. MATERIALS AND METHODS

A prospective exploratory randomized trial on swine was conducted using eight male Landrace pigs (77 kg, [72–88]). This animal experimental study was approved by the Université catholique de Louvain's ethics committee for animal experimentation (UCL, Brussels, Belgium) (ref 2013/UCL/MD/001 and ref 2015/UCL/MD/28). Formal approval was obtained for the use of the photographs taken during the experiments.

Intubation was performed on the animals using a cuffed endotracheal tube (7.5 to 8.0) 15 to 20 min after they had been pre-medicated with intramuscular injections of Tiletamine/Zolazepam (Zoletil® 100, Virbac© (6 mg.kg⁻¹) and Xylazin (Rompun® 2%, Bayer©) (2 mg.kg⁻¹). The pigs were sedated with a continuous intravenous administration of propofol (1.5 to 2.3 mg.kg⁻¹) in order to maintain spontaneous breathing throughout the study. Intubation was required to avoid obstructive apneic episodes during sedation and to record minute ventilation and end-tidal CO₂ levels during the experiment. In order to try to reproduce Non-invasive positive pressure ventilation (NiPPV), we did not connect the device directly to the endotracheal tube but to a Non-invasive naso-buccal mask (Fig. 1).



Fig.1: Experimental ventilation interface. The mask was adapted using an elastic strip to cover their labial commissure and limit air leaks.

The animals were then placed in the lateral decubitus position for the whole duration of the study. The propofol infusion rate was adjusted to obtain a balance between sedation and awakening, and pCO₂ values were maintained between 35 and 40 mmHg. Propofol was also titrated to control hemodynamic changes, spontaneous motor activity and palpebral reflexes, hypoventilation, and responses to noxious stimuli. Target levels of intoxication were obtained by administering a combination of 940 ppm (0.094%) CO gas, 20% O₂, and 79.906% N₂ (Pulmo1®, Air Liquide Medical systems©). This was administrated via a secured system using the endotracheal tube during spontaneous breathing. All exhaust gases from the respiratory circuit were scavenged and released outside the building (Fig. 2).

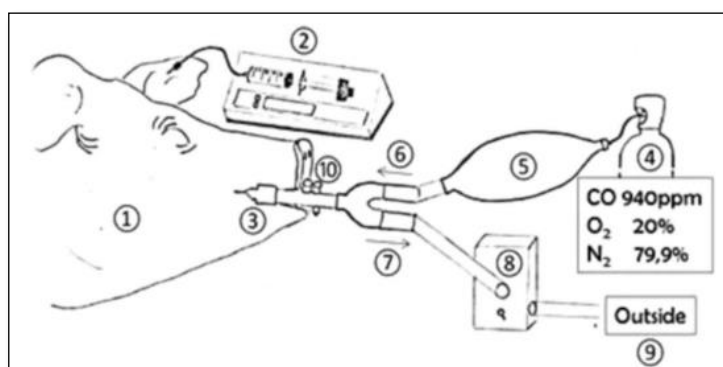


Fig. 2: CO intoxication phase. ① Pig. ② Anaesthesia with propofol 2%. ③ Spontaneous breathing through endotracheal tube. ④ Gas source containing CO 940 ppm. ⑤ Balloon with gas reserve for inhalation. ⑥/⑦ One-direction valve. ⑧ Exhaled gas scavenger. ⑨ Gas evacuation outside the room. 10 Pneumotachograph and FiO₂ measurement. After the intoxication phase, the endotracheal tube was kept in the same position and was covered by an adapted facial mask connected to the respiratory device (Fig. 1).

The CO concentration in the ambient air was continuously tested (PAC7000®, Dräger®, Germany).

Catheters were inserted into the carotid or femoral artery to continuously measure blood pressure and to determine COHb levels (%). All blood samples were analyzed for pH, pCO_2 (mmHg), pO_2 (mmHg), lactate (mmol l^{-1}), and COHb using the blood gas analyzer ABL90Flex® (Radiometer™, Radiometer Medical Aps, Denmark). For each experiment, minute ventilation ($\text{ml}\cdot\text{min}^{-1}$), invasive blood pressure (mmHg), FiO_2 (%), heart rate (min^{-1}), SpO_2 (%), end-tidal CO_2 (mmHg), and temperature (°C) were measured continuously using a pneumotachograph and a Datex-Ohmeda® S/5™ light monitor (Datex-Ohmeda®, Finland). Table warming was adjusted to maintain a target temperature of 37.5 °C.

Three different devices were investigated and compared to NRM: CPAP Boussignac® (CPAPb, Vygon®, Ecoen, France) and two PSV devices: Legendair® ventilator (Airox®, France) (PSV-Leg) and Vylife® (Vygon®, Ecoen, France) (PSV-Vy). The CPAPb system provides continuous positive pressure by injecting high-speed gas into a cylinder through angled side channels. This open system is fed by pure oxygen at 25–30 $\text{l}\cdot\text{min}^{-1}$ and delivers FiO_2 values from 0.7 to 1.0, depending on changes in respiratory rate and current volume [9, 10]. The pressure created from the flow of gas was monitored and maintained at 7.0–7.5 cm H_2O [11]. PSV-Vy is an open system, whereas PSV-Leg is a closed system. For both devices, positive airway pressure was set at + 12 cm H_2O for the inspiratory phase and + 4 cm H_2O for the expiratory phase, with pure oxygen, FiO_2 , at 1.0.

The complete experimental protocol was composed of four consecutive phases (Fig. 3): (I): the intoxication phase with determination of baseline COHb blood level followed by iterative sampling every 10 min until COHb levels reached 30% ($\pm 2\%$).

(II): the 'no intervention phase' with disconnection of the CO circuit. During that period, the animals were placed under ambient air (AA) for 10 min. This corresponded to the virtual delay between the extraction of the victim from the toxic environment and the arrival of the first responders in the context of human exposure. This delay was required for the installation of the respiratory device. (III): the treatment phase, with blood samples collected every 6 min until COHb returned to 5%. (IV): the post-treatment phase without any intervention for 60 min at AA in order to document the decrease in COHb or rule out a rebound.

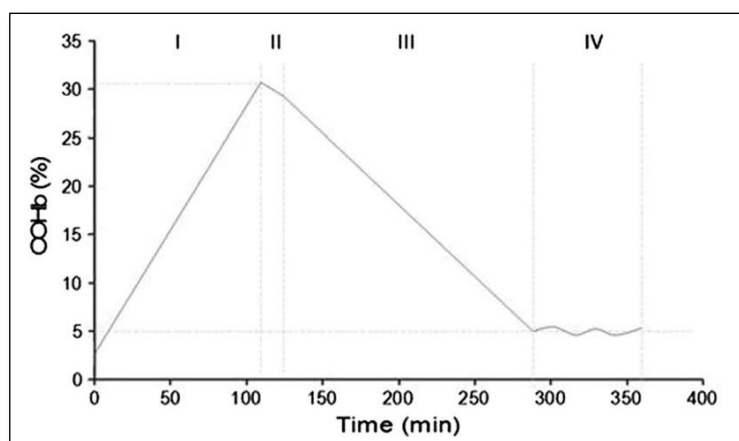


Fig.3: Experimental protocol. Phase I: intoxication; Phase II: no treatment (atmospheric room air); Phase III: treatment phase with different oxygenation/ventilation devices; Phase IV: no treatment (atmospheric room air) to assess COHb levels and verify the absence of a rebound.

Each day, one protocol was applied to a single pig, using a randomized treatment that was assigned after the intoxication phase. In order to limit the number of animals required and due to economic constraints, the same animal could be

exposed to a new intoxication phase and new randomized treatment using the same protocol the following day.

A minimum time of 12 h was established between experiments and we determined COHb levels before each experiment so as to exclude significant residual exposure, and we measured lactate levels so as to exclude end-organ damage.

Overall, four experiments were conducted with ambient air (AA), eight with NRM, five with CPAPb, five with PSV-Leg, and three with PSV-Vy.

The animals were euthanized at the end of the experiments once the study had been completed using a T-61[®] (embutramide/mebezonium iodide/tetracaine hydrochloride) injection.

The homogeneity of the intoxication phase was verified by comparing COHb levels and other parameters between the experimental groups at any time. The COHbt_{1/2} was assessed using non-compartmental analysis. The areas under the curve (AUCs) $\ln(\text{COHB}) \times \text{time (h)}$ were calculated. Results were expressed as medians [range], and continuous outcomes were compared using the Kruskal-Wallis test and Dunn's test for multiple comparisons using GraphPad Prism 4[®] software ($\alpha = 0.05$).

2.4. RESULTS

The clinical parameters were similar during all the phases of the experiment among the different intervention groups (Supplementary table, online resource or CH 7). There was no significant increase in lactate levels, and no rebounds in COHb levels were observed up to 1 h after the treatment had ended.

Measurements for delivered oxygen therapy were the highest in the PSV-Leg group in terms of median FiO_2 ($p < 0.05$ when compared to NRM, CPAPb, and PSV-Vy), minute ventilation ($p < 0.05$ when compared to NRM and CPAPb), and respiratory rate ($p < 0.05$ when compared to NRM and CPAPb) (Table 1). Median pO_2 values were significantly higher in the PSV-Leg group when compared to the CPAPb group ($p < 0.05$), and there was no difference in median pCO_2 values between the intervention groups (Table 1).

With regard to COHb toxicokinetic, the median terminal half-life of COHb was 251, 85, 82, 93, and 58 min for AA, NRM, CPAPb, PSV-Vy, and PSV-Leg, respectively (Fig. 4 and Table 1). All the interventions (NRM, CPAPb, PSV-Vy, and PSV-Leg) were superior to the reference group (AA) in terms of $\text{COHbt}_{1/2}$ ($p < 0.001$). However, there was no statistically significant difference between the intervention groups, with a trend only between PSV-Leg (58 min, $n = 5$) and NRM (85 min, $n = 8$) ($p = 0.18$).

The median AUCs for $\ln(\text{COHb}) \times \text{time (h)}$ were 170, 79, 83, 100, and 64 for AA, NRM, CPAPb, PSV-Vy, and PSV-Leg, respectively, with a statistically significant difference only between AA and PSV-Leg ($p = 0.002$).

	AA (<i>n</i> = 4)	NRM (<i>n</i> = 8)	CPAP (<i>n</i> = 5)	PSV-Vy (<i>n</i> = 3)	PSV-Leg (<i>n</i> = 5)
COHb t _{1/2} (min)	251 [130–273]	85 [46–116]	82 [40–94]	93 [92–113]	58 [52–79]
AUC (ln COHb x time (h))	170 [126–222]	79 [57–105]	83 [55–95]	100 [95–102]	64 [60–65]
FiO ₂ (%)	21	72 [59–83]	54 [49–73]	51 [51–53]	92 [76–93]
pCO ₂ (mmHg)	45 [30–58]	43.3 [39.2–52.5]	47.8 [46.5–48.3]	40.6 [37.1–46.7]	44.6 [39.4–48.6]
pO ₂ (mmHg)	88 [49–173]	369.4 [230.1–418.2]	281.0 [234.0–441.2]	286.2 [247.1–300.5]	475.0 [323.4–478.6]
MV (ml min ⁻¹)	6090 [1800–15,040]	6539 [5587–10,312]	7418 [6788–8460]	8096 [6695–8602]	9726 [9377–11,404]

Table 1: Median [range] respiratory parameters in swine during treatment. COHbt_{1/2} elimination half-life of carboxyhemoglobin, AUC area under the curve, FiO₂ fraction of inspired oxygen, pCO₂ partial pressure of carbon dioxide, pO₂ partial pressure of oxygen, MV minute ventilation in ml.min⁻¹. AA atmospheric air, NRM high concentration non-rebreathing mask, CPAP CPAP Boussignac, PSV-Vy PSV-Vylife, PSV-Leg PSV-Legendair.

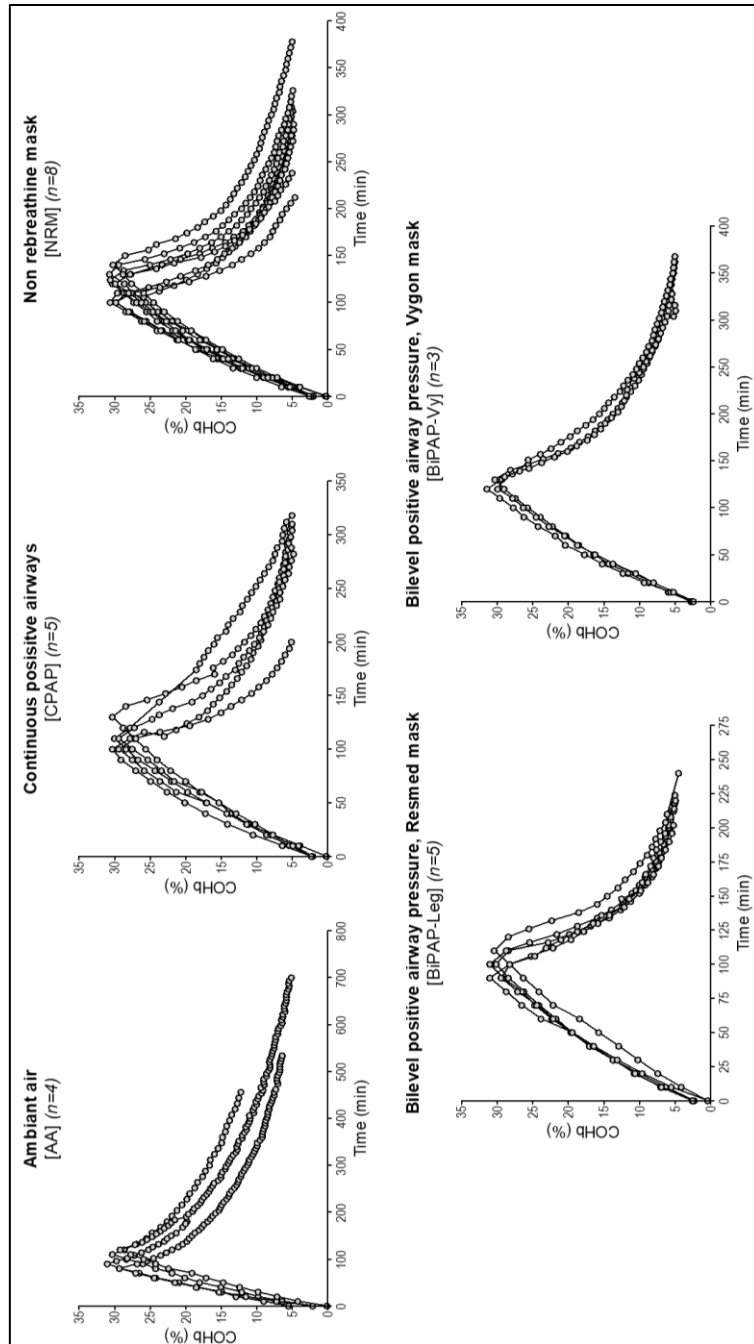


Fig. 4: Toxicokinetic profile of COHb for each treatment group. Atmospheric air (n = 4), CPAPb (n = 5), NRM (n = 8), PSV-Leg (n = 5), and PSV-Vy (n = 3).

2.5. DISCUSSION

Our pilot experimental study in swine on CO intoxication and Non-invasive pressure support ventilation with the closed Legendair® system showed promising results in terms of COHbt_{1/2} and the AUCs for COHb but did not indicate that there were benefits to using CPAP or PSV-Vy for CO elimination in comparison to NRM.

Non-invasive ventilation modes are widely used to treat acute respiratory failure in pre-hospital settings and emergency units, but, to our knowledge, our study is the first one to evaluate CPAP and PSV specifically in relation to CO intoxication. Several explanations can be hypothesized in light of our results and a few important limitations need to be acknowledged.

Firstly, our results confirm that NRM may not deliver 100% oxygen and, conversely to HBO, all ventilation supports can only lead to a limited value of pO₂ and a marginal increase in fractional atmospheric pressure [12]. Indeed, the Haldane equation for the reaction of CO in a blood sample is $[\text{COHb}]/[\text{O}_2\text{Hb}] = M \text{ pCO}/\text{pO}_2$, where $[\text{O}_2\text{Hb}]$ = oxyhemoglobin rate, pCO = partial pressure in CO dissolved, pO₂ = partial pressure in oxygen dissolved, and M = Haldane constant. Nevertheless, several in vivo studies showed the benefit of the influence of ventilation on COHb half-life [7]. Improving ventilation without excessive increasing pO₂ could limit the adverse effects of hyper-oxygenation. Moreover, our results suggest that ventilation therapies are not equal in terms of CO elimination. Oxygenation using NRM is considered the first standard treatment for CO poisoning. The FiO₂ delivered at 15 l.min⁻¹ using NRM is expected to reach up to 100%. However, due to unavoidable leaks around the mask and rebreathing, a maximum concentration of 85% is usually reached [13, 14]. In our study, the mean FiO₂ obtained was 72%. For CPAPb, the mean FiO₂ was limited to 58% for inspiratory flow in our experimental conditions, which runs contrary to the results of Templier et al.'s

bench laboratory test [10]. We observed similar results for PSV-Vy, with a mean FiO_2 of 51%. Both CPAPb and PSV-Vy are considered open systems. These open-system devices offer the advantage of more comfort for the patient, but the drawback of this is that atmospheric room air is inspired at the same time, thus resulting in lower FiO_2 levels in the gas mixture depending on inspiratory flow. Conversely, the PSV-Leg is considered a closed system and delivered higher levels of FiO_2 (92%) than NRM, CPAPb, and PSV-Vy. Interestingly, the PSV-Leg yielded the best results in terms of $\text{COHbt}_{1/2}$. Compared to NRM and CPAP, PSV is an effective ventilation mode for partly overcoming the potentially noxious effects of the sedation which decreases MV. It also improves pO_2 . Nevertheless, PSV-Vy and PSV-Leg used the same pressure levels and mode, yet the potential benefit of PSV-Leg mainly seems related to its higher levels of oxygen delivery. In addition, the trigger on PSV-Vy is unchangeable compared to that of PSV-Leg, so an inspiratory delay may lead to an increased inspiratory flow and thus decrease FiO_2 .

Secondly, our study in swine mimicked CO intoxication in humans to a certain extent but differed on several counts. Pigs were selected because they share some physiological cardiorespiratory characteristics with humans [15]. However, the relative affinity of swine hemoglobin for CO compared to O_2 (the Haldane constant [M]) at 37 °C is assumed to be 130, whereas the median (M) constant in humans is around 218 [16, 17]. This lower affinity for CO in swine hemoglobin may result in shorter laboratory experiments. We considered 30% COHb a significant level of intoxication because this level is supposed to induce clinically relevant symptoms in human intoxication and require HBO therapy [4]. The animals in the experiment had a body weight comparable to human (72 to 88 kg) and a facial size compatible with CPAP and PSV masks. Nevertheless, we needed to sedate them during the experiment and, as a first pilot experiment had shown numerous obstructive apneic episodes in pigs sedated under propofol, intubation was required. In order to

reproduce Non-invasive ventilation conditions, we did not directly connect the system to the endotracheal tube but to a naso-buccal mask (Fig. 1).

Thirdly, the power of the statistical analysis was limited by the small number of animals included in each intervention sub-group. This may explain why the difference observed between the PSV-Leg group (n = 5) and the NRM group (n = 8) was not statistically significant. Moreover, in an exploratory setting, we performed several analyses without considering alpha risk inflation. Finally, we cannot totally exclude the possibility that prior CO poisoning could have affected the terminal elimination half-life of CO in subsequent trials by virtue of end-organ damage. However, another experimental study found that long-term exposure to 200 ppm CO > 24 h did not lead to pathological changes in tissues or organs [18]. Moreover, before each experiment, we determined COHb levels so as to exclude significant residual exposure to CO and measured lactate levels for every COHb sample so as to exclude end-organ damage.

2.6. CONCLUSION

In conclusion, in our study on CO intoxication in swine, the closed PSV-Leg system gave the shortest COHbt_{1/2} (median 58 vs. 85 min with NRM) and the lowest AUC for COHb (median 63 vs. 79 with NRM). The results of our pilot study suggest that Non-invasive ventilation, such as the closed PSV-Leg system, can be more efficient than NRM at eliminating CO when HBO is unavailable and supports the design of a clinical study to evaluate this hypothesis.

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CHAPTER 3

CARBOXYHEMOGLOBIN HALF-LIFE TOXICOKINETIC PROFILE DURING AND AFTER NORMOBARIC OXYGEN THERAPY: ON A SWINE MODEL

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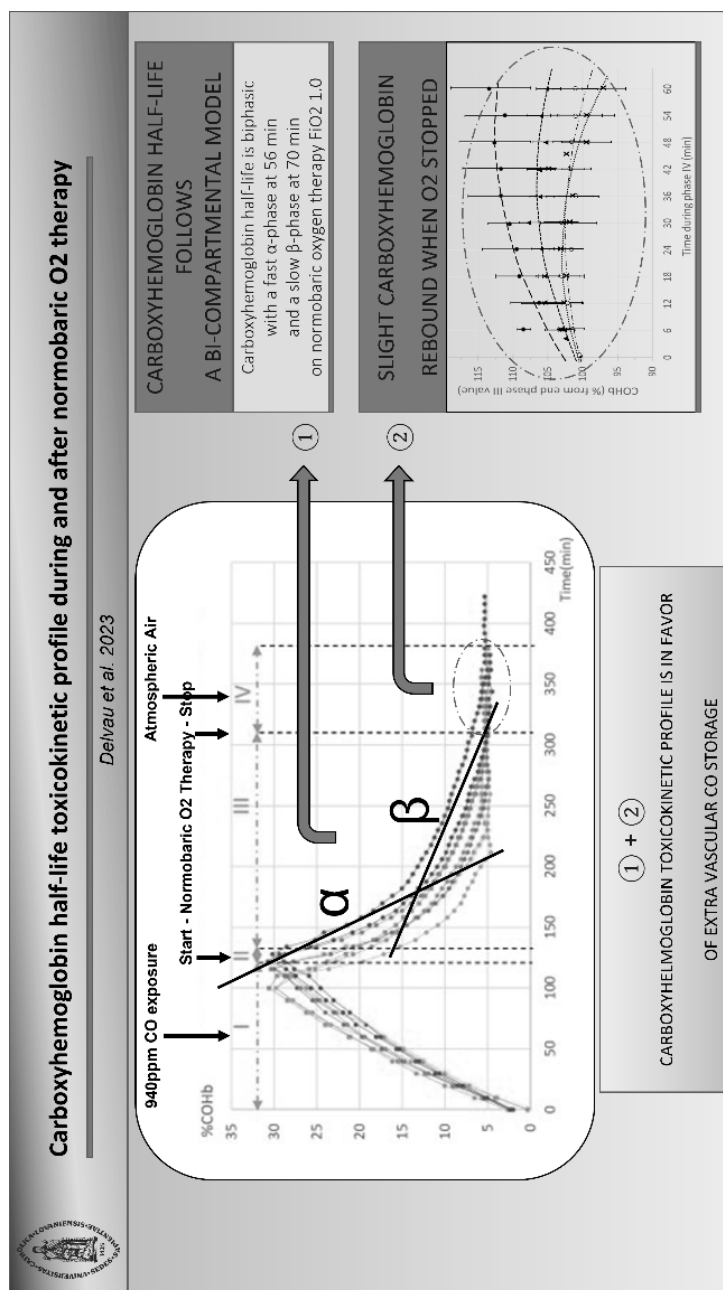
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3.1. ABSTRACT

Carboxyhemoglobin half-life toxicokinetic profile during and after normobaric oxygen therapy: on a swine model

After acute carbon monoxide (CO) poisoning, carboxyhemoglobin half-life (COHbt_{1/2}) toxicokinetic model is still controversial as it is mono- or bi-compartmental. Moreover, studies struggle to highlight a relevant discriminant criterion related to CO poisoning severity to predict complication as delayed neurological syndrome. In this context, it remains difficult to demonstrate the superiority of one method of oxygen (O₂) administration over others. Furthermore, the optimal duration of normobaric 100% oxygen (NBO) treatment is still the subject of debate. Myoglobin, as hemoglobin, are a potential binding site for CO, which could have an impact on clinical outcomes, but potential extravascular CO storage is inaccessible in clinical practice. Our aim is to focus on COHbt_{1/2} profile and on a potential COHb rebound after COHb has returned to 5% and NBO treatment stopped, as an indirect means of deducting this CO storage. On an experimental swine model exposed to moderate CO poisoning (940 ppm for ±118 min until COHb reached 30%), we first confirm that the COHb half-life follows a bi-compartmental model. Secondly, we observe for the first time a slight COHb rebound when COHb returns to 5% and oxygen therapy is stopped. On the basis of these two toxicokinetic characteristics in favor of extra vascular CO storage, we suggest considering the COHbt_{1/2} using the bi-compartmental model in future clinical studies comparing treatment effectiveness as a potential severity criterion to homogenize cohorts of the same severity. Moreover, from a general toxicokinetic point of view, we confirm that a treatment lasting less than 6 hours does not seem to be suitable for moderate CO poisoning. Future studies will be



necessary to confirm the impact of this COHbt_{1/2} criterion and/or a COHb rebound on the evaluation of CO poisoning severity.

Graphical Abstract

3.2. BACKGROUND

Carbon monoxide (CO) is estimated to be responsible for approximately 40,000 deaths and significantly affects more than one million people per year worldwide (2, 3). CO poisoning results in neurological sequelae at 6 weeks in 37% of cases (4). It has been a major unresolved medical and public health problem for over 120 years (3). Accidental CO poisoning in the US alone could cost society over \$1.3 billion a year, resulting from direct hospital costs and lost earnings (5).

While the exact role and accurate protocol of hyperbaric oxygen (HBO) therapy is still controversial in some categories of CO poisoned patients to avoid delayed neurological sequelae (DNS), normobaric oxygen (NBO) therapy is considered as a viable therapeutic option to treat moderate CO poisoning, leading to a comparable outcome with HBO therapy (3, 6-8). The American College of Emergency Physicians (ACEP) concluded that no clinical variables seemed to identify poisoned patients for whom HBO was most likely to provide benefit (9). One of the reasons why the superiority of one treatment over the other is difficult to prove is that there is too much heterogeneity in terms of severity among the cases of CO poisoning in the cohorts compared (8, 10).

Even though carboxyhemoglobin (COHb) level is easily measured in clinical practice, several publications confirmed poor correlations between the blood COHb level and the CO poisoning severity (11). It has also been shown that the same dose of CO administered in a different way (1 min at 7.500 ppm or 5 min at 1.500 ppm) could result in a similar COHb level ($\pm 2.06\%$) (12). In contrast, in guidelines, a COHb > 25% has been retained as potential severity criteria, but surprisingly, regardless of

when the blood is drawn after CO exposure has stopped, whatever post-exposure treatment is applied or of the CO exposure duration estimated (10, 13, 14). Other objective but not specific clinical selection criteria are used to judge severe CO poisoning such as transient or prolonged unconsciousness, abnormal neurologic signs, cardiovascular dysfunction, severe acidosis, or > 24 hours CO exposure. Nevertheless, they have not yet been able to demonstrate their effectiveness as truly discriminating criteria (15). It is thus necessary to re-examine early severe signs of CO poisoning to improve the homogeneity of cohorts compared to avoid failure of randomization (16). Studies comparing different treatments and/or assessing the severity of intoxication have not accurately assessed COHbt_{1/2} or the relevance of its estimation as an additional and more specific early means of identifying severe CO poisoning.

COHb level assessment alone does not take into account the complexity of CO pathophysiology (3, 17). Approximately 80% of the CO is bonded to hemoglobin (Hb) to form COHb. The remaining is bonded to other metalloprotein as myoglobin (Mb) for ≈15% to form carboxymyoglobin (COMb) and less than 5% to other compound stores as neuroglobin and cytochromes (18-21). This is considered as CO extravascular storage and could be correlated to CO poisoning severity, but it is not possible to technically evaluate this CO storage in clinical or experimental practice. In the light of results obtained using our swine model, we would like to discuss two potential indices that could provide an indirect estimation of this CO storage: first the COHbt_{1/2} profile and secondly a COHb rebound after COHb returns to 5% when treatment is stopped.

Although we don't have reliable specific biomarkers to assess the CO severity poisoning, we know that CO exposure time and exposure levels affect the severity of clinical symptoms, the CO extravascular burden and influence the COHbt_{1/2} (13, 21-23). Breathing in a high CO concentration during a short period results in a high %COHb level but a short COHbt_{1/2} due to low extravascular CO burden allowing for higher extravascular distribution during this post-exposure time (24). As a historical reminder, the famous physiologist Haldane experimentally intoxicated himself with COHb levels up to 50% for one hour. He developed acute symptoms, but didn't suffer neurological sequelae (25). Inversely, an exposure to a lower CO concentration but for a longer period will result in a lower COHb level, but allowing to reach a higher tissue CO burden with a longer COHbt_{1/2} (21). CO exposure estimated at least 24 hours at any level is actually considered as a severity criterion and is a current selection criterion for HBO (10). Although one of the keys to determining CO poisoning severity could result from the level and duration of CO exposure, they are most of the time hardly estimable in practice and are rarely reported in clinical studies - as opposed to experimental studies (16, 24, 26, 27).

First, as a preliminary to any evaluation of the role of COHbt_{1/2} as a severity criterion in future prospective clinical studies comparing different treatments, it is necessary to confirm the toxicokinetic model that corresponds to the COHb elimination profile. Using the right toxicokinetic model is necessary to evaluate COHbt_{1/2} as accurately as possible. Nevertheless, this COHbt_{1/2} toxicokinetic model is still controversial (16, 24, 26, 27). If the CO distribution in the body is not limited to only one compartment (the blood sector as COHb), the analysis of its toxicokinetic elimination (COHbt_{1/2}) should theoretically show a bi- or multi-compartmental profile and not a mono-compartmental one. Knowing myoglobin CO affinity, a second compartment of CO storage is expected, resulting in a biphasic COHb

elimination profile. This bi-compartmental model expressed by two $\text{COHbt}_{1/2}$ phases would be explained by: (1) a first α -phase of rapid COHb decay which correspond to CO elimination by expiration concomitantly with continued CO distribution in the extravascular tissue; and (2) a slower second β -phase, correlated to intravascular burden clearance and, because of the slow transfer of CO between Mb and Hb, a late CO release from potential extravascular storage (21, 23, 24).

Few studies have mentioned biphasic $\text{COHbt}_{1/2}$ elimination on CO poisoning and usually only on atmospheric air-treated (AA) such as Godin's study on dogs (1972), Myhre's on humans (1974), Wagner's on dogs (1975), Shimazu's on sheep (1990 and confirmed in 2000), and Croenenberg's on human smokers (2007) (28-30). Concerning experimental animal studies specifically using NBO, only two were published (on dogs) and contradict each other: Schwerma's older study in 1948 observing a biphasic $\text{COHbt}_{1/2}$ elimination, compared to Sasaki's 1975 study in favor of mono-compartmental decrease (30, 31). Since those two studies, accurate bi-compartmental elimination on CO poisoning NBO-treated in experimental conditions has never been accurately published. Only Bruce's mathematical model recently published data from an AA-treated human cohort modeled as 'NBO-treated' and demonstrated a bi-compartmental $\text{COHbt}_{1/2}$ elimination (21).

Secondly, in addition to the demonstration of a bi-compartmental $\text{COHbt}_{1/2}$ model, another objective argument in favor of CO storage would be to observe a significant COHb rebound after cessation of treatment once the COHb returns to <5%. This could also be a delayed CO release from extravascular storage. To date, COHb behavior evaluation after COHb returns to <5% is poorly explored in humans (32). Only Anderson in 1978 suggested that if exposure was long enough to reach equilibrium, COMb could be accounted for a rebound of COHb to significant levels

several hours after therapy, but without publishing any data (33). Dolan, citing Anderson, only noted that in patients who had received HBO therapy for severe CO poisoning until COHb was no longer detectable, COHb could be detected again several hours later, as a possible effect of slow CO release from tissular compartments. He claimed that severe CO poisoning patients could benefit from a new COHb sample several hours following HBO (33, 34).

No systematic review, randomized control trials, or cohort studies indicate the optimal duration of NBO 100% O₂ treatment after moderate CO poisoning (35). Even O₂ itself has not been rigorously evaluated as a therapy or for side-effects for CO poisoning (36-38). The duration of NBO treatment should therefore be adapted as accurately as possible to early poisoning severity identification in order to avoid DNS but without over-treating to avoid potential O₂-induced side effects. In clinical practice, to date, in NBO, the usual therapeutic approach for CO poisoning is empiric and recommends the use of continuous O₂ therapy for at least 6 to 12 hours or until COHb levels fall to < 5% or even ≤3%, considered as a safe level (7, 10, 13, 26, 35, 39-41). After this, further evaluation of COHb level is described as unnecessary, but hasn't been investigated (10).

In case of COHb rebound occurrence after treatment cessation, the clinical implication should be continuous 100% NBO beyond the time needed to return to 5% COHb, with an objective argument of opting for a treatment time of up to 6 or 12 hours in order to remove CO extra vascular storage that is clinically unmeasurable. This treatment duration might have an impact not only on the patient's outcome, but also on length of stay and costs incurred in the emergency departments. Optimizing the duration of NBO therapy is also supported by recent

studies indicating that CO, once stored in the brain, is more difficult to eliminate than from other tissues (42). Nevertheless, other authors have suggested that even NBO could have a detrimental effect (43). In this case, objective arguments to balance NBO dose (level and duration) are needed.

Thus, on our swine model, our goal was first to come to a conclusion on the COHbt_{1/2} mono- or bi-compartmental toxicokinetic model. Correlation between an accurately calculated COHbt_{1/2} and CO poisoning severity could then be explored in future studies. Our second goal was to study the COHb profile one hour after cessation of NBO treatment in order to explore a potential late release of CO after a return to normal COHb of <5%.

3.3. MATERIALS AND METHODS

Based on a previously published study on half-life COHb elimination ($\text{COHbt}_{1/2}$) using a model of male swine treated using several normobaric oxygenation (NBO) methods, we aimed first to investigate the COHb toxicokinetic model as mono- or bi- compartmental, and secondly the COHb late terminal behaviour when COHb has dropped below 5% and treatment has been stopped. The experimental conditions are outlined in a previous paper (1). The study was approved by the Université catholique de Louvain's ethics committee for animal experimentation (UCL, Brussels, Belgium) (ref 2013/UCL/MD/001 and ref 2015/UCL/MD/28). Briefly, eight pigs (21 experimentations) were acutely exposed to CO at 0,094% or 940ppm until COHb level reached 30%. Then they were treated, either with atmospheric air, or using NBO therapies with different devices (non-rebreathing mask (NRM), continuous positive airway pressure (CPAP), non-invasive positive airway pressure (NiPPV) on two different devices (NiPPV-V and NiPPV-L) delivering $\%\text{FiO}_2$ at 72 [59-83]; 54 [49-73]; 51 [51-53]; 92 [76-93] respectively. The objective was to assess the $\text{COHbt}_{1/2}$ until COHb level reached 5%. This toxicokinetic analysis was focused on phase III of the experiment as illustrated in Fig. 1.

Firstly, for a new assessment on data from the previous study, we assessed the toxicokinetic $\text{COHbt}_{1/2}$ model using our experimental COHb values obtained during washout under NBO in phase III (n=21). We plotted COHb% on a log scale vs. time on a linear scale, using the linear regression method with least square method (21). To decide on the best model, models are compared using the Bayesian information criterion (BIC).

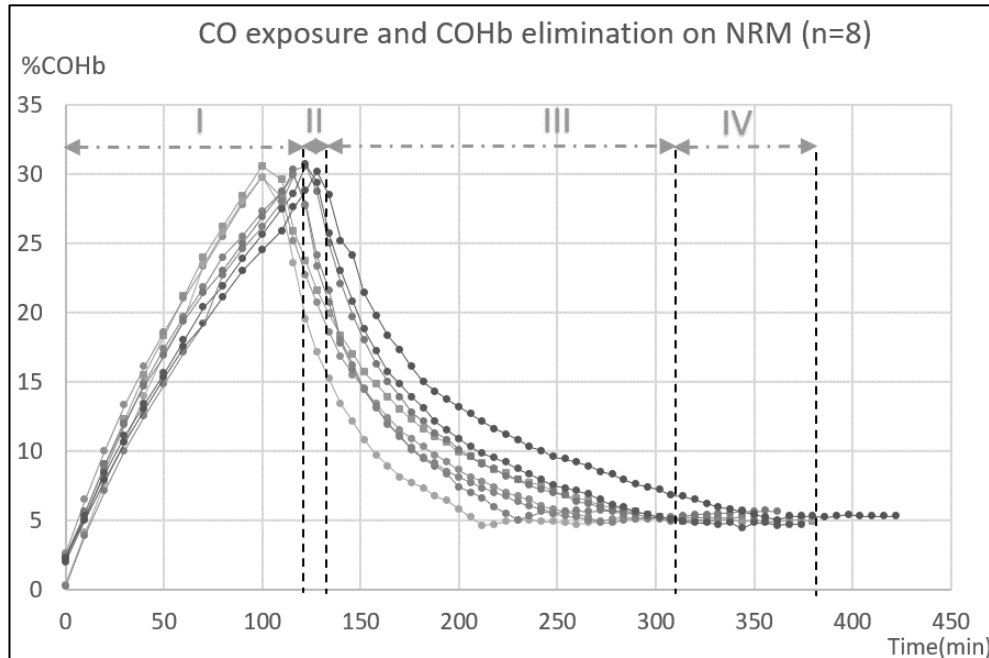


Fig. 1: COHb samples during CO exposure and during the NRM treatment arm on eight swine (1). As an example, on one of them (squares on the curve), phases are separated by dashed black vertical lines. Phase I: CO exposure time to reach COHb at 30% being 118min [± 10.4]; Phase II: no treatment (AA) for 10min; Phase III: time needed (min) to reach COHb at 5% varied according to the oxygenation/ventilation modalities: 110.15 [± 4.1]; 163.2 [± 15.3]; 168.7 [± 22.4]; 217 [± 5.9]; 647.9 [± 64.0]; 237.7 [± 38.7] for NiPPV-V; NRM; CPAP; NiPPV-L; AA; all treatments, respectively (p-value<0.0001); Phase IV: last hour only on AA to assess %COHb levels and verify occurrence of a rebound. From (1).

Theoretically, after cessation of CO exposure, a mono-exponential washout would be consistent with the concept that CO is being removed from either a single vascular compartment or a vascular compartment in rapid equilibrium with extravascular tissues (Fig. 2; Left) (44).

However, a bi-compartmental toxicokinetic model shows a biphasic COHb elimination profile. An initial rapid decrease (α -phase) with a steep slope (α -cst) correlated to CO removed from COHb in blood which can be at the same time exhaled by lungs and distributed to extra vascular tissues as myoglobin, followed by a slower phase (β -phase) with a softer slope (β -cst) correlated to CO exhalation from intra- and extra- vascular compartments (Fig. 2; Right).

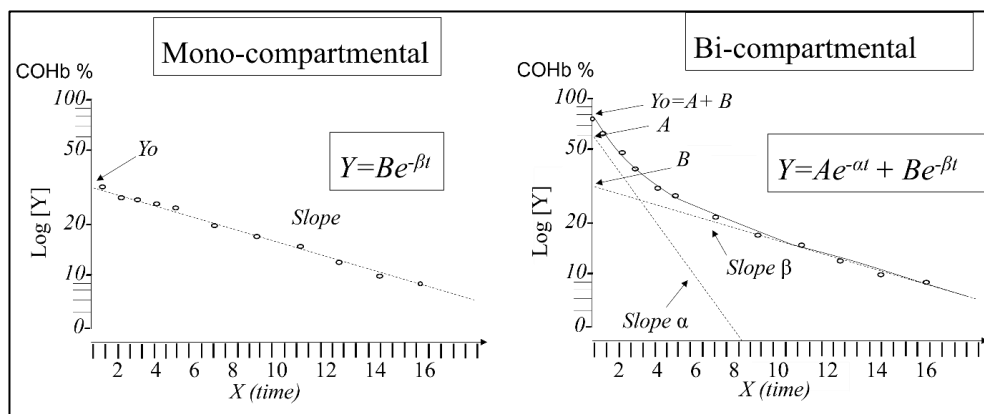


Fig. 2: COHb blood concentration vs time profiles during CO elimination on semi-logarithmic scale (mono- or bi- exponential equations) for CO which would exhibit a mono- or bi-compartmental model. Inspired by (16, 45), where ' α ' is the distribution constant, ' β ' is the terminal elimination constant. ' A ' is the y-axis intercept for the α -phase and ' B ' the y-axis intercept for the β -phase, ' t ' is the time after the end of intoxication. $COHbt_{1/2-\alpha} = 0.693/\alpha$ and $COHbt_{1/2-\beta} = 0.693/\beta$ (16).

Second, data were also collected during the last phase IV on AA (Fig. 1). When any therapy was stopped because COHb level target had reached 5%, blood was sampled every 6 min for 60 min (1). Analysis was focused on potential COHb rebound during phase IV. In the case of no COHb decay, $COHbt_{1/2}$ would be impossible to assess. Thus, we tested if data corresponded to a quadratic model

used to assess the importance of a potential rebound. For the rebound assessment, swine that were immediately on AA after CO exposure without NBO treatment (the control group; n=4) were not considered.

All analyses were performed using JMP statistical software version Pro 16.0.0 (SAS Institute Inc., Cary, NC, USA). To gain functional understanding of the COHb response following the different treatments stopped, polynomial regression models using the least square method were fitted to the data. Three mathematical expressions (i.e., linear, quadratic and exponential) were tested to mathematically shape the evolution during the phase IV. Corrected-Akaike criterion (AICc) and r^2 values were assessed to compare and evaluate the adequacy of the tested models. In addition, a visual inspection of the residuals was performed to exclude any potential bias. The best fit was selected based on a 2 AICc improvement (lower scores) when comparing 2 models. In any case, $p < 0.05$ was considered as statistically significant.

3.4. RESULTS

Concerning the first question on mono- or bi-compartmental toxicokinetic model observed on our swine model, overall, for all treatments, a bi-compartmental model fitted the data the best (Δ BIC compared to mono-compartment=-302.8) (Fig. 3). When analyzing the data for each pig individually, the bi-compartmental model was the most appropriate except for two experiments on two different pigs (Fig. 3).

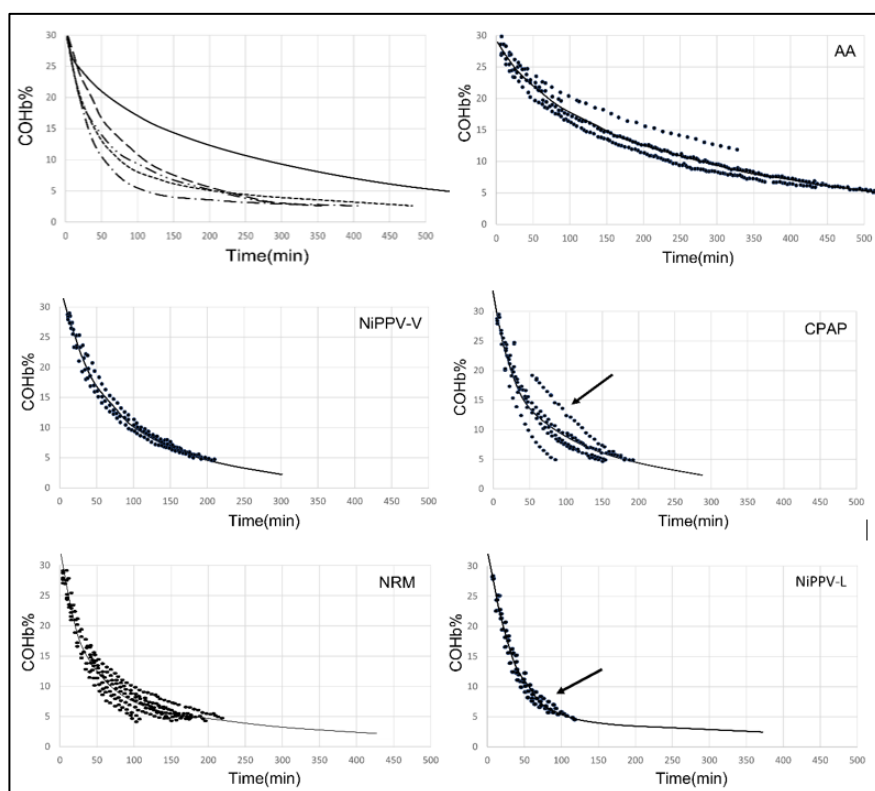
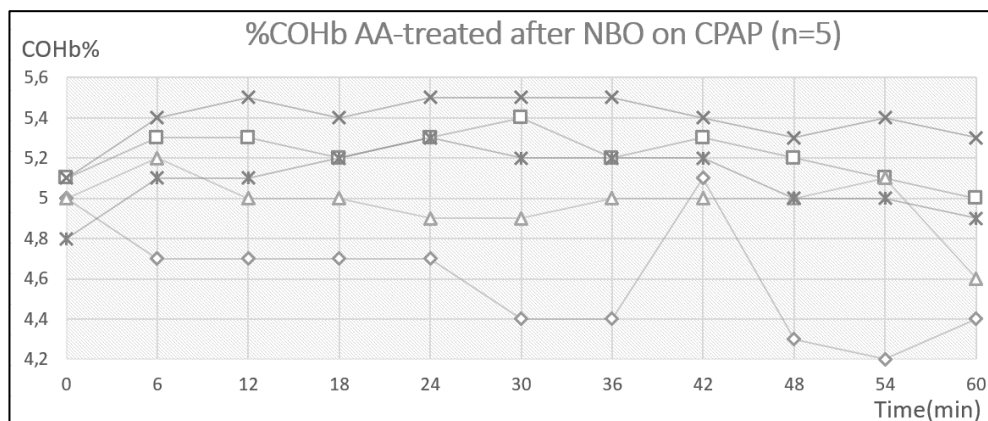
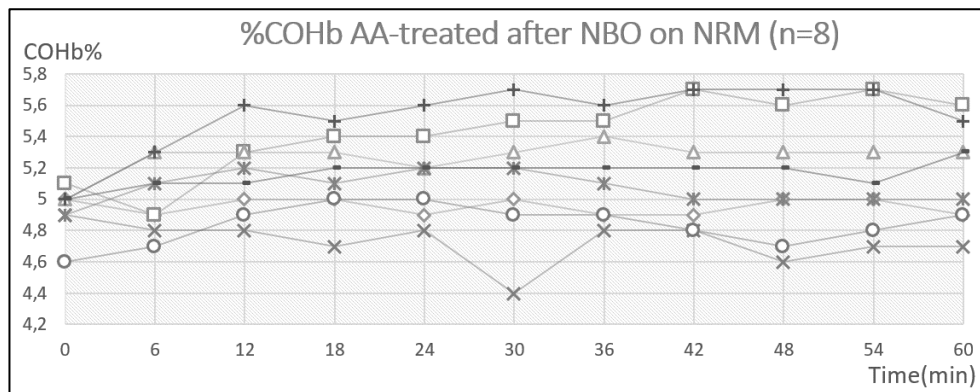


Fig. 3: Bi-compartmental $\text{COHb}_{t_{1/2}}$ elimination observed during treatment phase III with AA; NiPPV-V; CPAP; NRM; NiPPV-L from top to bottom. COHb elimination curves for each treatment suggests individually that the distribution and elimination of CO from the blood are best explained by a bi-compartmental model. Bi-compartmental model fit curves for each swine individually except for the two experiments marked by arrows.

Concerning the second question on COHb rebound on more than a thousand COHb assessments during all treatments over the phase III period (COHb decay from 30 % to 5%), absolutely no rebound was observed at any moment, not even 0.1% COHb (Fig. 1. Phase III) (1). However, over the first hour after termination of the different NBO methods, after COHb had returned to 5%, an apparent steady state of COHb level was observed or even a slight rebound. This profile curves methodologically did not allow for a calculation of COHbt_{1/2} value (Fig. 2 and 3).



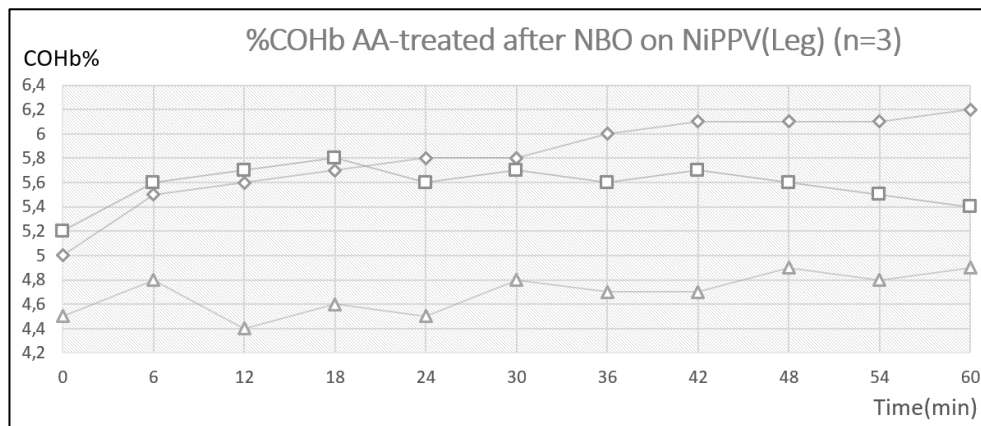
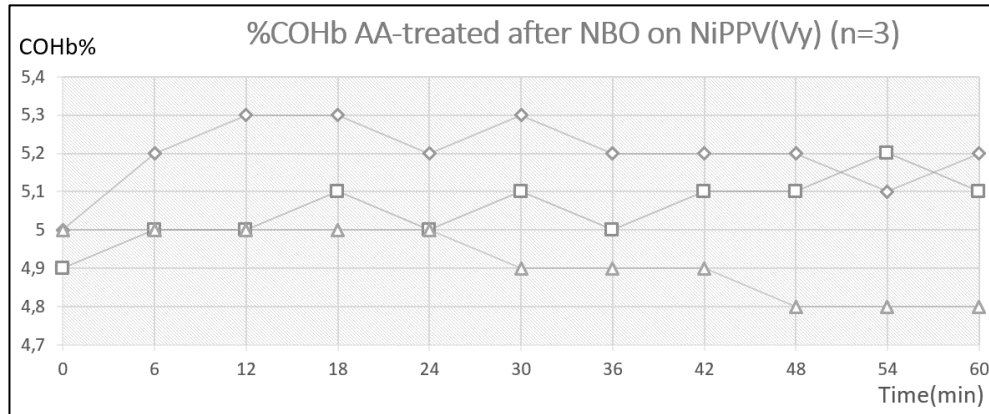


Fig. 4: After phase III as elimination phase on NRM, CPAP, NiPPV-V or NiPPV-L: %COHb evolution during phase IV, the additional hour without any treatment with blood samples taken every 6 min (11 samples) (n=19).

The quadratic model fitted the data the best ($\Delta AICc = -10.04$ compared to the linear model, $r^2 = 0.08$, $p = 0.0003$) and the plot of the residuals did not reveal any particular bias. This observation suggests that a parabola better fits our set of data compared to a linear or exponential model. The fit was improved when the treatment was introduced in the quadratic model as an independent factor ($\Delta AICc = -43.03$ compared to the model without covariates, $r^2 = 0.33$, $p < 0.0001$), reflecting that the

shape of the curve depends on the treatment previously received. Compared to other treatments, the strongest rebound was observed in NiPPV-L ($p<0.0001$; Fig. 1) (Fig. 4).

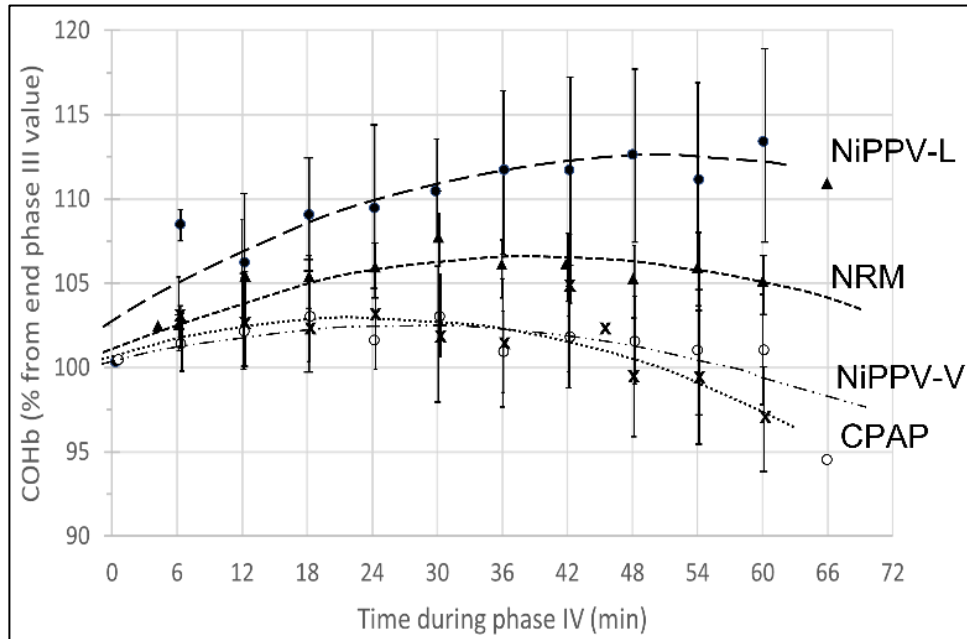


Fig. 5: COHb expressed in percentage from last measured value of Phase III (range 4.5 to 5.2% considered individually as 100%) according to time post phase III as Phase IV (min). Phase IV is the last hour without any intervention (on AA). Data are expressed as means $\pm$ SD. The lines represent the quadratic regression model according to each treatment. This allows firstly to homogenize the starting values, to compare curves and to better visualize the evolution of %COHb if it continues to fall as in treatment during phase III or if it stabilizes or even rises in this phase IV.

3.5. DISCUSSION

While analyzing the data on our experimental study of swine exposed to CO and treated on NBO, the $\text{COHbt}_{1/2}$ is biphasic following a bi-compartmental toxicokinetic model confirming the Shimazu data on sheep treated on atmospheric air and in contrast to Weaver's conclusion in a human retrospective clinical study. This suggests bi-compartmental CO distribution and a potential extravascular CO storage.

While NBO treatment for CO poisoning at COHb 30% brought COHb down to 5% and was stopped, during one hour on atmospheric air, COHb % failed to decrease. On the contrary, we observed a light COHb rebound also according with a potential extravascular CO storage, meaning that a further discussion on NBO treatment duration could be necessary.

3.5.1. On the bi-compartmental model

We considered in our previous study a non-compartmental assessment to avoid ambiguity regarding mono- vs bi-compartmental models and to compare our data with clinical studies suggesting the mono-compartmental $\text{COHbt}_{1/2}$ toxicokinetic model (26). In this much more detailed data analysis from our procedure including a large number of samples, the bi-compartmental model was confirmed for all experiments except for two where a mono-compartmental model was applied because it fitted the data the best (arrows in Fig. 3). Actually, in a very interesting way, one of the NiPPV-treated swine (NiPPV-L-4) showed such a fast early $\text{COHbt}_{1/2}$ that second β -phase didn't yet appear. And conversely, for one of the CPAP-treated swine (CPAP-1), due to a technical problem, CO values from 6 to 48min were not collected and the α -phase could not be modeled (arrow in Fig. 3). This is consistent with Weaver's clinical study where only few samples were made (26). As already

developed by Shimazu (bi-compartmental model observed on AA-treated sheep) and Weaver (mono-compartmental model observed in NBO-treated humans) and supported later by Bruce (bi-compartmental model observed in mathematical model developed from data on AA-treated humans), the argument of mono- vs bi-compartmental COHb elimination is rather methodological (21, 24, 27). An early rapid fall with shorter COHbt_{1/2} (α -phase) is probably not observed in Weaver's retrospective clinical study when early COHb measurements are missing, and is quickly followed by a second longer COHbt_{1/2} (β -phase) which is more significant (24). Early sampling is most often lacking in clinical practice during the α -phase, which is concomitant with the removal of the victim from the toxic environment, transport by ambulance, and/or admission to the emergency department. Usually, only two or three samples are taken from a CO poisoned patient, which does not allow us to observe a precise COHbt_{1/2} and even less so a biphasic character (4).

Other reasons could generate confusion. Mono-compartmental elimination could be observed in the case of bi-compartmental distribution if the intravascular compartment is in rapid equilibrium with the extravascular tissues (44). In the CO context, MbCO levels fall more slowly during washout than does %COHb even if CO links Mb with a lower relative affinity 'M' constant than for Hb (39 compared to 218 respectively). The slow rate of exchange of CO between blood and muscle tissues, compared to that of O₂, results from both the blood-tissue conductance for CO and the pressure difference driving this flow (23). Therefore, the mono-compartmental model is theoretically not expected (46-48). However, a bi-compartmental elimination can be observed in spite of a mono-compartmental elimination if some peripheral organs are poorly perfused (24). In our swine model, blood pressure, heart frequency and lactate were continuously monitored and physiological values

remained steady. Thus, our contribution fosters a discussion regarding the COHbt_{1/2} toxicokinetic model in favor of a bi-compartmental one.

In retrospective clinical studies, COHbt_{1/2} is calculated on the basis of only two COHb blood samples collected at random times (t₁ and t₂) where $COHbt_{1/2} = (t_2 - t_1) \times \ln(2) \div \ln(COHb_{t_1} - COHb_{t_2})$. This 'two-points method' assumes that COHb follows a mono- exponential elimination (4, 21, 49).

Bruce demonstrated on AA-treated humans (n=12) significant COHbt_{1/2} discrepancies which could be obtained from a 'two-point method' dependent on the sampling time. COHbt_{1/2} was assessed at 208±78min; 275±75min or 358±73min if the two blood samples considered were collected at five and 67 min (i.e. pre-hospital transport time estimated to be the rapid fall α-phase); 67 and 131min (i.e. moment of admission in emergency department) or 131 and 259 min (i.e. during potential waiting before entry in HBO chamber estimated to be the slow fall β - phase) respectively. Nevertheless, using a bi-compartmental model including all samples, reflecting thus the real COHb toxicokinetic, a biphasic COHbt_{1/2} is observed at 236.2 ±34.4min for first phase-α and 302.3±38.9 for second phase-β (21). In our swine model, we compared COHbt_{1/2} assessed on mono- and bi-compartmental model highlighting same discrepancies (Table 1). Different analyses are shown in this table to illustrate the variety of toxicokinetic comparative analyses possible between the different treatments studied. Our results for the α-phase could be underestimated because to mimic 'real' life, our protocol included an initial 10 minute post-exposure period without any treatment (AA) between the end of CO poisoning and the start of treatment, to replicate the victim's removal from the toxic environment and the arrival of emergency services (1).

	NRM		CPAP	NIPPV-V		NIPPV-L	AA	All	P-value
COHb_{t1/2} from (*)	85 [46-116]	82 [40-94]	93 [92-113]	58 [52-79]	251 [130-273]				0.18
COHb_{t1/2}-α	56.2 ±16.2	34.03 ±13.5	26.9 ±5.0	23.6 ±3.9	41.6 ±5.7	39.4 ±6.2			0.39
COHb_{t1/2}-β	70.0 ±22.1	99.6 ±17.3	120.6 ±10.0	174.7 ±63.8	310.5 ±23.5	141.8 ±22.4			0.0004
α-cst	0.022 ±0.0055	0.0032 ±0.0084	0.028 ±0.0047	0.0032 ±0.0042	0.018 ±0.0032	0.026 ±0.0027			0.4524
β-cst	0.026 ±0.0079	0.0081 ±0.0021	0.0058 ±0.00053	0.0053 ±0.0013	0.0023 ±0.00015	0.012 ±0.0034			0.06
A	15.7 ±0.8	16.0 ±3.4	12.3 ±1.8	21.6 ±2.0	6.6 ±0.4	15.1 ±1.2			0.001
B	12.7 ±1.0	15.1 ±1.4	17.5 ±1.7	8.7 ±1.2	21.2 ±1.1	14.5 ±1.0			0.0001
A/B ratio	1.33 ±0.18	0.90 ±0.20	0.73 ±0.15	2.56 ±0.64	0.32 ±0.036	1.21 ±0.19			0.0013
AUC_{0-∞}	2614.8 ±231.2	2716.3 ±368.0	3505.0 ±173.0	2263.6 ±340.4	9946.5 ±1032.6	3844.8 ±579.1			<0.0001
AUC_{0-t}	1849.1 ±169.3	2041.9 ±312.6	2661.2 ±144.5	1292.7 ±42.0	6574.5 ±525.4	2629.9 ±376.0			<0.0001
Time to reach 5%	163.2 ±15.3	168.7 ±22.4	217.1 ±5.9	110.15 ±4.1	647.9 ±64.0	237.7 ±38.7			0.0001

Table 1: Comparison between different toxicokinetic approaches on different O₂ therapy modalities compared to AA. (*)median terminal COHb_{t1/2} (min) using non-compartmental analysis in our previous study which despite a lack of statistical power(p=0.18) favored NIPPV-L favor (58min, n=5) as opposed to NRM (85min, n=8) (1).

Specific characteristics of the absorption phase influencing COHbt_{1/2} have been poorly studied and recently reviewed (50). While COHbt_{1/2} Peterson's contribution on AA (human experimental study, 1970) reports them at 320min and Weaver's study on NBO (human clinical study, 2001) at 74±25min (mean±SD), both present a very wide range from 128 to 409 min (n=39) and from 26 to 148 min (n=93), respectively (26, 51). For Peterson, variations in COHbt_{1/2} did not appear to be related to the duration of exposure or to the concentration inhaled but no assessment was reported (51). Weaver accepted that COHbt_{1/2} may be affected by CO exposure duration and dose but hasn't explored it (3, 27). Bruce, on modelling data estimated COHbt_{1/2}-α and -β to be 20 and 15% longer, respectively, for a long exposure to low CO concentrations compared to a shorter exposure to higher CO concentrations (21).

We know that during the elimination phase, COHbt_{1/2} value is mainly influenced by ventilation (or the ratio of blood volume to ventilation), by FiO₂ level and probably an individual variability (21). Nevertheless, in cases of moderate CO exposure, CO intrinsically does not seem to influence ventilation and accurate FiO₂ is seldom reported in clinical studies. Moreover, the adequate installation of the NRM has a great influence by varying the delivered FiO₂ and, an interval between end of CO exposure and O₂ treatment initiation is sometime delayed and estimated to be 1.2±2.5 hours (4). And, in our opinion, the COHbt_{1/2} ranges reported could be too wide to be exclusively explained by the inter-individual variability suggested in Benignus' study (21, 52). In addition, these wide ranges could be also explained not least by (1) the random sampling times or the lack of data inherent in retrospective studies, (2) the uncertain FiO₂ level administered or (3) the delay before setting NRM but also by (4) a different CO poisoning exposure characteristic, resulting in a different CO poisoning severity and therefore potentially suited to different

treatments. We therefore hypothesize that COHbt_{1/2} discrepancies could also correlate to the absorption phase due to duration and level of CO exposure heterogeneity (21). This involves, as a first step before assessing this COHbt_{1/2} relevance as a severity criterion, applying in future clinical studies the correct toxicokinetic model which is bi-compartmental, requiring multiple samples including early ones (53).

This bi-compartmental COHbt_{1/2} corroborates the hypotheses of an extravascular storage of CO notably linked to myoglobin. We observed a biphasic elimination after 118min SD±10.4 CO exposure considered as an intermediate time exposure, but this profile was also observed after short exposure (<6min) as shown in Wagner, Godin or Shimazu in experimental dog or sheep studies. In contrast, in cases of longer CO exposure, Wagner puts forward that the distribution phase could not be observed since CO distribution may have been almost completely in muscle tissue(53). However, COHb elimination after long CO exposure with moderate CO level (±500ppm) as seen in the studies on human smokers or sheep exposed for 5 or even 10 hours showed also a biphasic elimination even if it was more discreet (16, 21, 54). In our protocol, CO exposure time target didn't last the four to five hours necessary to reach the COHb plateau phase corresponding to the equilibrium between CO absorption and elimination (1). We had elaborated this protocol as assumed to be a moderate extravascular CO burden to avoid interpretation difficulties of an over- or under-expressed α -phase. In addition, this biphasic nature of the COHbt_{1/2} curve doesn't seem altered by various factors that might affect the COHbt_{1/2} such as peak COHb level, FiO₂ level supply, CO exposure concentration or CO duration (16, 21, 54). Our results confirm that the biphasic profile is not influenced either by the different devices used and FiO₂ supply.

Globally, a longer $\text{COHbt}_{1/2}$ would be observed in cases of longer CO exposure allowing higher CO tissue storage regardless of high or low CO exposure concentration and should be considered as more severe poisoning (50). It will be necessary to establish an average threshold of what would be considered a long or short $\text{COHbt}_{1/2}$. The current $\text{COHbt}_{1/2}$ reference is an average of 74 min but is assessed on the mono-compartmental model (26). And, at this stage, no $\text{COHbt}_{1/2}-\alpha$ or $-\beta$, references for human on NBO are known except for estimations using mathematical models with $\text{COHbt}_{1/2}-\alpha$ at $86.9 \pm 10.5\text{min}$ and $\text{COHbt}_{1/2}-\beta$ at $160.7 \pm 11.9\text{min}$ (21). It would seem that $\text{COHbt}_{1/2}$ difference issues arising from CO exposure conditions are more pronounced for the α -phase than for the β -phase (26, 50, 55). This is why it might be interesting to investigate in future clinical studies, not only the two elimination phases separately but also their $\text{COHbt}_{1/2}-\alpha/\text{COHbt}_{1/2}-\beta$; $\alpha\text{-cst}/\beta\text{-cst}$; A/B ratio (as shown in table 1). $\text{COHbt}_{1/2}-\alpha$ seems to be lower as the %COHb peak level increases, regardless of whether this higher %COHb level was due to a longer CO exposure or a higher inspired CO concentration (21). Fig. 5 provides an overview of the relationships between exposure conditions, perceived CO poisoning severity and COHb elimination profile.

CO exposure level:	LOW	HIGH	LOW	HIGH
CO exposure duration:	Short	Short	Long	Long
Severity expected:	±	+	++(+)	+++
%COHb peak level:	↓	↑↑	↓	↑↑
$\text{COHb}_{t_{1/2}}$ expected:	↓	↓	↑	↑
$\text{COHb}_{t_{1/2}-\alpha}$ expected: (Compared to $\text{COHb}_{t_{1/2}-\beta}$)	↓↓↓	↓↓↓	↓↓	↓

Fig. 6: Global overview of the impact of CO exposure criteria (duration x level) on the peak %COHb and COHbt_{1/2} expected. This could approximate severity CO poisoning in relation to potential CO storage. Pending future studies, the two reference thresholds that could be considered are COHb peak at 25% and COHbt_{1/2} at 74min (14, 26, 27).

Assessment of the COHb biphasic elimination could thus allow to open the field of new investigation. We encourage a systematic evaluation consideration of this COHbt_{1/2} from a several COHb venous samples during treatment, including very early samples from ambulance transport. COHbt_{1/2} could be accurate to reflect the CO burden, and so, the real severity of CO intoxication. Nevertheless, whatever the reason for having an extended COHbt_{1/2}, the treatment modalities or duration could be adapted considering higher COHbt_{1/2} as a severity CO poisoning criterion. This COHbt_{1/2} should be challenged as a potential inclusion and comparison criterion in future clinical studies considered as essential to homogenize the cohorts compared under different treatments (3).

3.5.2. On COHb rebound

In our previous study, visually and without statistical analysis on this secondary point, we prematurely concluded that there was no rebound. After statistical analysis was accurately focused on this point, a rebound up to 1h after the treatment ended was highlighted as significant (1). Preceding phase IV, during treatment phase III, on more than a thousand samples, no COHb rebound were observed, even for 0.1% and even for the AA-treated control group immediately after phase I. This new phase IV analysis has shown a systematic COHb rebound (n=19) after COHb returned to 5%. As discussed above and according to biphasic CO elimination, the extravascular tissue could be a significant storage site for CO

(48, 56, 57). In practice, these extra-vascular bindings could then result in a longer delay for complete CO elimination but once again, unfortunately, CO burden is not accessible in clinical investigations (21, 24, 48, 51). A rebound would therefore support the idea of a CO-releasing tissue (48) and suggests the use of ongoing treatment when COHb returns to 5% and for a minimum 6 hours post-exposure.

In our model, not having reached the five hours CO exposure time necessary to reach an equilibrium of a stable COHb state between absorption and elimination (16, 58), a stable COHb state after COHb returns to 5% or even a modest COHb rebound might not be expected. Nevertheless, rebound is systematically observed albeit discreet. Then, CO body burden seems to occur even from moderate CO exposure duration. This rebound could be more significant in case of longer exposure, even if this extravascular CO is only accounted for up to 15% of the global CO body burden. Nevertheless, we are not able to estimate the clinical impact of this CO burden on other metalloprotein binding CO as cytochromes or neuroglobin which could be significant, even in small amounts.

Another point of view could be that the fall of arterial pO_2 after switching to air (end of phase III) could allow more CO in the blood to bind to Hb and causes %COHb to increase. Bruce observed this phenomena in his mathematical model when switching from AA to O_2 therapy (21, 23). Nevertheless, Haldane constant (M-value) is the affinity of Hb for CO comparatively to O_2 with partial pressures of the two gases ($pCO=pO_2$) at 50/50 to assess the proportion of COHb and O_2 Hb formed. This M-value is at 218 meaning that Hb has 218-fold more affinity for CO than O_2 (47). More accurately, the overall affinity for a gas is the equilibrium constant of a gas (K_{Eq}) corresponding to the ratio between association and dissociation constant for a gas being respectively K_a and K_d . In vitro, the combination of CO with hemoglobin

is not especially rapid, but it is the dissociation which is very low. When, for example, blood is shaken in a 100 % CO atmosphere, only 26 % of the Hb is converted to COHb in 5 min; almost complete saturation takes place only in about 20 min (17). O₂ combines with Hb about 10 times as fast as does CO but the great affinity of CO for Hb is due to the fact that COHb dissociates ($k_d \approx 0.008 \text{ s}^{-1}$) 2.500-fold more slowly than HbO₂ ($k_d \approx 20 \text{ s}^{-1}$) (59, 60). For O₂, K_a is at $66 \mu\text{M}^{-1} \cdot \text{s}^{-1}$ and K_d at 20 for a $K_{eq}(K_a/K_d)$ at $3.2 \mu\text{M}^{-1}$. For CO K_a is at $6 \mu\text{M}^{-1} \cdot \text{s}^{-1}$ and K_d very small at 0.008 for a $K_{eq}(K_a/K_d)$ at $750 \mu\text{M}^{-1}$. And, M-value is the ratio $K_{eq} \text{ CO} / K_{eq} \text{ O}_2$ being ± 234 ($750/3.2$) close to 218. This means that what explains the high affinity of CO for Hb compared with O₂ is a slow COHb dissociation and not a fast linking of CO to Hb. Thus, ventilation which didn't change between the switching from O₂ therapy to AA could have enough time to eliminate the free CO already in the blood and the free CO slowly released from COHb on AA when treatment stopped. That's why this rebound would be an argument for a CO delayed release from an extravascular CO storage. Furthermore, the fall in tissue pO₂ will also allow more CO binding to Mb, potentially reducing the tissue to blood flux of CO when O₂ treatment stopped.

Moreover, as briefly suggested by Wazawa on AA-treated rabbits, we could hypothesize that this slight rebound may come from the endogenous CO production resulting from the hemoglobin catabolism which results in a physiological basal COHb rate from 0.4 to 0.8% (61). Nevertheless, this production is always present and contributing to COHb level in the order of at $6.1 \mu\text{l/h/kg}$ or 0.007 ml/min in humans (24). The swine used in our study had an average human weight of $\pm 80 \text{ kg}$, so they should therefore produce only $\pm 0.5 \text{ ml}$ CO during our hour of observation. So, it does not seem consistent to consider that the observed CO rebound corresponds only to the production of endogenous CO. Rebound from tissular storage is also observed in cases of lithium intoxication after hemodialysis

removes circulating lithium. In this context, serum concentrations often rebound, so repeated or prolonged treatment may be required (62). In our opinion, whatever the reason for this rise or stagnation of COHb, it might be wise to continue NBO beyond the time necessary to reach a %COHb level of 5% as long as COHbt_{1/2} does not start a normal decrease under AA.

This is in accordance with a theoretical complete clearance ($\pm 97\%$) of COHb expected after five-fold COHbt_{1/2} (74 ± 25 min [26 to 148]). Total O₂ treatment time could then be estimated at 370 min (± 6 h) but 12 hours if we consider the extreme range at 148min (63). Nevertheless, in clinical practice, the decision to stop O₂ therapy is taken when COHb is estimated to be below 5% and the patient is asymptomatic (35). The duration of 100% normobaric O₂ therapy may also vary largely depending on empiric decisions or residual subjective symptoms. Significantly, in half of the significant studies comparing NBO to HBO selected in meta-analyses assessing HBO efficiency, NBO was only administered for less than or equal to 6 hours (8, 41). This could be a significant bias in these comparative studies and highlights heterogeneity of treatment strategies. Moreover, among the few studies selected by meta-analyses which provided NBO >6h, they didn't find any benefit between NBO and HBO (41, 63, 64). Ideally, for severe CO poisoning, a homogenous NBO duration, probably 12 hours, should be achieved, which should be compared with HBO. To illustrate this issue, recent prospective clinical studies examined the influence of CPAP or NRM on COHbt_{1/2} for CO poisoned patients with a NBO treatment duration targeting only a COHb return close to 5%. It was reported that patients were discharged from ED after 127 minutes in the CPAP group and 201 minutes in the NRM group (65).

Based on (1) our COHbt_{1/2} bi-compartmental toxicokinetic evidence, (2) a rebound objectively observed for the first time, (3) in accordance with mathematical model suggesting extra-vascular CO store and, (4) waiting for future clinical impact

assessment of CO burden, we confirm the suggested method of providing a minimum of 6 hours NBO for all cases of CO poisoning. If the decision to use NBO and not HBO is made, conditions for stopping NBO after 6 hours could be: particular attention to delivered FiO_2 close to 1.0 (optimal NRM placement), CO exposure duration estimated less than five hours, $\text{COHbt}_{1/2-\alpha}$ very low compared to $\text{COHbt}_{1/2-\beta}$, $\text{COHbt}_{1/2-\beta}$ estimated less or close to 74 minutes and asymptomatic patient. Otherwise, there are arguments for examining higher CO body burden level and to then continue treatment for up to 12 hours.

3.6. CONCLUSION

On an experimental swine model subjected to moderate CO poisoning, first we confirm that the COHb half-life is biphasic and thus follows a bi-compartmental model. Secondly, we observe for the first time a slight COHb rebound when COHb returns to 5% and oxygen therapy is stopped. On the basis of these two toxicokinetic profiles in favor of extra vascular CO storage, we suggest that future studies might consider the $\text{COHbt}_{1/2}$, from multiple samples using a bi-compartmental model, as a potential CO poisoning severity criterion. Furthermore, COHb rebound extent needs to be assessed on other CO exposure protocols to be confirm and to state on a CO storage correlation. While waiting for further studies to emerge, we suggest that a treatment lasting less than 6 hours does not seem to be adapted for any case of moderate CO poisoning.

Nevertheless, the relationship between COHb elimination profile and/or $\text{COHbt}_{1/2}$ value as a reflection of CO storage and/or a potential CO poisoning severity criterion remains to be confirmed. The first goal is to homogenize cohorts of the same severity in further large multicenter randomized clinical trials regarding strategies for CO poisoning.

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CHAPTER 4

AFFINITY M CONSTANT OF ADULT AND FETAL HEMOGLOBIN FOR CARBON MONOXIDE IN HUMANS: A SYSTEMATIC REVIEW AND META-ANALYSIS

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4.1. ABSTRACT

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Relative Affinity M Constant of Adult and Fetal Hemoglobin for Carbon Monoxide in Humans: a Systematic Review and Meta-Analysis

The Haldane M constant represents the ratio of oxygen/carbon monoxide affinity to hemoglobin. Findings on M values appear to be quite variable among studies. A systematic review and a meta-analysis were conducted to set a reference of human M values according to experimental conditions. The searched included all the relevant articles published from 1985 to January 2016 and extracted from the major electronic libraries database (PubMed/Embase/JSTOR). For each article, the following items were collected: age, temperature, pH, atmospheric pressure, 2,3-diphosphoglycerate (2,3-DPG) or methemoglobin levels, and hemoglobin type. Statistical analyses were performed using JMP®Pro12 software.

Among the 1.683 articles analyzed, 15 were selected, which included 111 individual data, 21 mean values related to human adult hemoglobin (HbA) and 57 mean values for fetal hemoglobin (HbF) at known temperature and pH. The median M value at physiological conditions was 218 [191–257] (n = 19) for HbA and 175 [158–192] for HbF. There was a negative correlation between temperature and M value ($p < 0.001$), but no correlation between M and pH for HbA ($p = 0.33$), conversely to HbF ($p = 0.044$). Data were insufficient to perform a meta-analysis on the dependence between M and 2,3-DPG concentration, atmospheric pressure, pregnancy, methemoglobin, and sickle Hb.

Our analysis confirmed that the M value in humans at physiological conditions is 218 for HbA and 175 for HbF. Temperature influences M, but further studies are required to clarify pH influence.

P16-051

Relative affinity (M) constant of adult and fetal hemoglobin for carbon monoxide in humans: Systematic review and meta-analysis



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Background: The Haldane (M) constant represents the ratio oxygen/carbon monoxide bound to hemoglobin and is a key of the mathematical, physiological and pathological models exploring CO poisoning and endogenous CO production. However, there is a large discrepancy in (M) values among studies.

Aims and methods: To determine the different values of (M) according to experimental conditions, we performed a systematic literature review (PubMed, Embase and JSTORE) from 1895 to 2016. For each article evaluating human (M), we collected: temperature (T , °C), pH, atmospheric pressure, presence of 2,3-DPG or methemoglobin, type of hemoglobin. We grouped individual data or mean values to assess (M) according to experimental conditions. Linear regression analyses were performed using JMP/Pro12.

Results: No specific keyword entry was found for (M) in databases. Among the 217 articles screened, 15 addressed (M) constant including 102 individual and 21 means of (M) values concerning adults, and 57 for fetus at known T , °C and pH. Most data for (M) dated for more than 25 years. Median (M) value at physiologic conditions is found at 209 for adults and 178 for fetus. Our meta-analysis did not demonstrate any influence of pH due to a lack of data. The influence of T (°C) on (M) is confirmed, (M) increasing by

4.5 unit for each degree decrease in T (°C). Other factors did not seem to influence (M).

Conclusion: Human (M) value at T , 37 °C and physiological pH is 209 for adults and 178 for fetus. Insufficient literature data do not allow to discuss the influence of pH.

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Relative Affinity M Constant of Adult and Fetal Hemoglobin for Carbon Monoxide in Humans: a Systematic Review and Meta-Analysis

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Abstract—The Haldane M constant represents the ratio of oxygen/carbon monoxide affinity to hemoglobin. Findings on M values appear to be quite variable among studies. A systematic review and a meta-analysis were conducted to set a reference of human M values according to experimental conditions. The searched included all the relevant articles published from 1985 to January 2016 and extracted from the major electronic libraries database (PubMed/Embase/JSTOR). For each article, the following items were collected: age, temperature, pH, atmospheric pressure, 2,3-diphosphoglycerate (2,3-DPG) or methemoglobin levels, and hemoglobin type. Statistical analyses were performed using JMP®Pro12 software. Among the 1.683 articles analyzed, 15 were selected, which included 111 individual data, 21 mean values related to human adult hemoglobin (HbA) and 57 mean values for fetal hemoglobin (HbF) at known temperature and pH. The median M value at physiological conditions was 218 [191–257] ($n = 19$) for HbA and 175 [158–192] for HbF. There was a negative correlation between temperature and M value ($p < 0.001$), but no correlation between M and pH for HbA ($p = 0.33$), conversely to HbF ($p = 0.044$). Data were insufficient to perform a meta-analysis on the dependence between M and 2,3-DPG concentration, atmospheric pressure, pregnancy, methemoglobin, and sickle Hb. Our analysis confirmed that the M value in humans at physiological conditions is 218 for HbA and 175 for HbF. Temperature influences M, but further studies are required to clarify pH influence.

Keywords: carbon monoxide, affinity constant, hemoglobin – pH, temperature

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Carbon monoxide (CO) remains a significant concern and a subject of investigation, firstly due to the prevalence of CO poisoning and secondly because of its endogenous physiological role [1–4]. CO has a high affinity for all metalloproteins and particularly for hemoglobin (Hb), with which it forms the carboxy-hemoglobin complex (COHb). The Haldane constant, also called the M constant, represents the equilibrium constant for the reaction of CO + O₂Hb, resulting in COHb + O₂, i.e. the relative affinity of Hb for CO versus O₂ [5, 6]. The equation of this reaction in blood is $[HbCO]/[HbO_2] = M \cdot pCO/pO_2$, where $[COHb]$ = carboxyhemoglobin rate; $[HbO_2]$ = oxyhemoglobin rate; pCO = partial pressure in CO dissolved; and pO_2 = partial pressure in oxygen dissolved. Indeed, in 1912, Douglas and Haldane exposed human blood

in vitro to a mixture of O₂ and CO. Using tonometric assessment, they found that to convert half of the Hb to HbO₂ and half to COHb, the ratio of the O₂ to CO pressure in the mixture was 246 : 1 [5].

There after, other estimations of M ranging from 140 to more than 400 were published [5, 7]. Given that COHb rates vary directly in relation to M, using a widely variable M value may introduce a margin for error in determining COHb. Some experimental or physiological factors, such as temperature, pH conditions and type of Hb (adult or fetal) may influence the M value and explain at least in part these discrepancies.

In recent decades, researchers considered M values ranging from 210 to 245, but in most instances without justifying their choice [8]. Rodkey's and Engel's

4.2. INTRODUCTION

Carbon monoxide (CO) remains a significant concern and a subject of investigation, firstly due to the prevalence of CO poisoning and secondly because of its endogenous physiological role [1–4]. CO has a high affinity for all metalloproteins and particularly for hemoglobin (Hb), with which it forms the carboxyhemoglobin complex (COHb). The Haldane constant, also called the M constant, represents the equilibrium constant for the reaction of $\text{CO} + \text{O}_2\text{Hb}$, resulting in $\text{COHb} + \text{O}_2$, i.e., the relative affinity of Hb for CO versus O_2 [5, 6]. The equation of this reaction in blood is $[\text{COHb}]/[\text{O}_2\text{Hb}] = M \text{ pCO}/\text{pO}_2$, where $[\text{COHb}]$ = carboxyhemoglobin rate; $[\text{O}_2\text{Hb}]$ = oxyhemoglobin rate; pCO = partial pressure in CO dissolved; and pO_2 = partial pressure in oxygen dissolved. Indeed, in 1912, Douglas and Haldane exposed human blood in vitro to a mixture of O_2 and CO. Using tonometric assessment, they found that to convert half of the Hb to HbO_2 and half to COHb, the ratio of the O_2 to CO pressure in the mixture was 246: 1 [5].

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In recent decades, researchers considered M values ranging from 210 to 245, but in most instances without justifying their choice [8]. Rodkey's and Engel's experimental studies found, at 37°C and any pH, a M_{37} value of 218 ± 8 for HbA and 178 ± 10 for HbF; these appear to be the most extensive and detailed studies [9, 10]. Forty-five years later, our main objective was to determine whether more recent literature offered any new data related to this estimation in order to confirm

or improve the accuracy of the M value and, what is more noteworthy, under physiological conditions. We therefore performed a comprehensive and systematic review with a meta-analysis of the M values and we analyzed the factors influencing these results in humans. This updated M reference could be confidently used in all further studies, such as comparisons to findings on animals and/or to find the species with M values closest to humans.

4.3. METHODS

4.3.1. Search methods for the identification of studies

We performed a systematic search of electronic libraries (PubMed, Embase and JSTOR) and a manual search of references from 1895 to January 2016. All articles that provided an individual or mean M value, sometimes expressed by the letter K, obtained through the tonometric method or similar, were included. We excluded studies/results if M measurements were performed using a method very different from tonometry or using animal hemoglobin. The following keywords and search strategy were used:

(1) In the Embase library: (("carbon monoxide"/exp or "carbon monoxide") or "carbon monoxide poisoning" or "carbon oxide") and ("carboxyhemoglobin" or "carboxyhemoglobinemia" or "hemoglobin") and ("constants and coefficients" or "Haldane effect" or Haldane or "affinity" or "dissociation" or "linkage" or "half life time" or "toxicokinetics" or "kinetics" or "pharmacokinetics" or "releasing" or "elimination rate constant" or "elimination half-life" or "clearance lung" or "metabolic clearance rate" or "clearance" or "expired air" or "ligand binding") and ([English]/lim or [French]/lim) and [humans]/lim.

(2) In the PubMed library: ("carbon monoxide/[Mesh]" or "carbon monoxide poisoning/[Mesh]" or "carbon oxide") and ("carboxyhemoglobin/[Mesh]" or "carboxyhemoglobin" or "carboxyhemoglobinemia" or "hemoglobin") and ("constant" or "Haldane" or "haldane effect" or "affinity" or "dissociation" or "linkage" or "half-life/[Mesh]" or "half-life" or "toxicokinetics/[Mesh]" or "kinetics/[Mesh]" or "pharmacokinetics/[Mesh]" or "releasing" or "elimination" or "clearance rate metabolic/[Mesh]" or "ligand"). Filters: Human/English/French.

(3) In the JSTOR library: (from 1895 to 1960): ((“carbon monoxide”) or (“carbon oxide”)) and ((“hemoglobin”) or (“carboxyhemoglobin”)) and ((“affinity”) or (“Haldane”)).

We checked the reference lists of the studies retrieved and those of relevant narrative and systematic reviews to find additional potentially relevant studies.

4.3.2. Data extraction

Data extracted were: individual M values or, if not mentioned, data relating to mean, standard deviation (SD), median and distribution values; adult or fetal normal human Hb; pH and temperature conditions. We completed extraction by other experimental conditions: M assessment method, atmospheric pressure, presence of 2,3-diphosphoglycerate or methemoglobin, analyses performed on hemolyzed or whole blood.

Due to the time elapsed since the publication of some of the studies, we could not contact the original investigators to request missing data. We did not use statistical methods to model missing data. When authors mentioned that the assay was performed *in vivo*, we assumed that it was performed on whole blood under physiological conditions.

4.3.3. Data synthesis

We pooled all individual data extracted with defined conditions in terms of pH and temperature in Celsius (M_T). Individual M values are presented as median and extremes [minimum-maximum] due to non-normal distribution of data. When individual data were not mentioned, synthesis data (mean and SD) extracted from assays were used. For this purpose, we assumed that data followed a normal distribution.

To compare M data collected at different temperatures, we classified M data found in the following temperature ranges: $>0^{\circ}\text{C}$ and $\leq 5^{\circ}\text{C}$; $>5^{\circ}\text{C}$ and $\leq 10^{\circ}\text{C}$; $>10^{\circ}\text{C}$ and $\leq 15^{\circ}\text{C}$; $>15^{\circ}\text{C}$ and $\leq 20^{\circ}\text{C}$; $>20^{\circ}\text{C}$ and $\leq 25^{\circ}\text{C}$; $>25^{\circ}\text{C}$ and $\leq 30^{\circ}\text{C}$; $>30^{\circ}\text{C}$ and $\leq 35^{\circ}\text{C}$; $>35^{\circ}\text{C}$ and $\leq 40^{\circ}\text{C}$; $>40^{\circ}\text{C}$. We defined physiological conditions for adult Hb as a temperature $>35^{\circ}\text{C}$ and $\leq 40^{\circ}\text{C}$, and pH for whole blood from ≥ 7.35 to ≤ 7.45 and for hemolyzed or purified Hb from ≥ 7.10 to ≤ 7.20 [11]. For HbF, the range considered for whole blood physiological pH was from ≥ 7.15 to ≤ 7.45 and ≥ 7.0 to ≤ 7.15 for hemolyzed Hb [12–14]. All other conditions were considered as non-physiological.

4.3.4. Statistical analysis

All analyses were performed on JMP® pro12, statistical discovery™ software, SAS®, USA.

For individual data, due to the modest size of data for groups, non-parametric tests were considered. For comparison of M values between groups, a Mann–Whitney test (2 groups) or a Kruskal–Wallis test (3 or more groups) was performed. Simple linear regression was performed to analyze the correlation between M and temperature or pH.

4.4. RESULTS

4.4.1. Characteristics of included studies

We identified 1,683 articles in electronic libraries and 143 via references, with the first publication dating from 1895. After scanning the abstracts and titles, 217 articles were selected for full review, including 22 studies which reported M human values. Seven were ultimately excluded due to missing data (n, SD, temperature and/or pH) [5, 15–20]. We also excluded two assays in one study [21] because they were carried out based on a methodology very different to tonometry (“method Band C”). Finally, 15 assays were included in our meta-analysis (Fig. 1): 11 articles with individual M values [9, 10, 22–30] and 4 articles with M values mentioned as means and SD [21, 31–33]. These articles dated from 1936 to 1982.

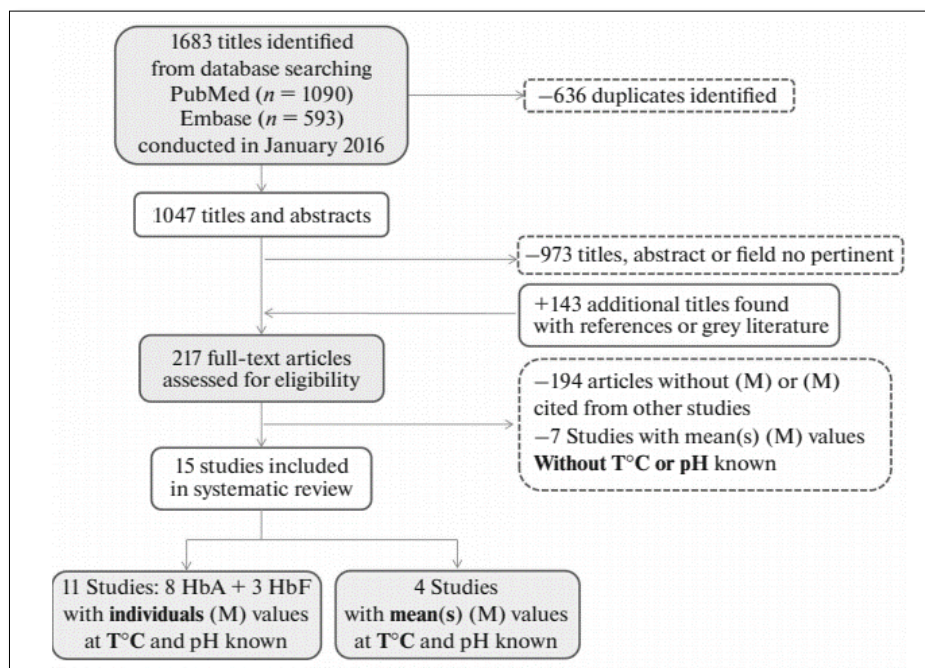


Fig.1: Flow diagram of studies selection in the systematic review.

Among the selected studies, eight studies contained individual M values on HbA (n = 111) with known temperature (19 to 37°C) and pH (6.78 to 9.30) [10, 22–24, 26–28, 30]. Among these data, some were performed under physiological conditions, on hemolyzed blood in the case of Rodkey's study (n = 3) and on whole blood in the case of others studies (n = 16) [9, 22–24, 28].

Three other studies focused on HbF with known pH condition and at 37°C [9, 30] or 22–25°C [26]. They included 57 individual M values on HbF, whereas 8 were at physiological pH, 4 on hemolyzed blood and 4 on whole blood [9, 30].

Four other articles reported mean M values (n = 21) only (unreported individual M values) from assays performed at known temperature (25°C to 37°C) and pH (7.3 to 9.1) [31, 32] or apparently physiological (in vivo) [21, 33].

4.4.2. M value for human hemoglobin (HbA)

The M median value under physiological conditions was 218 [191–257] (19 individual values from four articles) [9, 22, 23, 28] (2). In the physiological temperature range defined as >35°C and ≤40°C, all studies were performed at 37°C.

4.4.3. Influence of temperature on HbA

For other individual M values on HbA performed at other temperature ranges: M_{15-20} median = 321.50 [299–376] (n = 10 at 19°C) [27] and, M_{20-25} median = 252.50 [229–286] (n = 4 at 22°C; n = 18 from 22°C to 25°C; n = 2 at 25°) [9, 26, 29] (Fig. 2). No available data was found for HbA at temperatures ≤15°C; >25°C and ≤35°C; ≥40°C.

Among these 111 individual M values assessed at different conditions for temperature regardless of pH, there was a significant correlation between the M value for HbA and temperature ($p < 0.0001$). When temperature increased by one degree, M decreased by 4.5 units on average (Fig. 3a).

Moreover, for M mean data at any pH, we found the following different temperature ranges: M_{10-15} mean = 355 (SD 13; n = 2 at 15°C) [31]; M_{15-20} mean = 230 (n = 1 at 20°C) [32]; M_{20-25} mean = 274 (SD 9; n = 10 at 25°C) [31]; M_{25-30} mean = 295 (SD 3.5; n = 2 at 26°C) [33]; M_{35-40} mean = 228 (SD 3.5; n = 6 at 37°C) [21, 31, 33]. No available data were found for M means on HbA at the temperatures $\leq 10^\circ\text{C}$, $>30^\circ\text{C}$ and $\leq 35^\circ\text{C}$. Once again, based on mean data, we found a correlation between M and temperature (Fig. 3b).

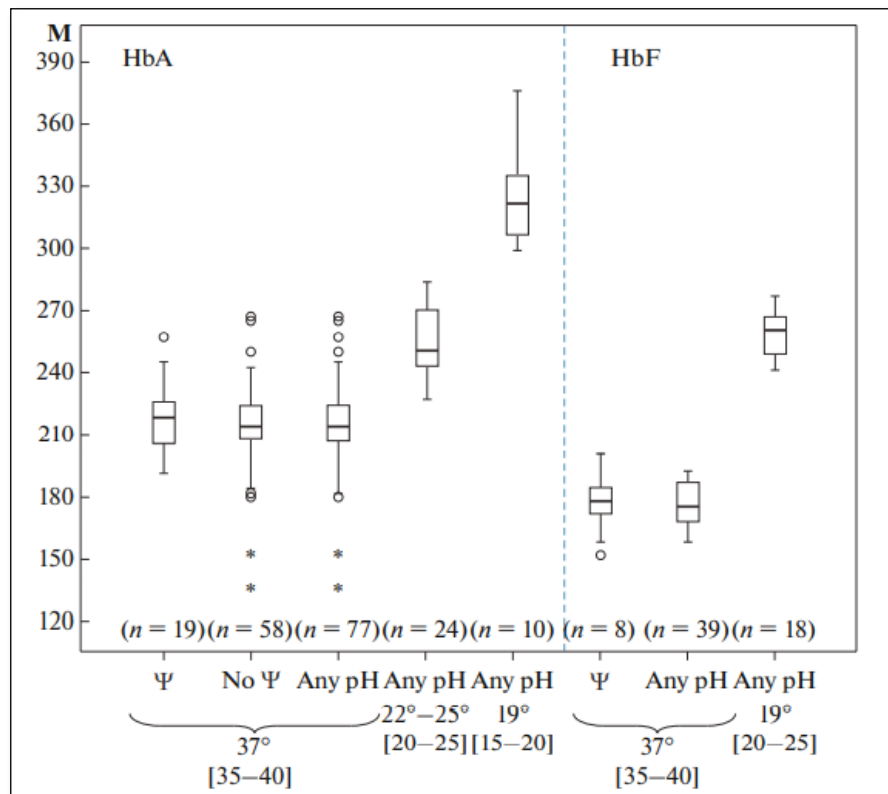


Fig.2: Individual M values performed at different temperature ranges or pH found for HbA and HbF (Ψ : physiologic pH and T°).

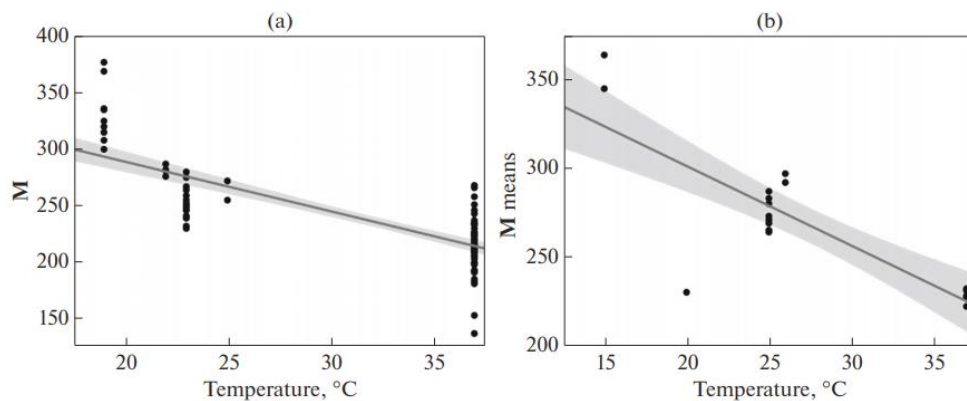


Fig. 3. Simple linear regression between M and temperature (at any pH). (a) From individual data ($n = 111$) ($r^2 = 0.62$). (b) From M mean data ($n = 21$) ($r^2 = 0.69$).

4.4.4. Influence of pH on HbA

The median for the M_{37} value under non-physiological pH conditions was 215 [136–267] (58 individual values excluding M values under physiological pH conditions) and, for the 77 individual M_{37} values including all pH conditions (6.78 to 8.85), the median M value was also 215 [136–267] (Fig. 2). There was no significant correlation between the M_{37} value for HbA and pH ($p = 0.33$).

4.4.5. M values in human fetal hemoglobin (HbF)

Two authors studied M_{37} on HbF, including the only 8 individual values carried out at physiological pH.

The median was at 175 [158–192] [9, 30] (Fig. 2). For other M median values from individual data performed at different temperature range at any pH: M_{20-25} median = 260.50 [241–277] [26]; M_{35-40} median = 178 [152–201] [9, 30] (Fig. 2). No available data were found for HbF at temperatures $\leq 20^\circ\text{C}$; $> 25^\circ\text{C}$ and $\leq 35^\circ\text{C}$; $\geq 40^\circ\text{C}$) or for M mean data.

4.4.6. Influence of pH on HbF

From all 39 individual data assessed at any pH, we calculated a very similar median value for M: 178 [152–201] [9, 30] (Fig. 2).

Simple linear regression analysis showed that M increased if pH increased ($p = 0.044$). For HbF, when pH increased by 1 unit, M increased by a mean of 5.5 units (Fig. 4). Nevertheless, this correlation was not statistically significant if we selected only the 8 M values at physiological pH ($p = 0.248$).

Moreover, for HbF, a significant difference was found between the M value and temperature (Fig. 2). We observed a statistically significant lower M_{37} compared to M_{22-26} ($p < 0.05$).

4.4.7. Comparing HbA and HbF

M_{37} for HbF at physiological pH (and at any pH), was significantly lower than M_{37} for HbA ($p < 0.0001$) (Fig. 2).

4.5. DISCUSSION

4.5.1. Strengths and limitations

Literature on M was limited and scattered. Only three eligible studies were published before Engel and Rodkey's studies in 1969. Subsequently, only two studies published new data [9, 10, 28]. Forty-five years later, this first systematic review and meta-analysis updated M_{37} values at 215 for HbA at any pH, 218 at physiological conditions and 175 for HbF, confirming Rodkey's reliability results of 218 ± 8 for M_{37} and any pH. Rodkey considered a pH between 6.8 to 8.8 as a physiologic extreme and concluded that pH had no effect on M values. If we consider our physiologic pH range from 7.35 to 7.45, Rodkey investigated only three values on hemolyzed blood and none on whole blood. We completed this with 16 new M values to confirm that M is independent of pH in the case of HbA. Conversely to HbA, M for HbF seems to depend on pH conditions, however this result must be viewed with caution due to the great dispersion of the data. Moreover, we confirmed that the M value for HbA and HbF depends on temperature conditions.

Limitation in our meta-analysis relates to the method used to measure M. No simple or direct method actually exists. Although tonometry is the more conventional way to measure M in vitro based on the pCO/pO_2 ratio [5, 22–25, 28, 34–36] or in vivo assuming an alveolar pCO value equal to pCO in blood [10, 16, 21, 22], authors used different protocols and were sometimes imprecise about the process they applied.

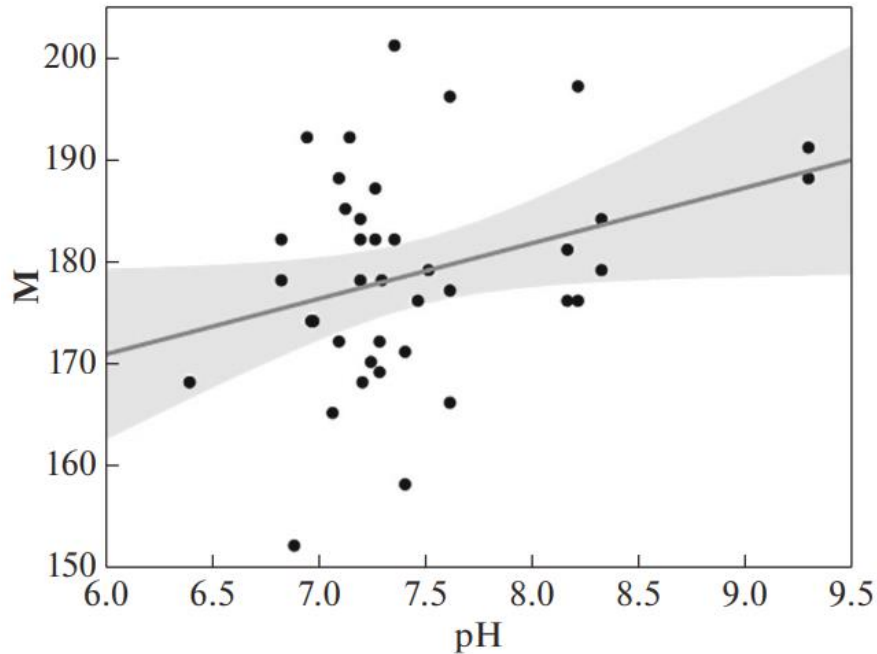


Fig. 4. Simple linear regression for fetal M value by pH (n = 39) ($r^2 = 0.105$).

4.5.2. Effect of pH and PaCO₂ on M HbA values

The possible influence of pH on M values was questioned in the case of studies performed under non-physiological experimental conditions. It should also be considered in the event of severe CO intoxication complicated by lactic acidosis and for transplacental CO exchange, as the pH in maternal and fetal blood varies slightly [37]. Moreover, in clinical studies, it is interesting to consider the PaCO₂ level relative to historical treatment with Carbogen, which was still considered up to 15 years ago. Carbogen is a gas mixture containing 95% O₂ and 5% CO₂. It is suggested to be used in cases of CO poisoning to increase ventilation by preserving normocapnia [38]. Another clinical study assessed physiological blood pH variation by non-invasive detection of endogenous CO content in breath assuming a high sensitivity of M to blood pH value [39].

Historically, no effect on M in HbA was detected by modifying the pH, over extreme values (6.8 to 8.8), even by modifying the PCO₂ level, by adding lactic acid or HCL buffer solution and/or Na₂CO₃ [5, 10, 40, 41]. Similarly, no pH-related effect has been reported on M in HbF [9].

Conversely, other authors suggested that the M value was affected by pH variation [22, 24, 27, 39]. However, these historical studies did not investigate intermediary pH or, especially, physiological pH [23, 24]. Moreover, studies were heterogeneous, modifying the pH by altering the CO₂ tension, while others used a buffer [9, 23, 24, 34, 42]. For Allen, M decreased if pH increased or decreased drawing a bell curve with a top for physiological range. This trend was constant in humans, dogs, and rats, and highlights the importance of exploring intermediate pH. Nevertheless, the author did not detail his statistical methodology [23].

In light of these discordant or inconsistent results, due to the lack of data, our meta-analysis was unable to confirm whether pH influences M for HbA. Nevertheless, given the limitations of accurate data, this result should be viewed with caution. It is likely that a nonlinear relationship between M and the pH of the blood exists. It could be detected using mathematical methods.

4.5.3. Effect of temperature on HbA

Conversely to pH, our analysis confirmed that temperature influences M values. This result correlates with previous studies [5, 9, 10, 31, 40, 41, 43–45]. When temperature increased by one degree, M decreased by 4.5 units on average, which is consistent with Rodkey's results [9, 10].

There are several possible explanations for the dependence of M on temperature. Rodkey suggested that temperature changes the conformation of the Hb molecular structure, modifying the nature of the link for CO or other ligands [10, 33]. Another explanation is to consider the proportionality of the variability level of gas solubility

at various temperatures. The dissolved gas in a liquid follows Henry's law using the Henry's law constant K and may be different for CO and O₂.

4.5.4. M for HbF

The nature and proportion of the different HbF and 2,3-DPG levels are variable through embryonal and fetal lives. HbA increases progressively to around 10% at 32 weeks. After birth, HbF still represents 70 to 85%; at 6 months after birth, HbF and HbA are equilibrated [46]. Hofmann, in 1998, was the only one to study M_{37} for embryonal HbF at pH 7.4, but expressed in genetically engineered yeast with a very low M value of 85 [47].

Clinical studies have shown that a fetus is probably very sensitive to neurological injury after CO poisoning [48]. A number of hyperbaric societies currently recommend hyperbaric therapy for pregnant women exposed to CO, even at low COHb [49]. Moreover, in an intoxication state, $[\text{COHb}]_f$ is 10 to 15% greater than $[\text{COHb}]_m$. Furthermore, M_f/M_m was shown to be 0.83 in humans [50], which is very similar to the average of literature values: 175/218 is 0.80.

The M_{37} median value found for HbF was significantly lower than M_{37} for HbA. The results, performed under physiological conditions, are based on only one study at 37°C, which included only eight fetal individual M values. These findings are confirmed on results obtained from non-physiological pH (Fig. 2) [9, 30]. However, two other studies, performed under non-physiological conditions or at pathological Hb, did not show a significant difference in M between HbA and HbF: M_{22-25} for maternal HbA at 245 and for HbF at 253 [26], and M_{37} on sickle Hb for HbA at 222 and for HbF at 225 [33].

Lower M_f values, therefore, do not seem to explain the higher $[\text{COHb}]_f$ compared to $[\text{COHb}]_m$ in physiological or intoxication states. Other specificities should be

considered, such as specific fetal metabolism and body mass, rate of exchange of CO across placenta and thus placental diffusing capacity for CO and endogenous production of CO [51].

Engel found M_f for pH to vary from 6.4 to 9.3 pooled in three pH ranges and concluded that the influence of pH on M_f was not significant. However, combining all available data, we found a significant linear correlation between pH and M_f , with M_f at 37°C increasing when pH increased. It is noteworthy that this correlation is opposite to the trend observed for HbA. This discordance, as well as the small number of available data, emphasizes the need to view results related to pH and M_f with caution. Although pH is known to influence the O₂ affinity of HbF or HbA, if M values are invariant, this would indicate that the Bohr effect is proportionately the same for CO and O₂ [9].

It is worth noting that Engel et al. studied M values at the termination of pregnancy and concluded that the pregnancy state does not alter the M value of maternal blood [9].

4.5.5. Methemoglobin, 2,3-DPG and atmospheric pressure influences

Available values are too limited to perform a meta-analysis of the possible influence of these parameters on M values. Nevertheless, previous studies provided some interesting information. The M value, on HbA or HbF, seemed to be independent of hemolyzed or fresh whole blood, in vitro or in vivo experiments, the presence of 2,3-DPG or otherwise. Likewise, the M value should be unaffected by methemoglobin [23].

Given that the level of 2,3-DPG in red blood cells varies between species and different fetal age, as well as between whole blood cells and hemolyzed blood, it is important to know if its level could influence the M value. Benesh et al. found that

M was unaffected by the presence of 2,3-DPG in HbA or HbF [26]. In addition, blood 2,3-DPG concentration had no effect on the CO dissociation constant of COHb and was unchanged by CO exposure [52, 53].

Studying the effect of atmospheric pressure on the M value is interesting when considering hyperbaric oxygen therapy, which is one of the most common treatments for severe CO poisoning [54]. Three studies assessed this question and all suggested that M was not influenced by atmospheric pressure ranging from 2.7 to 21.4 atm [9, 22, 55].

4.5.6. Sickle hemoglobin (HbS)

Rodkey's M_{37} value is 225 (95% HbS/2% HbF) or 232 (38% HbS/62% HbA), similar to normal HbA at 228 [33]. Engel et al. found the M value to be 191 in four children with major thalassemia (15 to 36% HbF), compared to 215 in normal adults (HbA) [9].

Few authors suggested that M could be not a constant but can depend on the level of COHb or O₂Hb saturation with, for example, a M value at 228 if COHb was between 30 and 80% and a M value at 185 if COHb was less than 20%; at 169 for COHb below than 2% compared to a global mean at 236 [24, 28]. However, this effect was not observed by Rodkey and in vitro data suggest that at least over the range of 5 to 90% COHb saturation, the value of (M) remains constant (10). Otherwise, M varied from 185 at 10% O₂Hb saturation to 230 at 70% [28, 56].

4.6. CONCLUSION

Our meta-analysis and systematic review provide up-to-date data for understanding and carrying out further studies on CO metabolism. A M_{37} value of 218 ± 8 traditionally considered in humans at any pH is also confirmed under physiological M conditions for adult hemoglobin (HbA), higher than for fetal hemoglobin (HbF) at 175. There is a negative correlation between temperature and M: when temperature increases by one degree, M decreases by 4.5 units. Conversely to results for HbA, M seems correlated to pH for HbF with more confidence. However, these results must be viewed with caution due to the low number of available data on this topic. Similarly, other factors like 2,3-DPG concentration in red cells, atmospheric pressure, pregnant state, methemoglobin, and sickle Hb do not seem to influence M, however there is insufficient data to perform a meta-analysis. Further studies are required to clarify the pH influence on the M value.

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CHAPTER 5

REPORT ON THE RELATIVE AFFINITY CONSTANT (M) OF HEMOGLOBIN FOR CARBON MONOXIDE IN THE ANIMAL WORLD: A COMPARATIVE REVIEW WITH A META-ANALYSIS BASED ON A SYSTEMATIC REVIEW

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5.1. ABSTRACT

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Reporting of the relative affinity 'M' constant of hemoglobin for carbon monoxide in animal world: an historic, systematic and meta-analysis review.

The Haldane M-value is the ratio of half-saturation pressures for O₂ and CO (PO₂/PCO) and is a key value of physiological and pathological models exploring CO metabolism or exposure to CO. Nevertheless, the M-values obtained from different studies and different species appear to be inconsistent. A better estimation of M-values and a consideration of interspecies variation will ensure the accuracy of CO study results and allow reliable models to be constructed.

We performed a systematic review and meta-analysis in order to determine and compare M-values according to species and experimental conditions. We used electronic libraries (PubMed/Embase/JSTORE) from 1895 to January 2016. For each article, we collected the following parameters: species, age, temperature, pH, atmospheric pressure, concentration of 2,3-diphosphoglycerate (2,3-DPG) or methemoglobin, and type of hemoglobin.

The systematic review found 25 articles addressing the M constant, including 135 with individual data and 51 with mean values concerning mammals, laboratory animals, fish, fetal hemoglobin and invertebrates. M-values at a physiological temperature and pH were assessed only for adult (137 [119–153]) and fetal (135 [112–168]) sheep, dogs (215 [193–218]), mice (194 [118–278]) and rats (191 [177–200]). A correlation between M-values and temperature was confirmed for sheep and horses. A correlation between M-values and pH could not be inferred because there was a lack of data. M-values varied according to species. Only the influence of temperature was confirmed. A formal measurement of the M constant in the given experimental conditions should be performed for each animal model study of CO. Further homogeneous studies are necessary to compare the M-values of different animals.



Reporting of the relative affinity (M) constant of hemoglobin for carbon monoxide in animal world: a systematic and meta-analysis review

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Introduction

The Haldane (M) constant represents the oxygen/carbon monoxide ratio bound to hemoglobin and is a key value of physiological and pathological models exploring CO metabolism or exposure. However, results on (M) values appear to be inconsistent among studies and species. A better estimation of (M) value and a species consideration will enforce accuracy of CO studies results and allow elaboration of reliable models.

Methods

We performed a systematic review and meta-analysis in order to determine and compare (M) values according to species and experimental conditions. We used electronic libraries (PubMed/Embase/JSTORE) and performed a manual search on references from 1895 to January 2016. For each article, we collected: species, age, temperature, pH, atmospheric pressure, presence of 2,3-DPG rate or methemoglobin, type of hemoglobin. Statistical analyses were performed with JMP[®] Pro12 software, SAS[®], USA.

Results

The systematic review found 25 articles addressing (M) constants including 135 individual data and 51 mean values concerning mammals, laboratory animals, fish, fetal hemoglobin and invertebrates. (M) values at physiological temperature and pH were assessed only for adult (137 [119-153]) and fetal (135 [112-168]) sheep, dogs (215 [193-218]), mice (194 [118-278]) and rats (191 [177-200]) (Figure1). Correlation between (M) values and temperature was confirmed for sheep (Figure 2) and horse. The lack of data did not permit to conclude about correlation between (M) and pH. No species presented statistically equivalent (M) values compared to human blood at 209 [190-257].

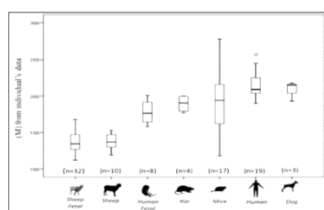


Fig 1: M_{35-40} medians from individual's data at physiological or supposed pH.

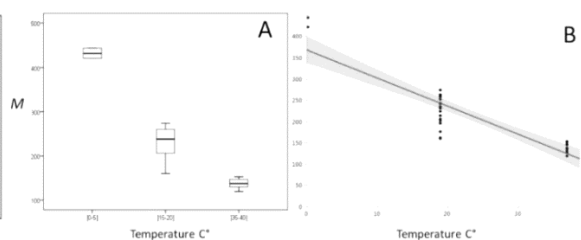


Fig 2: From sheep individual's data:

A. M values at different T° found (0.2°, 19° and 37°).

B. Significant correlation between M and T° ($r^2=0.79$; $p<0.001$), when T° decreases of 1°, M increases of 6.6 units.

Conclusion

Our results confirm that (M) values vary according to species. Only the influence of temperature is confirmed. A formal measurement of the (M) constant in the given conditions of experimentation should be performed for each animal model study on carbon monoxide. Further homogeneous studies are necessary to compare (M) values among different animals and to determine the species whose (M) value is most comparable to human blood.

REVIEWS

Report on the Relative Affinity Constant (M) of Hemoglobin for Carbon Monoxide in the Animal World: A Comparative Review with a Meta-Analysis Based on a Systematic Review

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Abstract—The Haldane M-value is the ratio of half-saturation pressures for O₂ and CO (PO₂/PCO) and is a key value of physiological and pathological models exploring CO metabolism or exposure to CO. Nevertheless, the M-values obtained from different studies and different species appear to be inconsistent. A better estimation of M-values and a consideration of interspecies variation will ensure the accuracy of CO study results and allow reliable models to be constructed. We performed a systematic review and meta-analysis in order to determine and compare M-values according to species and experimental conditions. We used electronic libraries (PubMed/Embase/JSTORE) from 1895 to January 2016. For each article, we collected the following parameters: species, age, temperature, pH, atmospheric pressure, concentration of 2,3-diphosphoglycerate (2,3-DPG) or methemoglobin, and type of hemoglobin. The systematic review found 25 articles addressing the M constant, including 135 with individual data and 51 with mean values concerning mammals, laboratory animals, fish, fetal hemoglobin and invertebrates. M-values at a physiological temperature and pH were assessed only for adult (137 [119–153]) and fetal (135 [112–168]) sheep, dogs (215 [193–218]), mice (194 [118–278]) and rats (191 [177–200]). A correlation between M-values and temperature was confirmed for sheep and horses. A correlation between M-values and pH could not be inferred because there was a lack of data. M-values varied according to species. Only the influence of temperature was confirmed. A formal measurement of the M constant in the given experimental conditions should be performed for each animal model study of CO. Further homogeneous studies are necessary to compare the M-values of different animals.

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5.2. INTRODUCTION

Carbon monoxide (CO) has a high affinity for all metalloproteins and in particular for hemoglobin (Hb), forming the carboxyhemoglobin complex (COHb). CO remains a key focus of interest, firstly, because of CO poisoning and secondly, due to its endogenous physiological roles [1, 2]. The Haldane (or M) constant represents the relative affinity of Hb for CO versus blood oxygen [3], which is presented in the following equation: $[\text{COHb}]/[\text{O}_2\text{Hb}] = M \cdot p\text{CO}/p\text{O}_2$ where $[\text{COHb}]$ is carboxyhemoglobin concentration (%), $[\text{HbO}_2]$ is oxyhemoglobin concentration (%), $p\text{CO}$ is the partial pressure of dissolved CO (mmHg), and $p\text{O}_2$ is the partial pressure of dissolved oxygen (mmHg).

Due to crucial ethical concerns and following Engel's and Rodkey's insightful human studies, human blood from volunteers has rarely been used to study CO behavior and COHb since the 1970s [4–7]. To extrapolate data relevant for humans, animal models were investigated, but we must consider species specificity, including species-specific M-values and factors such as pH or temperature (T) conditions, in order to explain the discrepancies between the M-values observed, at least in part. A similar meta-analysis on humans confirmed an M-value of 218 for adult Hb and 175 for human fetal Hb under physiological conditions, and also confirmed the T° influence [6, 8].

Our main objectives were to determine M-values among species, to analyze the factors that influence the results, to compare species, and to find the blood types most similar to those in humans. To do this, we performed a wide and systematic review with a meta-analysis of M-values in the animal world whenever data allowed.

5.3. MATERIALS AND METHODS

5.3.1. Search methods for identification of studies

We performed a systematic search of electronic libraries (PubMed, Embase and JSTORE) and a manual search of references from 1895 to January 2016. All articles that revealed an individual or mean animal M-value (sometimes denoted by the letter 'K'), as determined by tonometric or similar methods, were included. We excluded studies/results in which M measurements were taken using a method that was very different from tonometry or that used human Hb. The following keywords and search strategy were used.

In the Embase library: ('carbon monoxide'/ exp or 'carbon monoxide' or 'carbon monoxide poisoning' or 'carbon oxide') and ('carboxyhemoglobin' or 'carboxyhemoglobinemia' or 'hemoglobin') and ('constants and coefficients' or 'Haldane effect' or Haldane or 'affinity' or 'dissociation' or 'linkage' or 'half-life time' or 'toxicokinetics' or 'kinetics' or 'pharmacokinetics' or 'releasing' or 'elimination rate constant' or 'elimination half-life' or 'clearance lung' or 'metabolic clearance rate' or 'clearance' or 'expired air' or 'ligand binding') and ([english]/lim or [french]/lim) and [animals]/lim.

In the PubMed library: ('carbon monoxide/ [Mesh]' or 'carbon monoxide poisoning/[Mesh]' or 'carbon oxide') and ('carboxyhemoglobin/ [Mesh]' or 'carboxyhemoglobin' or 'carboxyhemoglobinemia' or 'hemoglobin') and ('constant' or 'Haldane' or 'Haldane effect' or 'affinity' or 'dissociation' or 'linkage' or 'half-life/[Mesh]' or 'half-life' or 'toxicokinetics/[Mesh]' or 'kinetics/ [Mesh]' or 'pharmacokinetics/[Mesh]' or 'releasing' or 'elimination' or 'clearance rate metabolic/ [Mesh]' or 'ligand'). Filters: Animal/English/ French.

In the JSTORE library (from 1895 to 1960): [(carbon monoxide) or (carbon oxide)] and [(hemoglobin) or (carboxyhemoglobin)] and [(affinity) or (Haldane)].

We checked the reference lists of retrieved studies and those of relevant narrative and systematic reviews, and completed this by a manual search (ND, AP, PH)

5.3.2. Data extraction

The data extracted were: individual M-values or, if not mentioned, mean data, standard deviation (SD), medians and distribution values, species, adult or fetal normal animal Hb, pH and T° conditions. If T° or pH were not mentioned, the article could be selected for discussion but data would be excluded from the statistical assessment. We completed an extraction based on other experimental conditions: M assessment method, atmospheric pressure, presence of 2,3-diphosphoglycerate or methemoglobin, analyses performed on hemolysis or whole blood.

A first reviewer (ND) read the full text of all articles including any data extracted. A second reviewer (PMR) confirmed the data extraction. Due to the average age of studies included, we could not contact the original investigators to request the missing data. We also did not use statistical methods to model the missing data. If the authors mentioned that the assay was performed *in vivo*, we considered that it was performed on whole blood at a physiological pH and T° for each specific species.

5.3.3. Data synthesis

We pooled all individual data extracted under defined conditions in terms of pH and temperature (M_T in Celsius). Individual M-values are presented as medians and extremes [minimum– maximum] due to non-normal distribution. When individual data were not mentioned, the synthesis data (mean and SD) extracted from assays

were used. For this purpose, we considered the distribution of data to be normal. We used the same method to assess M in animal fetal hemoglobin (HbF).

To compare M data obtained from blood at different T°, we classified M data found in the following T° ranges: > 0° and ≤ 5°, > 5° and ≤ 10°, > 10° and ≤ 15°, > 15° and ≤ 20°, > 20° and ≤ 25°, > 25° and ≤ 30°, > 30° and ≤ 35°, > 35° and ≤ 40°, > 40°.

If not mentioned, parameters considered as physiological for the whole blood experiments were similar for sheep, dogs, horses, mice, rabbits, rats, and swine: T° range > 35° and ≤ 40°, pH ≥ 7.35 and ≤ 7.45. We took a lower intracellular pH for hemolysis erythrocytes or purified Hb from 7.1 to 7.2 to be physiological [9].

5.3.4. Statistical analyses

All analyses were performed on JMP® pro12, statistical discovery™ software, SAS® or IBM® SPSS Statistics 24 (USA). For individual data, due to modestly sized data for groups, non-parametric tests were considered. To compare the different species, the Kruskal–Wallis test with the Bonferroni correction was applied to find out if there were interspecies variations. To compare animal species to humans, we used the M-value obtained from the previous meta-analysis (personal data, submitted). To find the most suitable species's blood to investigate with a view to approximating human blood types in terms of the M field, we tested equivalence using two unilateral tests, the Mann–Whitney U test and the Bonferroni correction, considering a physiological difference of M < 40 to be significant. To avoid excessive correction, we limited comparisons among species that were previously found not to be different by the Kruskal–Wallis test. Simple linear regressions were performed to analyze the correlation between M and T° or pH. If $p < 0.05$, the correlation line was accompanied by the 95% confidence interval.

5.4. RESULTS

5.4.1. Literature analysis and flow chart

We identified 661 articles from 1895 to 2016 in electronic libraries and 45 via references and the semantic web. After scanning the abstracts and titles, 142 were selected for full review but 117 were excluded because of missing data (n, SD, T° and/or pH). Finally, 25 articles were included: 7 studies revealing original individual data and 24 studies reporting original animal mean M-values from different species (Fig. 1). The 25 articles dated from 1910 to 1998 and revealed 135 individual data and 51 mean data on the animal world, including data on M-values for mammals, laboratory animals and other species.

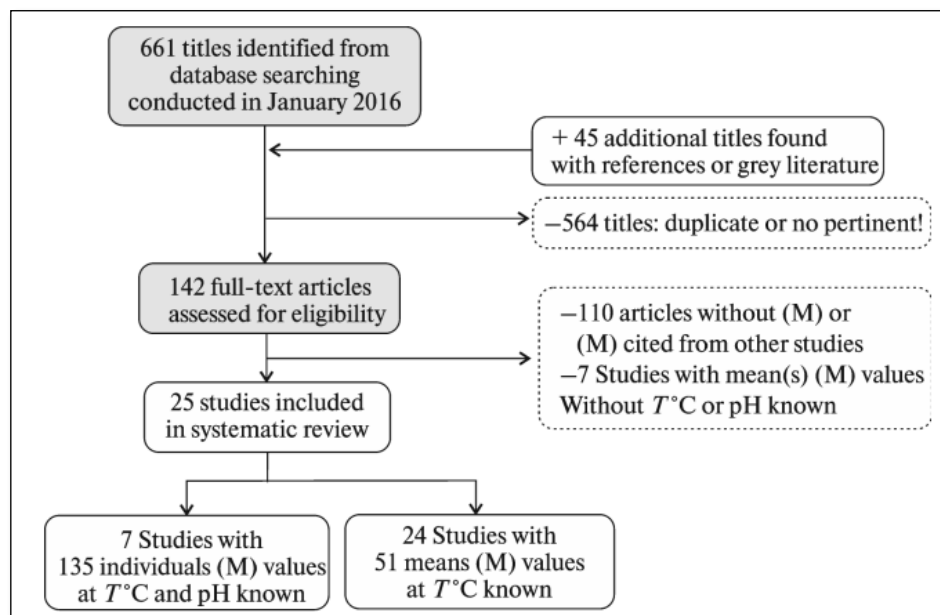


Fig. 1. Flow diagram of studies identified, excluded from and included in the systematic review.

5.4.2. M-value for animal hemoglobin

5.4.2.1. M-value for sheep

Individual data on sheep

- M_{0-5} median = 432 [421–443] ($n = 2$ at 0.2° and pH 9.1) (Fig. 2a) [10];
- M_{15-20} median = 238 [160–274] ($n = 22$ at 19°) (Figs. 2, 6), including the experiment performed at a pH of 7.10 ($n = 5$) with a median M_{15-20} of 176 [160–206] and pH of 9.10 ($n = 17$) with a median M_{15-20} of 252 [202–274] [10, 11];
- For M_{35-40} , we found 10 values which seemed to have been obtained at physiological T° and pH but without formal certainty. Nevertheless, if we assume that this was the case, M_{37} was 137 [119–153] ($n = 10$) (Figs. 2a, 4) [12].

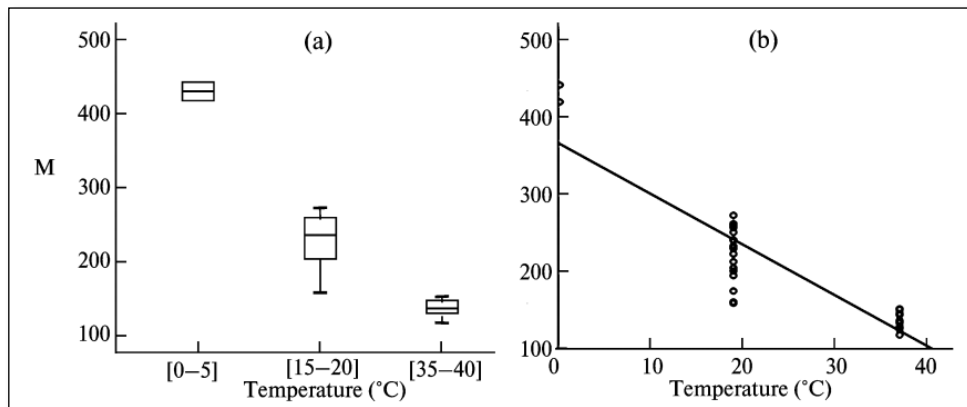


Fig. 2. Individual data on sheep: A. M-values identified at different T° (0.2° , 19° and 37°). B. Significant correlation between M and T° ($r^2 = 0.79$; $p < 0.001$): when T° decreases by 1°C , M increases by 6.6 units. No available data were found for $T^\circ > 5^\circ$ and $\leq 15^\circ$ or $> 20^\circ$ and $\leq 35^\circ$.

Mean data on sheep

We found 12 mean M-values at different known T°, including 6 pH values ranging from 6.2 to 11 and 6 unknown pH values. Due to a lack of pH data, we classified the 12 values found to be within T° ranges regardless of the pH (Fig. 3a): M_{10–15} mean was 195 (n = 1) [13]; M_{15–20} mean was 213 (SD 24; n = 7) [10, 14]; M_{20–25} was 179 (n = 1 at 24°) [15]; M_{30–35} mean was 155 (n = 1 at 34°) [13]. Among the studies on mean M-values, only two were conducted between > 35° and ≤ 40°, showing a M_{35–40} mean value of 156 (SD 8; n = 2) [16, 17].

The correlation between M and T° is statistically significant: when T° decreases by 1°C, M increases by 6.6 units for sheep from individual data (Fig. 2b) or 2.7 from mean data (Fig. 3b). Finally, no data on sheep are available since 1984.

Furthermore, from Roughton's data, we found a significant correlation between M and the two pHs tested ($r^2 = 0.673$; $p < 0.001$): when the pH increases by 0.1, M increases by 3.3 units [10, 14].

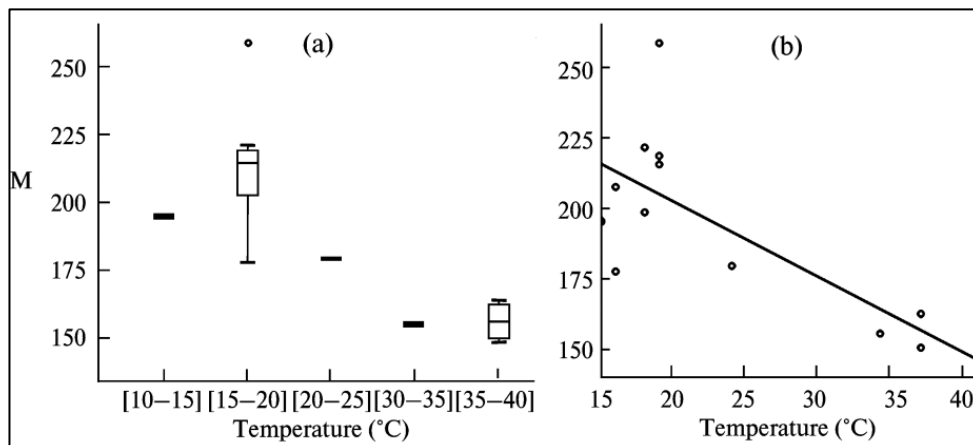


Fig. 3. Mean data on sheep. (a) Sheep M-values identified at different T°. (b) Significant correlation between sheep M and T° ($r^2 = 0.507$; $p < 0.009$), when T° decreases by 1°C, M increases by 2.7 units. No available data for T° ≤ 10°.

5.4.2.2. M-value for horses

Individual data on horses

No experiments between $> 35^{\circ}$ and $\leq 40^{\circ}$ were conducted on a horse. Only M_{15-20} was investigated with a median of 263 [208–288] ($n = 12$, individual data at 19°), including an experiment performed at a pH of 7.0 ($n = 4$; median M of 219 [208–231]) and at a pH of 9.1 ($n = 8$; median M of 267.5 [258–288]) [18] (Fig. 6). At this T° , we observed a correlation between M_{15-20} and the two pH extremes tested ($r^2 = 0.854$; $p < 0.001$): when the pH increases by 0.1, M increases by 2.5 units. This correlation warrants caution because intermediate pH and physiological T° were not investigated. Due to a lack of data, a correlation between M and T° could not be assessed.

Mean data on horses

On the other hand, an M_{15-20} mean value of 280 was found at 20° and a pH of 7.3 [19], however, this was very discordant with a previous study performed in the same physiological range, which revealed a value of 500 [20].

5.4.2.3. M-value for dogs

Individual data on dogs

M_{35-40} was only investigated in a single study with an M_{37} median of 193 [133–230] ($n = 9$), including the experiment performed at $pH < 7.35$ ($n = 4$; median M_{37} of 200 [141–230]), at $pH \geq 7.35$ and ≤ 7.45 ($n = 3$; median M_{37} of 215 [193–218]) (Fig. 4), and at $pH > 7.45$ ($n = 2$; median M_{37} of 140.5 [133–148]) [21]. No correlation was observed between M and pH ($r^2 = 0.074$; $p = 0.478$), in contrast to Allen's study in which a statistical analysis was not applied [21]. No available data were found for $T^{\circ} \leq 35^{\circ}$.

Individual data on dogs

We found M mean data obtained without specifying the pH for M_{10-15} (at 15°) of 320 [22] and for M_{35-40} (at 37–38°) of 239 (SD 11; n = 3) [16, 22, 23].

5.4.2.4. M-value for rats

Individual data on rats

We found 16 M_{35-40} individual data for rats (at 37°) and pH from 7.20 to 7.63 with an M median of 158 [116–200] compared to 191 [177–200], if we considered only the physiological pH (≥ 7.35 to ≤ 7.45 ; n = 4) [21] (Fig. 4). Based on the linearity hypothesis, we did not observe a significant correlation between M and pH ($r^2 = 0.025$; $p = 0.558$), in contrast to Allen's conclusion; however, he did not explain the methodology he used to construct his graph.

Mean data on rats

For mean data on rats, two studies found M_{35-40} (at 37°) of 141 and 162, regardless of the pH [24, 25]. The last study, which did not mention the pH and T°, showed an M of 150 [26].

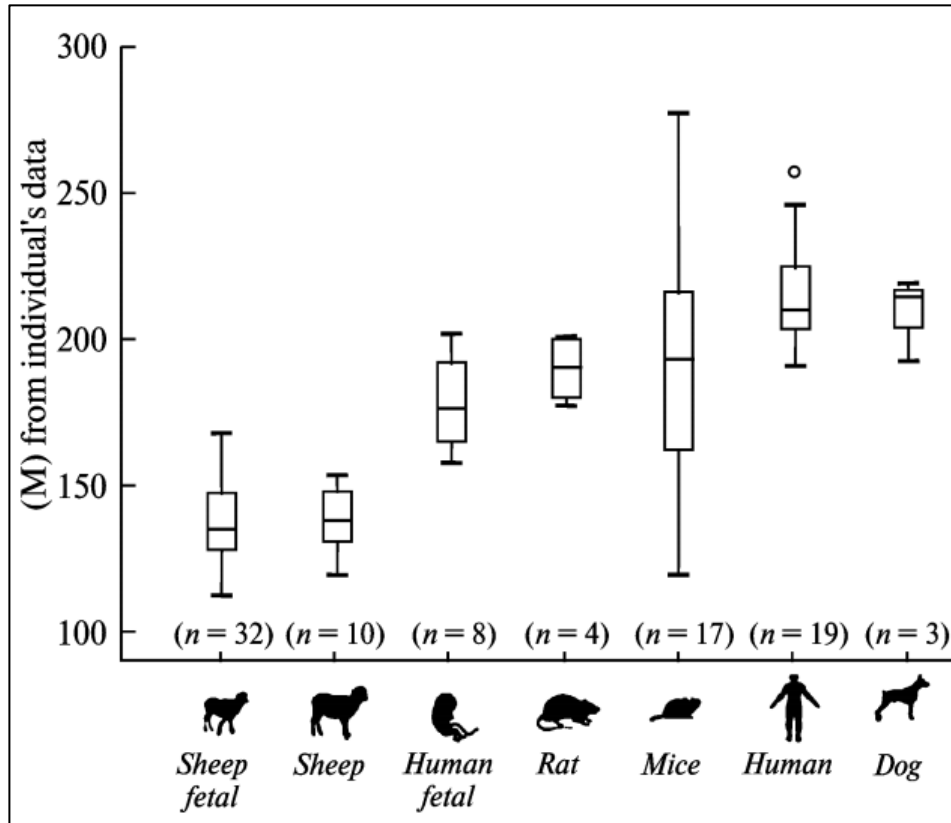


Fig. 4. M_{35-40} medians from individual data at physiological or supposed pH. The intraspecies variability in mice is noticeably greater than in humans, horses, and rabbits.

5.4.2.5. M-value for mice

Individual data on mice

We found a single study performed on mice with 17 individual M data performed between $> 35^\circ$ and $\leq 40^\circ$ (at 37°) and at a physiological pH due to analyses performed immediately after an animal's death. The M_{35-45} median was 194 [118–278] [27].

Mean data on mice

Three mean M-values were found. One was performed at 38° revealing an M mean of 148 and a pH that was probably physiological due to a blood PaCO₂ controlled at 40 mmHg. In the same study, M-values from 16 calculations were assessed at 134 to 306 [3, 28].

5.4.2.6. M-value for rabbits

Individual data on rabbits

No individual data were found for rabbits, and the pH was never investigated.

Mean data on rabbits

Five mean M-values were found, mainly without mentioning the pH. Only one M_{35–40} result was clearly reported at a physiological T° (supposedly > 35° and ≤ 40°) showing a value of 109 [24]. The others were obtained at 120 for M_{10–15} (at 15°) [29]; at 40 for M_{15–20} (at 20°) and pH 7.3 [19], and at 83 to 140 [30].

5.4.2.7. M-value for swine

Individual data for swine

No individual data were found for swine.

Mean data for swine

Only two articles investigated M for swine, both without mentioning the pH. In the first, the M_{10–15} (at 15°) was 220 [22], and in the second, the M_{35–40} was 130 (the authors cited that the protocol should be compared with that of another study where M was measured at 37°) [24].

5.4.2.8. M-value for oxen

Individual data on oxen

No individual data were found for oxen.

Mean data on oxen

The M_{35-40} value for an ox from 13 calculations (at 37°) was found to be 179 [31]. Another value of M_{38} from Krogh was cited by Killick to be 288 but not found historically in Krogh's article [30, 32, 33]. Haldane himself assessed the M_{15-20} (at 17°) of oxen as 140 but questioned his procedure in his later article [3, 34].

5.2.4.9. M-values for hamsters, opossums, monkeys, guinea pigs and miscellaneous vertebrates

Only one M_{35-40} value (at an assumed temperature of 37°) was found at 181 for hamsters [24], 247 for opossums, 195 for monkeys [16], and 117 for guinea pigs [25].

5.2.4.10. M--value for fish

Individual or mean data were not distinguished from each other in the articles on fish that we managed to find. Only one M-value was found (at a temperature of 20°) and with a known pH (of 7.0) where the M_{15-20} was measured at 92 for a carp and the pH varied between 6.5 and 9.0 without changing the M-value [35]. The following M values were found without specifying the T° or pH: 60.5 for carps, 100 for pikes, 99–114 for eels [36], 89 for lampreys [37], 63 for goldfish, and 210 for the common roach [38, 39]. The physiological parameters of fish blood depend on the ambient environment. The M_{15-20} parameters found were in the range of > 15° and ≤ 20°, while pH was observed at 7.3 for eels and 6.6 for lampreys.

5.4.2.11. M--value for invertebrates

Descriptions of M-values were too imprecise to certify values from individual or mean data. We found M-values for nine different invertebrates.

Annelids

Arenicola marina M-values were found to be 150 from 5 to 11 calculations performed at a pH of 7.30 and temperature of 20° [19, 29]; M₂₀ assessments for the Tubifex, Planorbis corneus, and *Branchiomma vesiculosum* were performed at a pH of 7.30 resulting in values of 40, 40 and 570, respectively [19] (Fig. 6); trematode M₂₅ values were measured at 1 for a pH of 7.0 [40]; M-values for *Glycera dibranchiate* were measured between 4.5° and 24.5° with a pH of 5.8 to 9.0 resulting in a mean of 20,000 [41].

Annelids presented extreme values whereby the most recent values found for invertebrates were from 1998 and showed that Trematodes have an extremely low M-value of less than 1, due to an increased O₂ affinity rather than a decreased CO affinity [40]. *Glycera dibranchiate*, which possesses a monomeric Hb characterized by an extremely high CO affinity, has a much greater M-value (≈20,000) [41]. Physiological parameters for *Arenicola*, *Planorbis* and *Tubifex* range from > 15° to ≤ 20° for T° and, for pH, the physiological parameter for *Arenicola* is 7.3 [42].

Insects

Two *Chironomus* M₂₀ data were obtained at a pH of 7.0 and 7.3 with M-values of 300 and 400 [19, 43]; two data on *Gasterophilus* were obtained at 22° and 25° and a pH of 7.3 with M-values of 0.5 and 0.67, respectively [39, 44]. The physiological temperature is 17° for *Chironomus* and 39° for *Gasterophilus larvae* living in equine intestines.

5.4.3. M-value comparison between species

Three figures are proposed to give a graphic summary of the data found: the first shows all experimental animal studies performed from > 35° to ≤ 40° and with a

physiological pH or derived from individual data on sheep, dogs, rats and mice compared to humans (Fig. 4); the second refers to data obtained at 37° and any pH from individual data and mean data on miscellaneous species (Fig. 5); and finally, the third shows the data obtained from > 15° to ≤ 20° and any pH on humans, sheep and horses (Fig. 6).

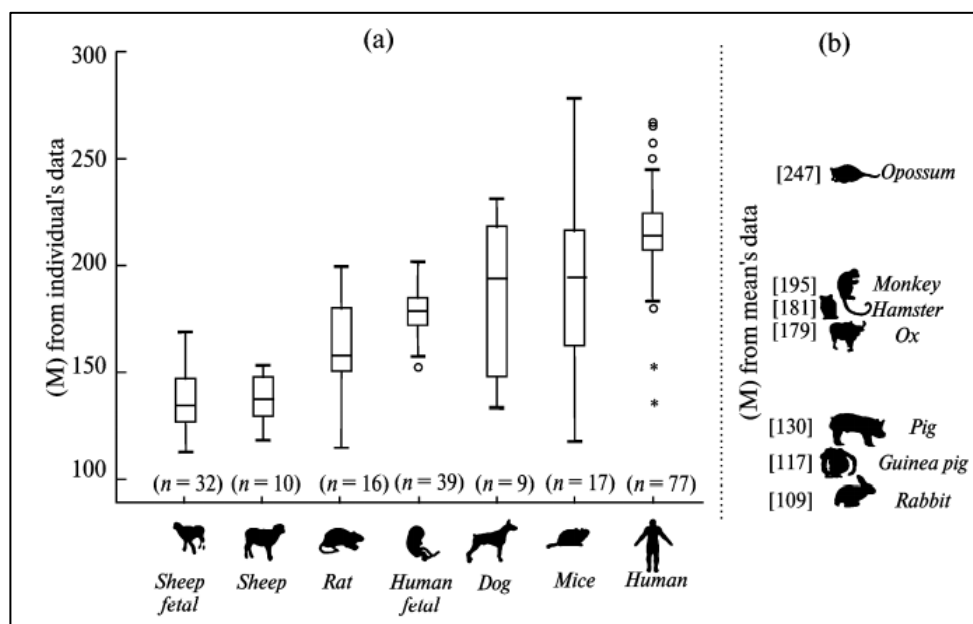


Fig. 5. (a) M₃₅₋₄₀ at any pH: M₃₅₋₄₀ medians from individual data. (b) M₃₅₋₄₀ medians from mean data.

Data availability allowing a comparison of Hb in adult species.

– At 19°: there were only three species for which we could find M₁₉ medians obtained at any pH: sheep M₁₉ = 238 [160–274], horses M₁₉ = 263 [208–288] and humans M₁₉ = 322 [299–376]. A Kruskal–Wallis test showed a significant median difference between humans and sheep, and between humans and horses ($p < 0.001$). No significant difference was shown between sheep and horses (Fig. 6).

– At 37° and a physiological pH: the Kruskal–Wallis test makes it possible to detect at least one median difference among the seven species ($p < 0.001$): adult humans $M_{37} = 218$ [191–257], fetal humans $M_{37} = 175$ [158–192], adult sheep $M_{37} = 137$ [119–153], fetal sheep $M_{37} = 135$ [112–168], dogs $M_{37} = 215$ [193–218], rats $M_{37} = 191$ [177–200], and mice $M_{37} = 194$ [118–278] (Fig. 4). Using the Bonferroni correction, it was possible to identify significant interspecies differences between fetal sheep and adult human Hb ($p < 0.001$), dog Hb ($p = 0.042$) or mouse Hb ($p < 0.001$), and between adult sheep and adult human Hb ($p < 0.001$) or mouse Hb ($p = 0.023$). Moreover, we tested the equivalence to humans using the Mann–Whitney U test with the Bonferroni correction: no equivalence was found, assuming a significant physiological difference of $M < 40$.

– At 37° and any pH, including data obtained at the physiological pH: seven species have significantly different M_{37} medians: adult humans $M_{37} = 214$ [136–267], fetal humans $M_{37} = 178$ [152–201], adult sheep $M_{37} = 137$ [119–153], fetal sheep $M_{37} = 135$ [112–168], dogs $M_{37} = 186$ [133–230], rats $M_{37} = 162$ [116–200], and mice $M_{37} = 194$ [118–278] ($p < 0.001$). Note that all data found were at the physiological pH for fetal sheep and adult sheep Hb. We then also used these values as “any pH”. The Kruskal–Wallis test allows us to detect at least one median difference among groups ($p < 0.001$). The Bonferroni correction detects significant differences between fetal sheep Hb and human adult Hb ($p < 0.001$), fetal human Hb ($p < 0.001$), mouse Hb ($p < 0.001$) or dog Hb ($p = 0.006$), between adult sheep Hb and human adult Hb ($p < 0.001$) or mouse Hb ($p = 0.015$), between adult human Hb and rat Hb ($p < 0.001$), and between adult human Hb and fetal human Hb ($p < 0.001$). In addition, we tested the equivalence using the Mann–Whitney U test with the Bonferroni correction. Again, no equivalence was found, assuming a significant physiological difference of M at $\Delta < 40$. No species has a similar M median to that of the adult human Hb.

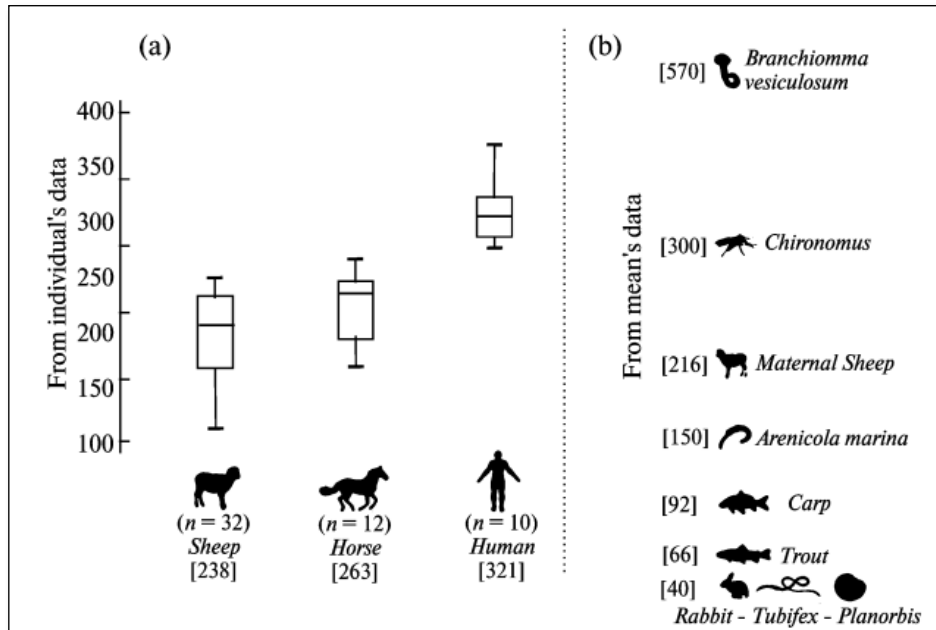


Fig. 6. M_{15-20} at any pH: M_{15-20} medians from individual data. The differences between sheep and horses ($p = 0.14$) are not statistically significant, but both are statistically significant compared to humans ($p < 0.001$) (left) or M_{15-20} means from mean data (right). Note that species investigated at 15° were not included.

5.5. DISCUSSION

5.5.1. Strengths and limitations

Literature on the M-value in the animal world appears to be limited and scattered. Previous reviews on this topic were incomplete and no meta-analysis or statistical comparison between species has ever been performed [33, 45–47]. As far as we know, our systematic review is the widest on this topic. The most recent references date back to 1984 [17]. Moreover, despite the fact that mice are the living CO alarms (detectors) for miners, their M-value was poorly investigated with only a single study performed so far. The lack of recent studies on M could be explained first, by a lack of expertise in tonometry and second, by of the large amount of time these studies require. In addition, the reason for the difficulty in finding the M-value using a classical literature search strategy was that most of the M-values derive from older studies and the keywords are non-specific, requiring a semantic web search strategy.

In the literature reviewed, there is evidence that Hb affinity for CO is species-specific, but statistical methods were rarely applied. The absence of weight, species, race, number of experiments performed in each study, number of analyses completed, inaccuracies of means lacking standard deviations, and heterogeneous or inaccurate experimental conditions (pH and T°) made it impossible to pool data and conduct a classical meta-analysis for most of the species. In addition, most historical studies observed a clinical specificity of species towards the same CO exposure but did not particularly highlight or estimate the M-value [48–50]. However, we still performed a systematic and narrative review, pointing out the M-values found but with methodological limitations for the means calculated. Nevertheless, data pooled on sheep, horses or dogs allows the statistical assessment to test a correlation between M and T° or, more unusually, with the pH.

Moreover, the only fetal Hb in the animal world was investigated in sheep with a M_f -value (fetal M-value) of 135 [112–168] calculated on 32 individual data from the same author. His protocol suggested that results were obtained at a T° of 37° , but the exact T° and pH conditions were not described. This must explain why his M-value was 40% lower than that of the only other M_f -value, which was found at 19° and a pH of 9.1, measuring an M_f -value of 216 [51]. Furthermore, in both studies the maternal and M_f -values were similar, and no statistical difference was found between adult and fetal sheep Hb ($p = 0.673$) (Fig. 5). As for humans, no correlation between the fetal age and M-value was observed [12] (Delvau, 2018, submitted).

5.5.2. Effect of temperature on animal M-values

In contrast to human studies, studies on animals were often conducted at a non-physiological T° . Many M-related differences in CO studies derive from experimental conditions in which blood T° was reached at various different room T° in order to increase control over the other parameters, such as solubility or Hb osmotic pressure [52].

A review of animal M-values confirmed that M increased when T° decreased [22, 23, 33, 53]. With the available data, the relationship between M-value and T° could only be assessed and determined for sheep. The correlation between M and T° was, as in humans, confirmed to be statistically significant (Delvau, 2018, submitted). In sheep, another T° coefficient of 1.3 to 4.0 per 10°C was found by Roughton [14, 54]. Moreover, a fall in the COHb saturation by 0.5% for every 1°C rise in T° is consistent with a lower M-value when T° increases [55]. In humans, this coefficient was found at 4.5 between 19° and 37° [6] (Delvau, 2018, submitted).

In rabbits, Fox's M-value of 40 at a T° of 20° conflicts with the M-value of ≈ 110 for temperatures of $> 35^\circ$ and $\leq 40^\circ$ from other studies. Holland extrapolated this M_{37} value at 29, using Rodkey's correlation for humans [19, 46, 53], and other studies

used Holland's result of 29 for rabbits [56]. No clear explanation can be found, and the correlation drawn from the human experiment between 22° and 37°, as applied to rabbits, seems to be unfounded. Holland made the same uncertain conversion for horses from 280 at 20° to 206 at 37°. There is no evidence that a linear relationship (the conversion of 4.5 unit/°C for humans) exists between T° and animal M at any T° and for any species. Consequently, it seems impossible to propose a universally validated correcting factor for T° .

In clinical practice, M modified by T° may be taken into account for hypothermia. Moreover, hypothermia reduces the rate of CO uptake, increasing resistance for chickens or rats, and the maintenance of hypothermia after CO exposure noticeably increases mortality in rats [57]. In addition, this temperature effect is probably the reason for COHb remaining stable for several months in a blood test if kept at a classical low T° (4°C) [58].

5.5.3. Effect of pH on M-values

The influence of pH on M-values has to be considered for species that have different physiological blood pHs and in studies conducted under different experimental conditions.

Before 1940, results obtained by Haldane or Hartridge in 1912, and by Roughton in 1934, demonstrated that M is practically independent of pH, salt content, or the level of Hb purification [33]. Lilienthal first suggested in 1946 that a human M-value of 204 is lower at a higher pH [59]. In a previous study on human Hb, it was not possible to confirm that pH influences M for adult Hb. Nevertheless, given the limitations of the data that could be evaluated, this result should be taken with caution.

Roughton investigated the effect of pH on M_{19} in sheep and found that M rose by an average of 35%, when the pH was increased from the extreme values 7.1 (M =

180) to 9.1 (M = 243) by a borate buffer solution [10, 54]. We found a significant correlation between M and the two pHs tested ($r^2 = 0.673$; $p < 0.001$): when the pH increases by 0.1, M increases by 3.3 units. This correlation warrants caution because only two extreme pHs were tested. This change in M is at least similar in direction and degree to the results for M presented in Joels's study on humans [45]. However, neither Roughton nor Joels investigated intermediate pH values. As such, it is impossible to reach a conclusion on M behavior between these extreme values. We must therefore state that we lack the data to postulate the existence of a correlation.

In addition, using sheep, Engel showed no effect of pH on M_f -values at 172 or on the maternal M-value at 217 for pH variation from 6.4 to 9.8 [6]. Furthermore, for carp Hb, Tan found M-values to be equal at 92 and invariant with a pH ranging from 6.5 to 9.0 [35].

Finally, we did not observe a correlation between M-values and pH when assessing the only dog study focused on this topic. The author proposed a bell curve to express the relationship between M and pH with a peak M-value at the physiological pH, close to 225, and 30–40% lower at a pH of 7.1 and 7.6. He thus suggested that pH does influence M-values but did not use statistical methods to support his conclusion. Nevertheless, he observed the same trend in the experiments conducted on dogs, humans and rats [21].

Based on these conflicting reports and the lack of data, it is impossible to affirm or refute the suggestion that pH significantly influences the value of M.

5.5.4. Hemoglobin environment adaptation

Evolution of the Hb structure followed the living beings' need for adaptation [60]. *Arenicola* is an annelid that lives buried in the sand. *Planorbis* is a gastropod mollusk with a shell. *Tubifex* is a worm that lives in colonies in the sediments of lakes or

rivers. In addition, Hb is infrequent in the insect world, occurring in a few species or their larvae, e.g., in *Gasterophilus* [39, 44, 61]. With a similar T° , the highest value found was for *Chironomus* at 400, given a monomeric Hb. In contrast, the lowest value was an extreme of 0.67 for the *Gasterophilus* larvae, given a specific dimeric Hb without cooperativity between its two subunits. *Gasterophilus* lives as a parasite immersed in the gastric juice of the horse. Furthermore, if combination velocity constants are about 20 times slower for CO than for O₂, the only exception to this rule is the Hb of *Chironomus*, an insect that resembles mosquitoes. The combination velocity constants show the same consistency as was seen in O₂ binding [19, 39, 43, 44]. In contrast, cockroaches do not use Hb to transport O₂, so they can tolerate very high exposure to CO for more than 18 days without developing any pathological symptoms [34, 62].

One of the expressions of how these Hb conformations have adapted to their microenvironment is the M constant [44]. Moreover, adaptation to a specific way of life can also come from the Hb microenvironment. In the majority of mammalian red blood cells, 2,3-DPG is the most abundant organic phosphate. Millimolar concentrations of 2,3-DPG decrease the affinity of Hb for O₂, shifting the dissociation curve for O₂ to the left [63].

5.5.4.1. Effect of 2,3-diphosphoglycerate on M-values

No single study has investigated the influence of 2,3-diphosphoglycerate on animal M-values. Human M-values are unaffected by the presence of 2,3-DPG [64, 65].

In fact, studies on M can be performed on whole blood cells or hemolysis blood. Thus, it is important to know if the presence of 2,3-DPG in red blood cells changes the M-value. Furthermore, the 2,3-DPG levels in red blood cells are variable in different animals and variable in different fetal ages [66]. It is worth noting that the

2,3- DPG concentration in the blood was unchanged by CO exposure in Penney's study [57]. Without exploring the COHb dissociation or M-value, another study showed that 2,3-DPG caused a 15% decrease in the CO combination velocity [67].

5.5.4.2. Effect of methemoglobin on M-values

Fire smoke inhalation often causes cyanide and CO co-intoxication [68]. Antidotes to treat cyanide poisoning may use methemoglobin inducers (amyl nitrite, sodium nitrite, etc.) and thus may influence M-values. With only one study, this topic has received little academic attention; however, Allen concluded that M-values should not be affected by methemoglobin [21].

5.5.4.3. Effect of atmospheric pressure on M-values

No single study has investigated M at different atmospheric pressures in animals. This variable was only partially investigated in humans, and it was shown that barometric pressure does not influence the M-value [6, 59, 69]. Nevertheless, Rose studied the acute toxicity of CO under hyperbaric conditions in rats, mice and guinea pigs [70].

5.5.4.4. Effect of sickle hemoglobin (HbS) or splenectomy on M-values

No single animal study has investigated sickle Hb (HbS), although the M-value for human sickle Hb seems to be similar [6, 8]. Allen (1957) also studied dogs that had undergone splenectomies without revealing significant differences in M [21].

5.5.4.5. M-values for fetal hemoglobin and pregnancy

As for small animals, clinical studies showed that the fetus is probably also very sensitive to neurological injury following CO poisoning [71]. Many hyperbaric societies recommend hyperbaric therapy for pregnant women exposed to CO, even at low COHb [72]. The nature of fetal Hb changes during pregnancy, and the level

of 2,3-DPG varies. The affinity of fetal Hb for O₂ is higher than that of adult Hb. Thus, we specifically reviewed M-values with regard to pregnancy, searching for maternal and fetal blood levels. The results showed that sheep were the only species investigated for M in fetal Hb.

Independently of M, the interest in CO and pregnancy is not a new phenomenon. First, Fehling [73] and Högyes [74] investigated the effect of CO on the blood of pregnant rabbits or rabbit fetuses. Later, Nicloux (1901) was a pioneer, studying the placental diffusion of CO from mother to fetus in guinea pigs [75, 76]. Later, a few other historical studies observed specific CO kinetics in a fetus: the ratio between fetal and maternal COHb in sheep, dogs, and rabbits being 2.2/3.2 [51, 77] or the M_f/M_m ratio of 0.99 in sheep and 0.83 in humans [77]. Moreover, differences in CO uptake or COHb clearance have been shown. After exposing sheep to CO, the half-life of fetal [COHb] is 2–5 times that of maternal [COHb] [78, 79] and continued to grow to be twice as high as maternal [COHb] after 4–6 h. Moreover, pCO in fetal blood is slightly higher than in maternal blood due to the endogenous CO production by the fetus [80].

The difference between M_m and M_f was found to be statistically significant for humans at 218 and 175, respectively ($p < 0.00001$) but not for sheep at 135 and 137 ($p > 0.5$). If a difference exists, M_m is higher than M_f . It is worth noting that human HbF at 175 is statistically significant compared to sheep HbF at 135 ($p < 0.019$).

The difference between maternal [COHb] versus [COHb] levels and between maternal versus fetal COHb half-lives in physiological or intoxicated states should be explained in another way than the difference in M on either side of the placenta. Firstly, we previously discussed the contribution of specific metabolic indices in small animals. This specificity might equally be applied to fetuses. Secondly, the rate of exchange of CO across the placenta due to the placental diffusion capacity

should be considered [78, 80]. In ewes exposed exogenously to CO, the CO diffuses slowly across the placenta with an equilibrium reached between fetal [COHb] and maternal [COHb] in 1–2 h, approaching a steady state only after 7h, when maternal [COHb] is 4.1% and fetal [COHb] is 8.4% [77, 81]. The maternal-to-fetal transfer rate of CO is slow, taking more than 30 min, and depends on the extremely low plasma proportion of CO compared to that of COHb [82]. There is an interspecies difference: the rate of fetal CO uptake averaged 0.034 mL/min in sheep and 0.0035 mL/min in dogs during the hour after exposure to CO [81]. Thirdly, physiological hyperventilation during pregnancy or the influence of endogenous production of CO must be considered.

Furthermore, after birth, there is a changeover from fetal to adult Hb, and each species challenges this adaptation in its own specific way, particularly by increasing 2,3-DPG. The 2,3-DPG does not modify M-values for fetal human Hb. We did not find any relevant studies on other fetal species [63, 65].

5.5.5. Specific CO sensitivity and M-value

5.5.5.1. Discrepancies between CO sensitivity and M-values

In 1874, Gréhant proposed keeping birds in apartments where gas was used for heating [83]. Later, he exposed birds, dogs and rabbits to CO: the birds died within 4 min, the dogs within 15 min, whereas, amazingly, the rabbits remained free of symptoms even after 20 min [84]. At the same time, Bernard already showed a faster CO elimination in rabbits [85]. Additionally, mice and birds are well known to be more sensitive to CO, as demonstrated by their use in coal mines as living alarms until 1987 [86]. At the end of the 19th century, Haldane and Krogh were the first to point out species-specific differences in M. In mice, the Hb saturation with CO was about 20 times faster than in humans [3]. Haldane calculated the M-value at 250

for humans and at 140 for mice [3, 30, 55, 87]. Killick cited a mean of 204 from Douglas's study but with unspecified pH and T° (probably 38° and a PaCO_2 level of 40 mmHg) [27]. The most recent mean M-value for mice was reported as 130, without specifying the pH and T° [26]. In our review, with a median M-value of 194 [118–278], mice, as well as rabbits, seem to have higher intraspecies variability. However, due to limited data, we cannot confirm a lower M for birds or a significant difference between M for mice and humans ($p > 0.05$). Nevertheless, a significantly lower M, compared to humans, was found for rats, presenting less intraspecies variability compared to mice ($p < 0.001$). A slower rate of blood saturation with CO was observed in large laboratory animals [24, 33, 34]. In our review, nothing allows us to demonstrate that M-values obtained from smaller animals were lower than those from larger ones. The paradox of being more sensitive to a toxic substance with a lower affinity to it was already discussed by Haldane himself [88]. Moreover, in coal mines, men have more strenuous work than mice, and CO absorption increases with activity [55, 89]. One explanation proposed for this was that small animals have a higher basal metabolic rate which favors CO absorption and causes symptoms of poisoning before gas levels become critical for humans or larger animals [3, 57]. Under normal physiological conditions, mice actually have higher rates of O_2 consumption per unit weight than larger animals and some other metabolic specificities, such as a heart rate as high as 325 bpm and a respiratory rate of 80 to 250 min^{-1} . The relationship between the respiratory exchange per unit weight and the physiological range of PaCO_2 was not constant for all species, being lower than 40 mmHg for some small animals [24, 49, 66]. Large laboratory animals, such as swine, which have a lower respiratory minute volume per unit weight, have a slower rate of blood saturation with CO. Klimisch noted that preliminary experiments on domestic swine showed no difference in M, as evaluated in vitro, compared to miniature swine [24]. In the Coburn–Forster–Kane equation (CFKE),

Coburn described the factors that influence CO absorption and excretion. In his equation, the most important species-dependent variables were the following: CO diffusion capacity ($\text{mL}/(\text{min mmHg})$), alveolar ventilation rate (mL/min), blood volume (mL) and M [90]. Furthermore, CO is also linked with myoglobin (another metalloprotein) with a lower affinity than for Hb, as evaluated at 39 [91]. The myoglobin content in skeletal muscles varies from one species to another, with a wet mass (in mg/gr) of 8 in rabbits, 1.9 in mice, and 2.1 in humans [92].

Small mammals have a relatively higher hypoxia tolerance compared to large ones. This was explained by a lower affinity of their Hb for O_2 which facilitates O_2 unloading in the tissue [57]. Among the small laboratory vertebrates, guinea pigs have the lowest sensitivity to CO, which increases successively in mice, chickens, rats and quails. Interstrain differences were observed in all these species except guinea pigs. Moreover, genetic intraspecies difference could be an important aspect when considering CO sensitivity [50, 93]. Once again, the order of different small animals and birds from highest to lowest CO sensitivity was shown to be as follows: (1) canary, (2) mouse, (3) chicken, (4) small dog, (5) pigeon, (6) sparrow, (7) guinea pig, and (8) rabbit [48]. When comparing a number of species exposed to CO, rabbit blood was found to have the lowest affinity for CO, with swine being similar to rats, and hamsters having the highest affinity [24, 33]. Thus, rabbits have the lowest M-value (≈ 110) and CO sensitivity in vertebrates. In rabbits, the CO sensitivity and M-values are concordant. Canaries proved to be most sensitive to CO in small quantities without being affected by repeated exposure and, helpfully in coal mines, these birds fall from their perch in their cage, displaying an unmistakable symptom and making them a perfect CO alarm [48].

Other studies confirmed the specificity of M-values for species, age, sex, weight, T, O_2 , and diet [57]. The COHb rate in dog blood was higher than in pig blood under the same CO exposure [22, 23]. In hamsters, the saturation equilibrium was

reached twice as rapidly as for swine [24]. Hamsters and rats showed a similarly steep rise in the COHb saturation curve. In 1975, Klimisch showed that under the same CO exposure, COHb saturation in swine was about 30% and in rabbits even 40% lower than that in hamsters [24]. Data on swine were scant, but the M-value reported in the same study for swine was higher than for dogs [22]. Finally, swine seemed to have a lower M_{37} compared to dogs. Moreover, swine seem therefore to have a similar value at 37° and 15° as sheep. In fish, all M-values found (less than 100) seemed to be lower than in other species, but there was no sound evidence for the T° conditions.

5.5.5.2. Choosing the right animal as a model

In the first part of the 20th century, dogs, widely available in animal pounds for stray animals, were believed to have a similar physiology in terms of CO behavior and M-value. Thus, they were used as a model for acute experimental intoxication. The median M-value of 215 seemed to be similar to the human median of 218 [21] (Delvau, 2018, submitted). Nevertheless, in this review, due to a lack of data, no statistical equivalence can be shown between human and canine M-values. Later, from the 1960s to the 1980s, for physiological, cardiorespiratory, technical and ethical reasons, sheep were used more frequently to analyze the kinetics of CO association and elimination after acute CO poisoning. The sheep lungs and their lack of physical activity appeared to be more closely related to those of humans and thus became a suitable model to study the effects of CO [17, 57]. Additionally, sheep Hb is believed to have a lower M for CO affinity (≈ 137) than most of the other 'large' species like dogs (≈ 215) or humans (≈ 218). Therefore, sheep can be used successively for repeated experimental poisoning; similar studies in dogs would require more time [94]. An M-value of 156, drawn from mean data, seems to be in accordance with the average from individual data, evaluated at 138. Nevertheless, the intermediate pH, partial pressure of carbon dioxide (PaCO_2), or atmospheric

variations were never investigated in sheep. In the literature, we failed to show an equivalence or statistically significant difference between human adult, sheep and other animal M-values.

A practical demonstration of the relationship between CO poisoning and the species can be made based on two recent reports. A mother, a 3-year-old child, and a bird were accidentally exposed to the same concentration of CO at the same time.

The first affected was the bird, which fell unconscious, then the child developed relevant symptoms and finally the mother [95]. Another similar story described how the 'Sudden death of a bird alerted a family to danger' [86].

Thus, in in vivo experiments, where test subjects are exposed to CO and blood is collected afterwards to be analyzed, the body mass, metabolic rate, respiratory exchange and M-value may explain the differences in sensitivity for absorption and elimination of CO. In in vitro experiments, where only the blood was exposed to the CO, only M-values and the experimental conditions, including T° and perhaps pH, should make a difference. In in vitro studies, COHb drawn from experiments at equal CO concentrations showed COHb values that were 40% lower than those found in in vivo experiments on guinea pigs, rats, hamsters and rabbits but showed a relative affinity of the same order of magnitude [24].

5.6. CONCLUSIONS AND PERSPECTIVES

This is the largest review of M-values collecting data in the animal world. Over 19 years, no new evaluations of M have been published. Haldane's constant has proved to be inaccurate for most species. In the different mammals explored, M_{37} is between 109 and 215 under physiological conditions. Sheep are the most studied species but far less commonly at a physiological T° . There is also some intraspecies variability, especially among mice. Our analysis did not provide evidence for the existence of a species-specific Hb that would approximate the human M-value sufficiently enough to propose it as a replacement for human blood in relevant or future studies.

Only T° provides evidence of influencing M, but no general T° correlation is applicable for all species. The available data are insufficient to show whether the pH influences M. In red blood cells, the 2,3-DPG concentration, atmospheric pressure, pregnancy, methemoglobin and sickle Hb do not seem to influence M. Sheep are the only species studied for fetal Hb and, in contrast to humans, their fetal Hb is similar to their maternal Hb. The sensitivity of fetuses or small animals does not result from a high Hb affinity to M for CO. In the future, animal investigations on CO need to evaluate M_{37} under physiological conditions to better fit the extrapolation of data obtained from animals to humans.

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CHAPTER 6

UNEXPECTED CARBOXYHEMOGLOBIN HALF-LIFE DURING CARDIOPULMONARY RESUSCITATION: A CASE REPORT

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6.1. ABSTRACT

Presented at BESEDIM Congress, 2015, Brussels, Belgium

Unexpected carboxyhemoglobin half-life during cardiopulmonary resuscitation: a case report

Background: Cardiac arrest (CA) following CO poisoning (CO-induced CA) exposes patients to an extremely high risk of mortality and remains challenging to treat effectively. Terminal carboxyhemoglobin elimination half-life ($\text{COHbt}_{1/2}$) is critically affected by ventilation, oxygen therapy, and cardiac output, which are severely affected conditions in cases of CA.

Case presentation: Asystole occurred in an 18-year-old woman after unintentional exposure to CO in her bathroom. Cardiopulmonary resuscitation (CPR) was started immediately, including mechanical ventilation with a fraction of inspired oxygen (FiO_2) of 1.0 and external chest compressions with a LUCAS® device. CPR was stopped after 101 min, as it was unsuccessful. During this period, we calculated a $\text{COHbt}_{1/2}$ of 40.3 min using a single compartmental model.

Conclusions: This result suggests that prolongation of CPR time needed to back COHb at 10%, a level more compatible with successful return of spontaneous circulation (ROSC), could be compatible with a realistic CPR time. Calculating $\text{COHbt}_{1/2}$ during CPR may help with decision-making regarding the optimal duration of resuscitation efforts and further with HBO₂ or ECLS. Further evidence-based data are needed to confirm this result.

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Introduction

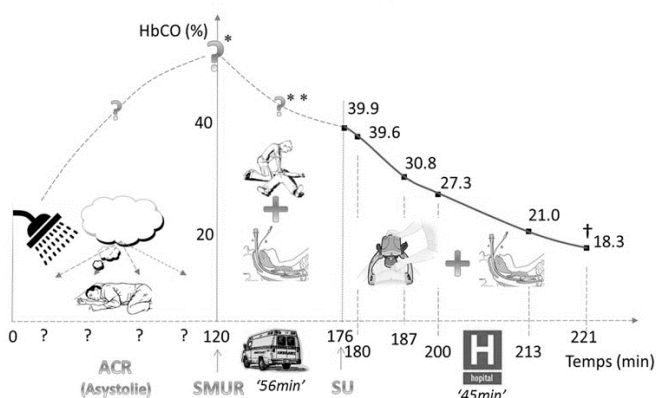
Le monoxyde de carbone (CO) est la 1^{ère} cause de mortalité par intoxication aiguë. Le pronostic après arrêt cardiorespiratoire (ACR) est très sombre, indépendant du recours à l'OHB, étroitement lié au délai de 'No Flow' et à la toxicité du CO. Le temps de demi-vie de l'HbCO (HbCO T_{1/2}) est de 5h à l'air ambiant, 80 min sous oxygène (FiO₂ ± 100%) et 23 min sous oxygénothérapie hyperbare (OHB). L'HbCO T_{1/2} pendant une réanimation cardio respiratoire (RCP) ne semble pas décrit dans la littérature.

Cas clinique

Le SMUR intervient pour une fille de 18 ans, sans antécédent, retrouvée dans sa salle de bain en asystolie après intoxication au CO via un chauffe-eau. Un massage cardiaque externe (MCE) et une ventilation mécanique (VM) (FiO₂ ± 100%) sont réalisés. Après 45 min de RCP infructueuse, le SMUR est dirigé vers notre service d'urgence où un relais par MCE pneumatique (LUCAS®) est démarré. Des gaz du sang, comprenant l'HbCO, nous ont permis de calculer l'HbCO T_{1/2}.

Toxicocinétique

Six HbCO veineux montrent un temps d'élimination, HbCO T_{1/2}, de 40,3 min.



Discussion

L'HbCO T_{1/2} est influencé par la durée d'exposition au CO, la ventilation et la pO₂. Une hémodynamique inadéquate pourrait compromettre l'élimination du CO. Ce HbCO T_{1/2} de 40,3 min est plus rapide qu'en situation hémodynamique normale et en ventilation spontanée. Il est surprenant en comparaison à d'autres résultats obtenus sous VM (92+-40min).

Cette élimination rapide de l'HbCO suggère la pertinence d'une prolongation de la RCP pour atteindre une HbCO < 10%, plus favorable à la récupération d'une activité circulatoire spontanée. Le HbCO T_{1/2} observé se rapproche de l'HbCO T_{1/2} sous OHB et ne plaide pas pour le recours à l'OHB en cas de RCP. L'OHB reste à discuter en cas de RCP, tout comme elle est controversée, voire délétère, pour l'intoxication au CO en dehors de l'ACR. D'autres prônent son intérêt dans les 24h, pour favoriser le décrochage du CO mitochondrial et diminuer la cascade inflammatoire potentiellement responsable des séquelles neurologiques.

Conclusions

Ce résultat suggère l'intérêt d'une RCP prolongée au-delà de 30 min en cas d'intoxication au CO visant une HbCO < 10%. Par ailleurs, sur base de la toxicocinétique de l'HbCO, l'OHB précoce ne serait pas indispensable pendant une RCP.

CASE REPORT

Open Access

Unexpected carboxyhemoglobin half-life during cardiopulmonary resuscitation: a case report

Nicolas Delvau^{1*}, Andrea Penaloza¹, Véronique Franssen¹, Frédéric Thys², Pierre-Marie Roy³ and Philippe Hantson⁴

Abstract

Background Cardiac arrest (CA) following CO poisoning (CO-induced CA) exposes patients to an extremely high risk of mortality and remains challenging to treat effectively. Terminal carboxyhemoglobin elimination half-life (COHbt_{1/2}) is critically affected by ventilation, oxygen therapy, and cardiac output, which are severely affected conditions in cases of CA.

Case presentation Asystole occurred in an 18-year-old woman after unintentional exposure to CO in her bathroom. Cardiopulmonary resuscitation (CPR) was started immediately, including mechanical ventilation with a fraction of inspired oxygen (FIO₂) of 1.0 and external chest compressions with a LUCAS[®] device. CPR was stopped after 101 min, as it was unsuccessful. During this period, we calculated a COHbt_{1/2} of 40.3 min using a single compartmental model.

Conclusions This result suggests that prolongation of CPR time needed to back COHb at 10%, a level more compatible with successful return of spontaneous circulation (ROSC), could be compatible with a realistic CPR time. Calculating COHbt_{1/2} during CPR may help with decision-making regarding the optimal duration of resuscitation efforts and further with HBO₂ or ECLS. Further evidence-based data are needed to confirm this result.

Keywords Carbon monoxide poisoning, Cardiac arrest, Cardiopulmonary resuscitation, Carboxyhemoglobin half-life, Case report

Background

Carbon monoxide (CO) poisoning remains the main cause of unintentional, intentional, and fire-related poisoning deaths around the world [1–3]. The overall

mortality rate is marginally affected by the level of medical care provided, with a fatality rate of approximately 3% [4]. Commonly identified factors that are strongly correlated to a higher mortality rate include concomitant cyanide intoxication, loss of consciousness (LOC), COHb levels > 25%, arterial pH, and the need for endotracheal intubation. Moreover, the mortality rate of CO-induced CA may be as high as 100% [5]. However, in certain cases, some patients with CO poisoning could benefit from more aggressive or prolonged treatment [6].

Current evidence shows that oxygen (O₂) therapy reduces blood carbon monoxide elimination half-life (COHbt_{1/2}), but its influence on neurological outcome is inconsistent [7–11]. Despite a lack of high-level evidence, hyperbaric oxygen (HBO₂) therapy is recommended by

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6.2. BACKGROUND

Carbon monoxide (CO) poisoning remains the main cause of unintentional, intentional, and fire-related poisoning deaths around the world [1–3]. The overall mortality rate is marginally affected by the level of medical care provided, with a fatality rate of approximately 3% [4]. Commonly identified factors that are strongly correlated to a higher mortality rate include concomitant cyanide intoxication, loss of consciousness (LOC), COHb levels > 25%, arterial pH, and the need for endotracheal intubation. Moreover, the mortality rate of CO-induced CA may be as high as 100% [5]. However, in certain cases, some patients with CO poisoning could benefit from more aggressive or prolonged treatment [6].

Current evidence shows that oxygen (O₂) therapy reduces blood carbon monoxide elimination half-life (COHbt_{1/2}), but its influence on neurological outcome is inconsistent [7–11]. Despite a lack of high-level evidence, hyperbaric oxygen (HBO₂) therapy is recommended by many scientific societies for severe CO poisoning [12–15]. There are currently no specific recommendations that deal specifically with cardiac arrest (CA) following CO poisoning (CO-induced CA) [16–18]. A review conducted in 2001 concluded that there was an extremely poor survival prognosis for CO-induced CA, with or without HBO₂ [5]. To our knowledge, there have been no more recent publications on this topic, and none of the studies mentioned COHbt_{1/2} during CPR. COHbt_{1/2} is affected by ventilation, oxygen therapy, and cardiac output, all of which are conditions severely impaired by CA [11, 19]. As such, we investigated COHbt_{1/2} during prolonged CPR in a case of CA following unintentional CO exposure.

6.3. CASE PRESENTATION

An 18-year-old woman with no previous medical history was unintentionally exposed to carbon monoxide (CO) from a water heater at home and was discovered unconscious on her bathroom floor. According to her family, she had gone into the bathroom 1 h earlier. The mobile emergency and resuscitation service (SMUR) found her pulseless in asystole and CO poisoning was confirmed via a bedside CO detector (alarm without a displayed value) (Fig. 1). Advanced cardiac life support (ACLS) was applied at home by the emergency physician according to European Resuscitation Council (ERC) guidelines [20]. Intubation was immediately performed on site and mechanical ventilation was initiated with a fraction of inspired oxygen (FiO_2) of 1.0 for the entire duration of resuscitation (Fig. 1). Following evidence of unsuccessful CPR after 30 min with sustained asystole, the decision was made to transport the patient to a tertiary referral hospital while ongoing CPR was performed.

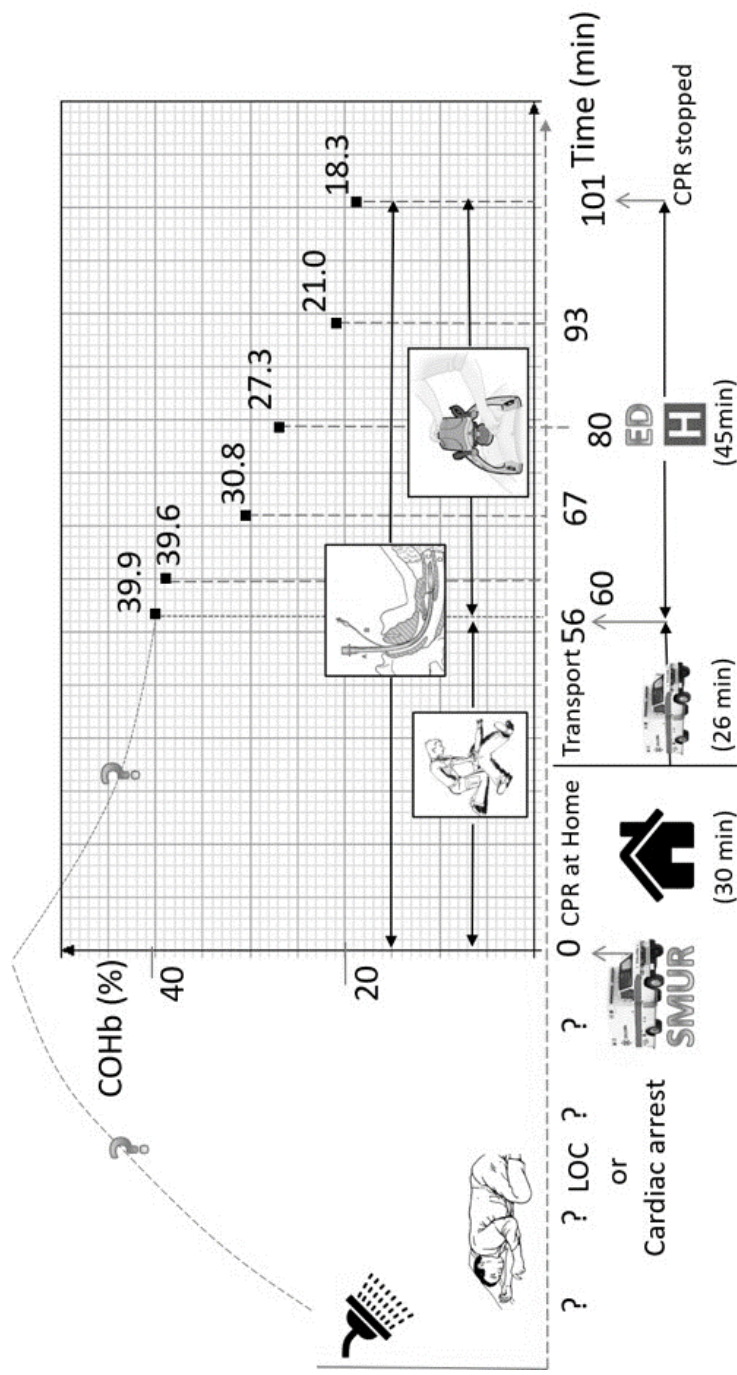


Fig. 1: Timeline of our case report on cardiac arrest following CO poisoning and COHbt_{1/2}. LOC, loss of consciousness; CPR, cardiopulmonary resuscitation; LUCAS® external chest compression device for the last 45min; SMUR, mobile emergency and resuscitation service.

CPR was continued when the patient arrived at the referral hospital. During CPR, repeated arterial blood gas analysis was performed using a point-of-care ABL90Flex® blood gas analyzer (Radiometer™, Radiometer Medical Aps, Denmark). Our secondary focus for the six blood samples analyzed during CPR was arterial COHb. In total, the unsuccessful resuscitation efforts lasted for 101 min. The patient was ventilated with a FiO₂ of 1.0 during this period and chest compressions were performed with an external LUCAS® thoracic compression device (Fig. 1) during the last 45 min of CPR. Assuming that the elimination of CO followed a single compartmental model, we calculated a COHbt_{1/2} of 40.3 min from the six data points [11].

6.4. DISCUSSION AND CONCLUSIONS

In this patient with CO-induced CA, we observed a $\text{COHbt}_{1/2}$ of 40.3 min during CPR with external chest compressions and controlled mechanical ventilation with an FiO_2 of 1.0. This was much lower than expected and closer to the figure reported with HBO_2 therapy with spontaneous breathing [11, 21–24]. This finding suggests that, in cases of CO-induced CA, the duration of resuscitation may be extended up to the conventional recommendation of 30 min. This is with an empirical objective of reaching a COHb level lower than the 10% typically found in smokers and considered as a non or low COHb level toxicity [20, 25].

For patients with spontaneous breathing with normobaric oxygen therapy and a non-rebreathing mask, $\text{COHbt}_{1/2}$ is 74 ± 25 min [26 to 148] [11]. For normobaric oxygen therapy, Levasseur et al. reported a $\text{COHbt}_{1/2}$ of 92 ± 40 min in fire victims with controlled ventilation with a FiO_2 of 1.0 [21]. Conversely, with HBO_2 therapy, the $\text{COHbt}_{1/2}$ is estimated to be around 23.3 min or even > 43 min [22, 26]. However, these values were assessed in the absence of severe cardiopulmonary dysfunction and may be underestimated in cases of CA [26]. It seems that no other case report regarding $\text{COHbt}_{1/2}$ in a pure CO-poisoning and CA patient has been published. The reasons for this unexpectedly short CO half-life during CPR merit discussion. First, we can hypothesize that it is, at least partly, due to early intubation and mechanical ventilation with a FiO_2 of 1.0 and sustained external chest compressions. Second, $\text{COHbt}_{1/2}$ in clinical practice is typically quoted on normobaric oxygen therapy at $74 \text{ min} \pm 25$ [26–148], which reflects a very wide range that includes our low value [11]. In fact, the elimination time of exposure to CO for a short duration may enable lower CO diffusion to extravascular compartments. For a short duration at high-level exposure, $\text{COHbt}_{1/2}$ is shorter than

a long duration at low-level exposure [27]. In addition, $\text{COHbt}_{1/2}$ may be slightly shorter for females due to total hemoglobin and muscle myoglobin mass [28, 29]. Furthermore, despite the fact that we applied the ERC guidelines [20], arterial pH was less than 7.0 during CPR. With this acidity level, we suspect a lower affinity of Hb for CO, even if we did not confirm this hypothesis in a recent meta-analysis [30].

High COHb levels are a marker of exposure but unrelated to clinical presentation or outcome [14]. Even in the absence of strong evidence, we suggest that COHb levels should be monitored regularly during CPR with the objective of reaching COHb levels lower than 10% before deciding to stop CPR. Indeed, COHb levels lower than 10% are frequently observed in smokers and are considered to be non-toxic [25]. This target could be reached within a reasonable period compatible with ROSC [31]. However, CO binds to cardiomyoglobin and to cytochrome-c oxidase, which interferes with cellular respiration [32, 33], but is not available in clinical practice to estimate the CO body burden. Thus, the CO burden impact could be prolonged beyond %COHb back to 10% [27, 34].

Unfortunately, the patient did not survive. We stopped CPR without reaching the COHb level at 10% because the “no flow” before CPR was considered too long to hope for a CPR success. This adverse outcome is not surprising [5]. The current survival rate of all-cause out-of-hospital CA is less than 10% [35]. In cases of CO-induced CA, brain injury is worsened by the absence of O_2 delivery and by the other biochemical mechanisms of CO toxicity [36]. After O_2 supply therapy, mitochondria respiration inhibited by CO is restored more slowly compared to mitochondria simply deprived of oxygen [5]. These mechanisms lead to consider HBO_2 therapy for CO-induced CA. Hampson et al. reported that 100% of the directors of HBO_2 therapy medical centers would recommend HBO_2 therapy for extremely

unstable CO poisoning, predicting a 74% chance of survival after HBO₂, and a 28% chance of complete recovery without neurologic sequelae [5]. However, in Hampson's case series, the mortality rate among 18 patients with CO-related CA treated by HBO₂ was 100% [5]. Indeed, patients who developed CO-induced CA rarely survived, even if a return of spontaneous circulation (ROSC) was achieved [37]. No study considers the COHb% threshold in a protocol to assess the chances of survival. Ideally, HBO₂ therapy should be initiated as soon as possible in the first 6 h. Six hours corresponds to the "golden hour" that could decrease the incidence of delayed neurological sequelae from CO poisoning [38]. Unstable patients requiring multiple vasoactive drugs are not easily transferred safely to an HBO₂ chamber. Despite this, some authors suggest speeding up the transfer to HBO₂ chambers due to the fear of impending cardiac arrest [39]. It should be noted that patients who require chest compressions, intubation, or positive pressure ventilation during their resuscitation are at an increased risk of an HBO₂-induced tension pneumothorax [39]. On the other hand, extra-corporeal life support (ECLS) has been applied with success in some selected cases as during cardiovascular failure but not after cardiac arrest [40–42]. ECLS was not pursued because in our Center ECLS protocol is only considered in cardiac arrest with a certainty of less than 10 min "no flow". It appears that ECLS and HBO₂ may have complementary effects: ECLS improves oxygenation to target organs independently from heart and lung failure, while HBO₂ is more effective in ensuring high O₂ dissolution in brain and heart cells [43]. The 2015 guidelines of the European Resuscitation Council concluded that there is an unproven benefit of transporting patients with ROSC to an HBO₂ therapy facility and that such a decision must be considered on a case-by-case basis [37]. Transfer to a tertiary referral hospital could be partly motivated by the possible consideration of organ donation [44, 45].

The vast majority (88%) of patients who achieved sustained ROSC after CA from any cause did so within 30 min of CPR, and CPR longer than 20 min after asystole is generally accepted as futile [46]. However, in specific cases of presumably reversible causes (airway obstruction, hypothermia, poisoning, etc.), the optimal CPR duration remains a case-by-case bedside decision that may, for CO-induced CA, take into account the rate of CO elimination [46, 47]. Interestingly, in Hampson's fatal CO poisoning retrospective review, the duration of CPR ranged from 19 to 45 min [5]. The lack of information about the duration of the "no flow" period could also affect the physician's attitude to the prolongation of CPR. The difference between patients with signs of life before they go into cardiac arrest and those already found with no pulse is an important factor to consider in assessing hypoxic brain injury. Indeed, we only found two clinical descriptions of patient survival with good neurological recovery for patients that underwent mechanical ventilation and hypothermic therapy, without HBO₂ therapy. Both patients presented with CA in the emergency department (ED). ROSC was achieved after a few minutes without a "no flow" period [48–50]. The determination of the "no flow" period appears to be particularly difficult in patients with CO poisoning as a loss of consciousness may precede CA and may be erroneously considered an absence of cerebral blood flow. In our opinion, CPR should be initiated in any case of CO-induced CA and extended when circulatory collapse or CA is witnessed by responders. CPR could be prolonged until a COHb rate < 10% is reached, a level expected to allow ROSC occurrence, especially if the CO body burden is suspected to be low in case of short CO exposure time.

In our case report of CO-induced CA with prolonged CPR including controlled mechanical ventilation at a FiO₂ of 1.0, we observed a COHbt_{1/2} much lower than expected. This result suggests that prolonged CPR may lead to a COHb level which

could be compatible with successful ROSC. Calculating COHbt_{1/2} during CPR may help in decision-making regarding the optimal duration of resuscitation efforts and further therapy with HBO₂ or ECLS. Further evidence-based data are needed to confirm this result.

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CHAPTER 7

EARLY CARE MANAGEMENT AFTER
CARBON MONOXIDE POISONING BASED ON
CARBOXYHEMOGLOBIN TOXICOKINETIC

DISCUSSION
PERSPECTIVES
AND
CONCLUSIONS

7.1. INTRODUCTION

The previous five chapters were the foundational part of this thesis. Those chapters addressed the five research questions and examined whether the hypotheses we have specified appear to hold true.

We will draw attention to the strengths and limitations of our five investigations and the precautions to be taken when interpreting our results. We take up elements of the discussion from the published articles to connect them together and integrate some supplemental unpublished data.

All this discussion naturally leads to future perspectives and a new study design. We suggest some hypotheses to be drawn from our findings and some recommendations. While some hypotheses may be considered premature at this stage for immediate application in clinical practice, we provide relevant arguments for investigating them in future studies.

The final conclusion restates our contribution to the evolution of knowledge in this field.

7.2. RESEARCH QUESTIONS AND ANSWERS

7.2.1. Research Question 1

Question: What is the COHbt_{1/2} on NIV (NiPPV or CPAP) compared to NRM on NBO? In our study on CO intoxication in swine, the closed NIV system gave the shortest COHbt_{1/2} (median 58 vs. 85 min with NRM). We discourage the use of CPAPb for this indication demonstrating a COHbt_{1/2} at 82min. A review of the literature shows no evidence of a significant positive effect with more conventional CPAP. The only certain positive effect with conventional CPAP is the optimization of FiO₂ by using the hermetic mask in comparison with the NRM.

Aim: To identify if NIV (NiPPV or CPAP) could be an effective first-care treatment after CO exposure considering only the COHb elimination. The results of our pilot study suggest that NiPPV can be more efficient than NRM at eliminating CO when HBO is unavailable or delayed, and supports the design of a clinical study to evaluate this hypothesis.

Secondary aim: To identify any adverse effect of NiPPV or CPAP applied after acute CO exposure on a pilot study. We have not identified any side-effects attributed specifically to NIV. Variations in pH and capnia, which remained within acceptable physiological limits, were attributed to the necessary sedation of animal protocol.

Hypothesis: As NiPPV or CPAP improved ventilation and oxygenation in other acute respiratory diseases, providing positive pressure and better sealing in order to improve FiO₂, we expected a COHbt_{1/2} improvement compared to NRM. NiPPV improves COHbt_{1/2} compared to NRM, but the part attributed to the mask's airtightness, the removal of potential hypoventilation attributable to sedation or a positive effect of intrinsic positive airway pressure needs to be confirmed clinically.

7.2.2. Research Question 2

Question: Does COHb toxicokinetic fit with a mono- or a bi-compartmental model? COHb half-life is biphasic and thus follows a bi-compartmental model.

Aim: To make statements around the current controversy between experimental and clinical studies. The argument of mono- vs bi-compartmental COHb elimination observed is rather methodological. We come down in favour of the bi-compartmental profile. We encourage a systematic evaluation of this COHbt_{1/2} from several COHb venous samples during treatment, including very early samples from front-line ambulances.

Secondary aim: The bi-compartmental model is an argument in favour of CO storage. If this model is confirmed, it allows us to compare COHbt_{1/2} for the first and second toxicokinetic phases in studies of treatment efficiency. Moreover, the proportion from one phase to the other could reflect CO storage, severity of exposure, or treatment efficiency. In addition, we seek to highlight clinical practice in NBO duration, currently estimated to range from 6 to 12h or longer. Potential extravascular CO storage is inaccessible in clinical practice. Muscle biopsies were done during the intoxication and detoxification phases of our protocol but were impossible to assess for analytical reasons. Bi-compartmental elimination is an argument in favour of CO storage. Moreover, COHb being bi-compartmental, we show that it is possible to assess the two elimination phases separately, as we assessed on NRM with COHbt_{1/2}- α at 56.2 and COHbt_{1/2}- β at 70.0, compared to NiPPV-L with COHbt_{1/2}- α at 23.6 and COHbt_{1/2}- β at 174.7 in minutes respectively - but also their COHbt_{1/2}- α /COHbt_{1/2}- β ; α -cst/ β -cst; A/B ratio. We show that COHbt_{1/2} assessment using mono- or bi-compartmental models impacts the results with COHbt_{1/2} at 85 for NRM and 58 for NiPPV-L in minutes respectively with mono-compartmental analyses. Even if the hypothesis is relevant in view of our

results, we have not been able to demonstrate in practice that the proportion from one phase to the other could reflect CO storage and/or CO severity poisoning.

Hypothesis: COHb toxicokinetic fits with a bi-compartmental model; in clinical practice we must consider this model, assessing COHbt_{1/2} to improve NBO duration. Treatments compared could have different efficiency depending on whether phase - α or - β is considered. COHb half-life is biphasic and thus follows a bi-compartmental model. We are now able to stress the importance of early and regular blood sampling to confirm this toxicokinetic profile in clinical studies. The ultimate goal will be to assess whether this precisely-estimated COHbt_{1/2} could be a criterion of CO poisoning severity, and to discuss case by case NBO duration or oxygen therapy modalities. According to this toxicokinetic COHb profile it might be wise to continue NBO up to 12h if COHbt_{1/2} is estimated to be longer than the current reference at 74 min. Distinguish COHbt_{1/2}- α and COHbt_{1/2}- β provides toxicokinetic data which could be helping to differentiate treatment efficiency.

7.2.3. Research Question 3

Question: Is there a %COHb rebound after COHb backed at 5% and NBO cessation? We observe for the first time a slight COHb rebound when COHb returns to 5% and oxygen therapy is stopped.

Aim: To argue in favour of delayed CO release from extravascular CO storage as COMb. Potential extravascular CO storage being inaccessible in clinical practice, and our muscles biopsies analyses being unsuccessful, we could consider this second toxicokinetic profile in favour of extra vascular CO storage.

Secondary aim: To argue in favour of the multi-compartmental toxicokinetic model. To emphasise the importance of establishing pertinent duration after CO exposure. To be more cost-effective regarding the in-hospital monitoring duration. COHb rebound is an argument for the multi-compartmental model. Rebound extent needs to be assessed in other CO exposure protocols before being confirmed and to claim any precise CO storage correlation. While waiting for further studies to emerge, we suggest that a NBO treatment lasting 6 hours for any case of moderate CO poisoning doesn't seem futile.

Hypothesis: There is a COHb rebound after NBO stopped up to 5% in favour of the multi-compartmental toxicokinetic model. This rebound is expected to be moderate because the extra vascular CO storage itself is estimated at 15%. A COHb rebound occurred when COHb returns to 5% and oxygen therapy is stopped. This rebound is slight, but this could be explained by a CO exposure duration design proposed in our study of less than 5 hours. Therefore, a COHb rebound must be confirmed in clinical practice or under other experimental procedures. Nevertheless, this rebound is too systematic and too significant to be ignored or attributed to endogenous CO production. To this toxicokinetic COHb profile, while waiting for further studies to emerge, it might be wise to continue NBO beyond the

time necessary to reach a %COHb level of 5% - as long as COHbt_{1/2} does not start a normal decrease under AA.

7.2.4. Research Question 4

Questions: What are the accurate M-values for humans and animals? What is the impact of species and various physiological conditions on M? Our meta-analysis and systematic review provide up-to-date data with human M-value at 37°C at 218 $\pm$ 8 at any pH. Moreover, we processed the largest review of M-values data in the animal world. Animal M-values are inaccurate for most species. In the different mammals explored, M-value is between 109 and 215 under physiological conditions. Only temperature appears to influence M.

Aim: To estimate the time required for CO intoxication and elimination to develop the study protocol. To extrapolate results from our experimental swine study to humans. M-values reported for swine are very limited. The value found is 130. This validates our swine model for the planned design of our protocol, with an expected procedure duration (intoxication 'phase I' and detoxification 'phase III') compatible with the laboratory's opening hours.

Secondary aim: To confirm or improve the M-value accuracy to avoid error in determining COHb from Haldane's equation. To find the best animal blood model matching humans for M value. Our M-value reviewed at 218, and not at 240 as often reported in literature, and should become the reference used in CO studies. In mathematical modelling, this difference seems to modify the %COHb reached, or COHbt_{1/2} expected, but only slightly. Our analysis did not provide evidence for the existence of a species-specific Hb that would approximate human M-value sufficiently to suggest it as a replacement for human blood in relevant, or future studies.

Hypothesis: M-value for humans is similar to mammals. M-value differed according to physiological conditions, explaining the discrepancies in previous studies. M-value in the animal world was thoroughly investigated. It was found

that the M-value for swine is almost half of the M-value for humans, but swine M-value is insufficiently studied. Only temperature appears to influence M. The available data are insufficient to show whether the pH influences M. The 2,3-DPG concentration, atmospheric pressure, pregnancy, methemoglobin and sickle Hb do not seem to influence M. Most M-values on animals have been evaluated at non-physiological temperatures, making extrapolation to humans difficult. M discrepancies comes from intraspecies variability and variable physiological conditions.

7.2.5. Research Question 5

Question: What is the COHbt_{1/2} during CPR on CO-induced CA? In a case report of CO-induced CA with prolonged CPR including controlled mechanical ventilation at a FiO₂ of 1.0, we observed a COHbt_{1/2} much lower than expected at 40.3min.

Aim: To assist in decision-making during CPR in CO-induced CA management. If COHbt_{1/2} is allowed to reach infra-toxic COHb level in a time close to realistic CPR time, CPR could be continued beyond standard recommended CPR time. This result suggests then that prolonged CPR may lead to a COHb level which could be compatible with successful ROSC. Calculating COHbt_{1/2} during CPR may help in decision-making regarding the optimal duration of resuscitation efforts and further therapy with HBO or ECLS.

Hypothesis: COHbt_{1/2} being perturbed by alveolar ventilation, alterations in gas exchange efficiency, or variability in cardiac output, COHbt_{1/2} during CO-induced CA is expected to be largely prolonged compared to COHbt_{1/2} on NBO and spontaneous cardiac rhythm, estimated in literature at 74min. This is not the situation in our case report, with a COHbt_{1/2} observed at 40.3min compared to humans unaffected by cardiac arrest where the standard reference is 74min.

7.3. STRENGTHS AND LIMITATIONS

In this important part, we now draw attention to the strengths and limitations of our five investigations and the precautions to be taken when interpreting our results. We link elements of the discussion from the published articles with additional unpublished data.

7.3.1. On research question 1

For ethical reasons, it is no longer possible to study CO in human models (1). The animal model and the number of animals we investigated have obviously limits but it allowed us to demonstrate the feasibility of the technique and produced encouraging results in favour of NiPPV on human (Fig. 1, 2 and 3).

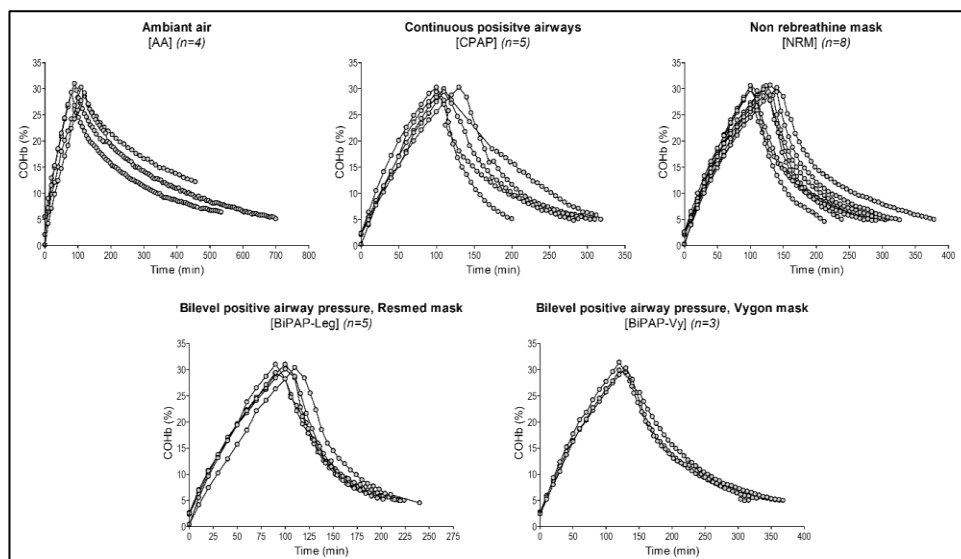


Fig. 1: Toxicokinetic profile of COHb for each treatment group. Atmospheric air (n = 4), CPAPb (n = 5), NRM (n = 8), PSV(BiPAP/NiPPV)-Leg (n = 5), and PSV(BiPAP/NiPPV)-Vy (n = 3) (2). The last phase IV is not illustrated in this figure.

The breed of swine investigated has a cardiopulmonary physiology and weight relatively similar to humans and we performed 25 experimental procedures over 23 days on eight swine. In addition, treatments were randomized (3). In terms of pure toxicokinetic, our sampling was done frequently over very accurately-measured periods, with over a thousand analyses. The radiometer blood gas analyser was calibrated every day at the beginning of the procedure by the technician. All biostatistical analyses were performed by biostatisticians specialized in toxicokinetic.

All the interventions (NRM, CPAPb, NiPPV-Vy, and NiPPV-Leg) were superior to the reference group (AA) in terms of COHbt_{1/2} ($p < 0.001$). However, there was no statistically significant difference between the intervention groups, with a trend only between NiPPV-Leg (58 min, $n = 5$) and NRM (85 min, $n = 8$) ($p = 0.18$) (2).

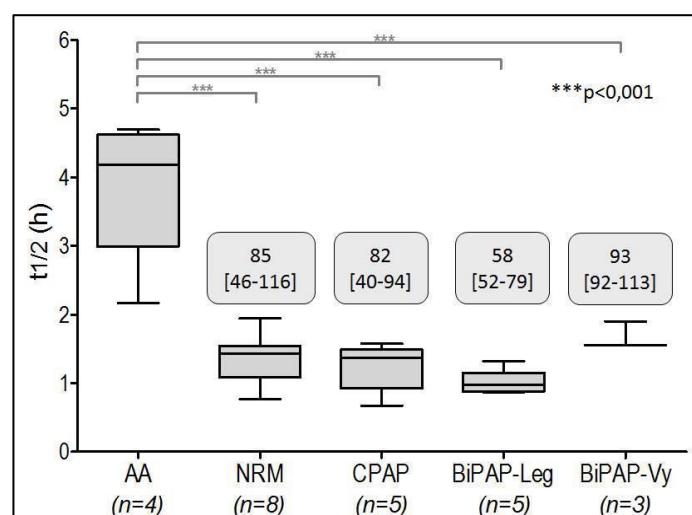


Fig. 2: COHbt_{1/2} (min) compared with box plots from following Fig. 3 (2).

To verify this trend for NiPPV-Leg despite the lack of statistical power, we therefore completed the analysis of the results by estimating the AUCs for each treatment (Fig. 3: Additional unpublished box plots). The median AUCs for $\ln(\text{COHb}) \times \text{time}$ (h) were 170, 79, 83, 100, and 64 for AA, NRM, CPAPb, NiPPV-V, and NiPPV-L, respectively, with a statistically significant difference only between AA and PSV-Leg ($p = 0.002$).

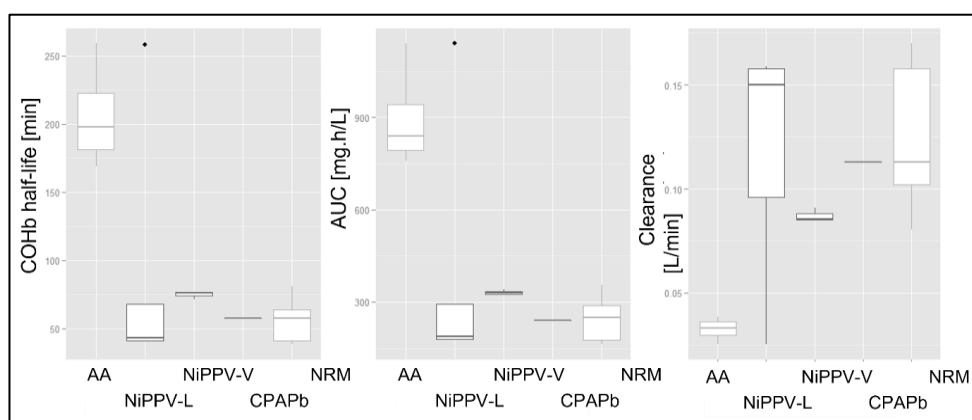


Fig. 3: $\text{COHb}_{1/2}$ (min); AUC (mg.h/l); CO Clearance ($\text{l} \cdot \text{min}^{-1}$) related to each treatment.

Moreover, we also checked that %COHb level between each phase (I to IV) of the protocol for all investigations to be comparable (Fig. 4).

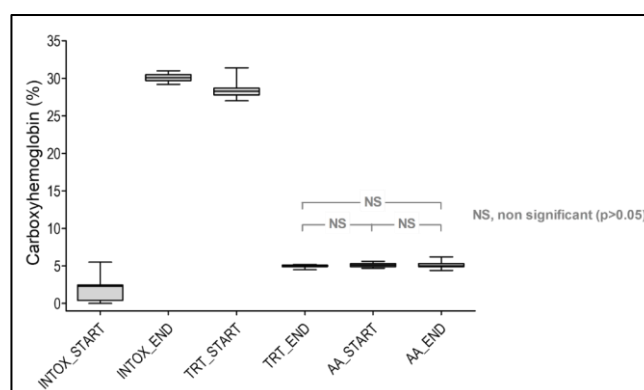


Fig. 4: %COHb level between each phase of the protocol for all investigations are comparable (unpublished graphic).

In addition, we assessed the duration of treatment to back to COHb 5% (TRT Time to reach 5%). Once again, NiPPV-Leg showed better performance in term of TRT time to reach 5% (Table 1).

	NRM (<i>n</i> =8)		CPAPb (<i>n</i> =5)		NiPPV-Leg (<i>n</i> =5)		NiPPV-Vy (<i>n</i> =3)	
TRT time (min)	168	[102-228]	168	[90-210]	114	[102-120]	222	[210-228]
COHbt_{1/2} (min)	85	[46-116]	82	[40-94]	58	[52-79]	93	[92-113]
FiO₂ (%)	72	[59-83]	54	[49-73]	92	[76-93]	51	[51-53]
pCO₂ (mmHg)	43.3	[39.2-52.5]	47.8	[46.5-48.3]	44.6	[39.4-48.6]	40.6	[37.1-46.7]
pO₂ (mmHg)	369.4	[230.1-418.2]	281.0	[234.0-441.2]	475.0	[323.4-478.6]	286.2	[247.1-300.5]
VE (ml.min ⁻¹)	6539	[5587-10312]	7418	[6788-8460]	9726	[9377-11404]	8096	[6695-8602]

Table 1: Median [range] respiratory parameters on swine during different NBO treatments. Minute ventilation (VE) was the best for NiPPV-Leg and, moreover, measurements for delivered O₂ therapy were the highest in the NiPPV-Leg group in terms of median FiO₂ ($p < 0.05$ when compared to NRM, CPAPb, and NiPPV-Vy). In comparison, for paO₂ point, paO₂ on AA-treated pigs (control group) after CO exposure was checked being 87,39 SD±13,19 for a standard up 80 to 100 mmHg.

In addition, we provide four schematized procedures (AA-treated, NRM -treated and two NiPPV-treated procedures) with supplemental data where %COHb evolution and the respiratory parameters Vt, paCO₂ and pH are overlapped to better visualize the interconnection of their variations, see Appendix (Fig. 1 to 4) (unpublished design).

These three approaches, COHbt_{1/2}, AUC and 'TTT time' are therefore in favour of NiPPV. Nevertheless, on analysis of these results in favour of NiPPV-Leg, several limitations are identified, and others approaches of minimising them are put forward.

- A. What is the sedation impact on CO absorption and elimination?
- B. The highest (VE) for NiPPV-Leg (Table 1) is a real ventilation improvement or only a hypoventilation sedation-induced compensated by the non-invasive ventilation?
- C. Does COHbt_{1/2} improvement on NiPPV come from the positive effect of FiO₂ only or possibly combined with positive effect on positive pressure?
- D. For financial and logistical reasons, together with the ethics surrounding large mammal use, the number of swine investigated was limited. Obviously, in our swine model, the strength of the statistical analysis was limited by the small number of animals used in each intervention sub-group. This may explain why the difference observed between the NiPPV-Leg group (n = 5) and the NRM group (n = 8) lacks statistical evidence. After confirming bi-compartmental COHbt_{1/2} elimination, are we able to develop new toxicokinetic strategy to address this ethical limitation and to strengthen our previous results?

A. What is the sedation impact on CO absorption and elimination?

Our most important limitation was the obligation to sedate the pigs with a view to limiting their motor activity and to accept non-invasive interfaces, possibly impacting absorption and elimination COHb toxicokinetic. As explained in the *Methods* part, we provide the most minimal sedation possible, using continuous Propofol infusion. During CO absorption (Intoxication phase I), we expected the impact of significant sedation on ventilation to increase the time to reach COHb 30% target. This should be added to the expected increase in absorption time, attributed to the lower M value on swine. Nevertheless, the time required to reach 30% COHb in our sedated swine model on 940ppm CO exposure was relatively similar (± 110 min) to the human data in the literature and to the predicted CO uptake of CFKE on unsedated resting humans (Fig. 5).

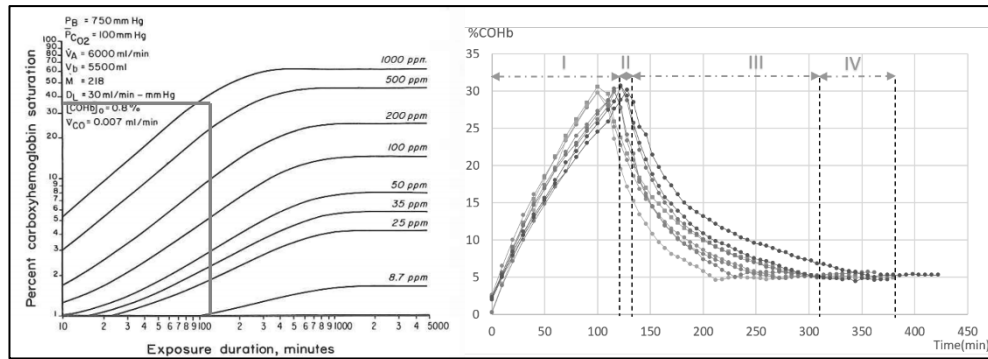


Fig. 5: (Left) COHb at $\pm 35\%$ expected with the CFKE human model after ± 110 min CO exposure compared with our COHb% level observed in our swine model study at 30% on the same conditions (Right).

Similarly, under significant sedation, the $\text{COHbt}_{1/2}$ during phase III treatment should be longer, whereas on NRM it is found to be ± 85 min, i.e., relatively similar to the $\text{COHbt}_{1/2}$ at 74 min Weaver reference in humans treated with NRM. Nevertheless, the smaller M-value for swine during the elimination phase, which should reduce $\text{COHbt}_{1/2}$, could be offset by the negative impact of sedation, which increases $\text{COHbt}_{1/2}$.

At the end, these two considerations, on absorption and elimination durations, lead us to suggest that our sedation, titrated to a minimum, would have had little impact on the ventilation of our swine model.

B. The highest (VE) for NiPPV-Leg (Table 1) is a real ventilation improvement or only a hypoventilation sedation-induced compensated by the non-invasive ventilation?

We have just highlighted that '*Time to reach 30%*' and '*return to 5%*' are similar to humans on NBO (NRM) without sedation. We are therefore examining other arguments to suggest that sedation has only slight impact on ventilation. First, COHbt_{1/2} on our control group (n=3) AA is at 273 (SD 28 min), similar on human without sedation. Second, at no time, irrespective of the treatment, was apnoea observed despite a close visual monitoring with administration of titrated Propofol ranged from 1,5 to 3 (mg.kg⁻¹).h⁻¹. Third, minute ventilation (VE) during Phase I and Phase IV on NRM or NiPPV are stable despite sedation increasing (Fig. 6). Fourth, another way to estimate the impact of sedation on ventilation, was to compare respiratory parameters during our procedures on sedation to normal physiologic ventilation rates on swine. Physiologically respiratory parameter for human being 6 to 8ml.kg⁻¹, tidal volume (Vt) extrapolated for non-sedated 77kg swine is 540ml ((±7ml.kg⁻¹) x (±77kg)) and minute ventilation (VE) is therefore estimated at 6480ml.min⁻¹ (540ml x RR at 12min⁻¹). In comparison, (VE) during our non-treatment phases are very similar, i.e. for NRM, phase I was 6500 (SD1381) on Propofol 151 mg.h⁻¹ and (VE) during phase IV was 6175 (SD697) on Propofol 160 mg.h⁻¹ (Fig. 6).

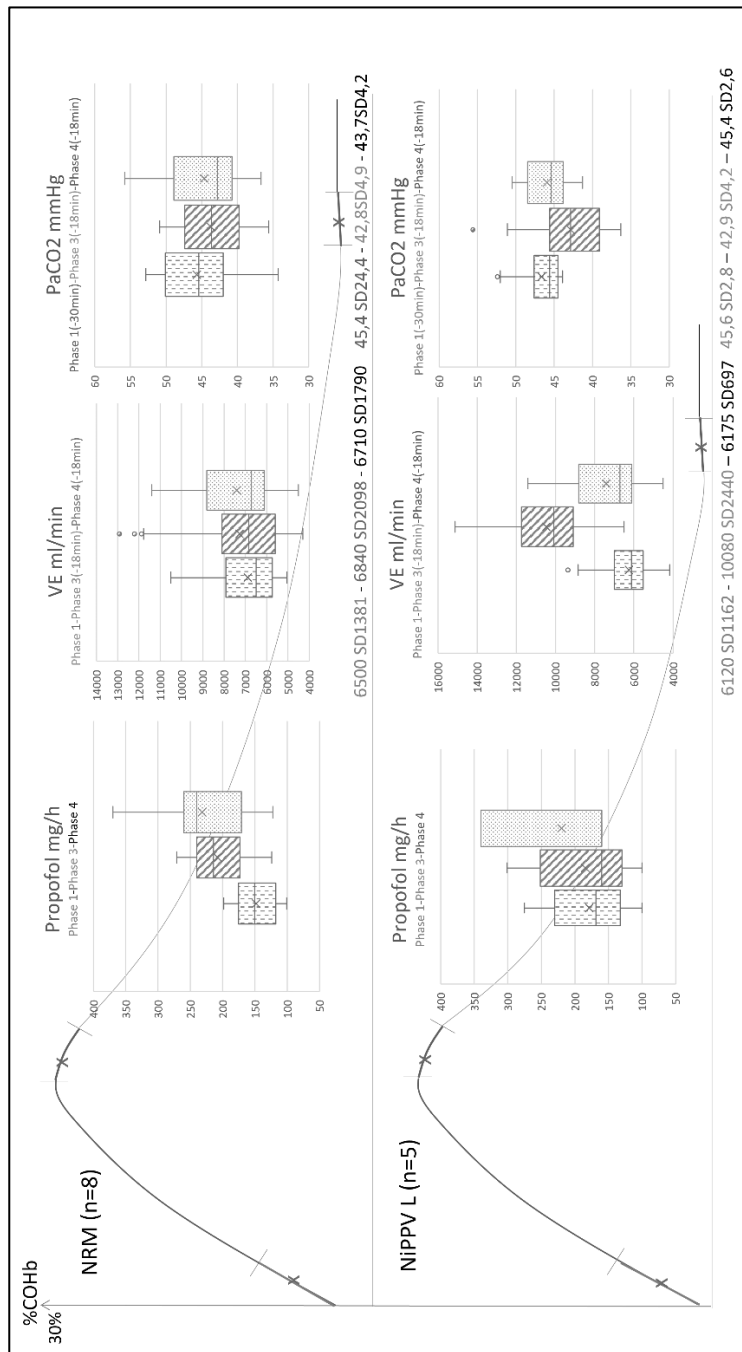


Fig. 6: Evolution of sedation, minute volume (VE) and PaCO₂ parameters during phase I, III and IV of NRM compared with NiPPV-Leg. In order to consider only stable respiratory values, in phases III and IV we did not take into account the first 18 min of adaptation following the fitting or removal of the respiratory device when estimating the (VE). Similarly, in order to consider only stabilised PaCO₂ we did not consider the first 30 min of phase I, the first 18 min of phase III and the first 18 min of phase IV.

As our swine maintain a PaCO₂ close to physiological values despite the sedation, we considered that the baseline ventilation of the pig is consistent with a (VE) at $\pm 6500 \text{ ml} \cdot \text{min}^{-1}$ and that if the protocol using Propofol had caused hypoventilation, it was only very slight. What we can only observe is that compared to humans, the respiratory rate of swine is faster and its Vt less, but with at the end a similar (VE) being $V_t \times \text{RR}$.

In addition, only a few clinical experimental studies have evaluated adverse respiratory effects of CO exposure in relatively small numbers of healthy subjects (4, 5). Results of these studies indicate that brief CO exposure to relatively higher levels (e.g., $\geq 500 \text{ ppm}$) may decrease ventilatory performance. In retrospect, we concluded in Chapter 1 that unlike CO₂, CO had very little impact on ventilatory parameters. If this was the case, NiPPV should bring a benefit on COHbt_{1/2}.

We monitored the paCO₂ level in real time. While observing PaCO₂ upper limits at 44,6mmHg, we were tempted to increase the NiPPV pressure levels, fixed in our protocol at PS 10mmH₂O and PEP 2 to 4mmH₂O, which had resulted in an increase of even more COHbt_{1/2} but we refrained to avoid a potential respiratory discomfort to patients not presenting respiratory distress in the real life.

In the appendix we provide electronic supplemental data on our swine study published: heart frequency; systolic and diastolic blood pressure; carboxyhemoglobin; hemoglobin; pH; lactate; temperature (Table 1; Fig. 5) (2). We present unpublished data on lactate values (Fig. 6 and 7), and we provide box plots for individual respiratory data on NRM and NiPPV-Leg (Fig. 9 and Fig. 10) with their medians ($\pm \text{SD}$) in Tables 2 and 3.

C. Does COHbt_{1/2} improvement on NiPPV come from the positive effect of FiO₂ only or possibly in combination with positive effect on positive pressure (alveolar recruitment, clearance of dead space, increase in Vt or proportionate decrease in respiratory frequency)?

Our study investigated for the first time NiPPV on animals; the very principle of NIV is the preservation of spontaneous breathing which we wanted to respect as much as possible. This is the strength of our study, but simultaneously its limitation. Indeed, to make it possible we had no other choice than to sedate the pigs in order to accept the setting of the O₂ therapy with NIV hermetic interfaces. Before that, we had to catch the swine in its cage. The swine was premedicated with the usual sedative dose through intramuscular injections of Zoletil®100, Virbac© (6 mg.kg⁻¹) being a combination of Tiletamine (dissociative anaesthetic similar to Ketamine) and Zolazepam (benzodiazepine looking like Diazepam) with a duration of action up 20 to 60min and low respiratory repercussions. This is directly associated with another intramuscular injection of an analgic, sedative and myorelaxant, Rompun® 2%, Bayer© (2 mg.kg⁻¹), being Xylazin, a α-2 adrenergic receptor agonist, with duration of action also up 20 to 60 min. This allows the swine to be placed on the procedure table, to set up a first venous access for the Propofol infusion of a sedative dose (1.5 to 2mg.kg⁻¹) and to start the neck arterial access surgery. This preliminary procedure before starting the intoxication phase lasts ±2 hours. Therefore, no side-effect of the pre-medication on ventilation is expected, in contrast to a continuous Propofol infusion which might slightly decrease normal ventilation during the four phases assessed. The target was a sedation level just to the level of what was required to accept the procedure, adjusted progressively to be as minimal as possible, but modified in case of eyelash reflex response, spontaneous movements, stability of heart rate and blood pressure or respiratory asynchrony. Our choice of Propofol allowed us to sedate on-and-off, and to adjust

the dosage for the lightest-possible sedation. Ketamine, known for its low respiratory impact, was an alternative, perhaps reducing potential hypoventilation. However, even if there had been less respiratory depression with Ketamine, it would still have been difficult to distinguish the lifting of this ventilatory depression from the positive effect of increased FiO_2 .

First, measurements for delivered O_2 therapy were the highest in the NiPPV-Leg group in terms of median FiO_2 ($p < 0.05$ when compared to NRM, CPAPb, and NiPPV-Vy) (Table 1). We checked that from the specific FiO_2 provided, it results P_{O_2} in blood gas analyses itself correlated to total CO exposure on the different treatments (Fig. 7 and 8). Median P_{O_2} values were significantly higher in the NiPPV-Leg group when compared to the CPAPb group ($p < 0.05$).

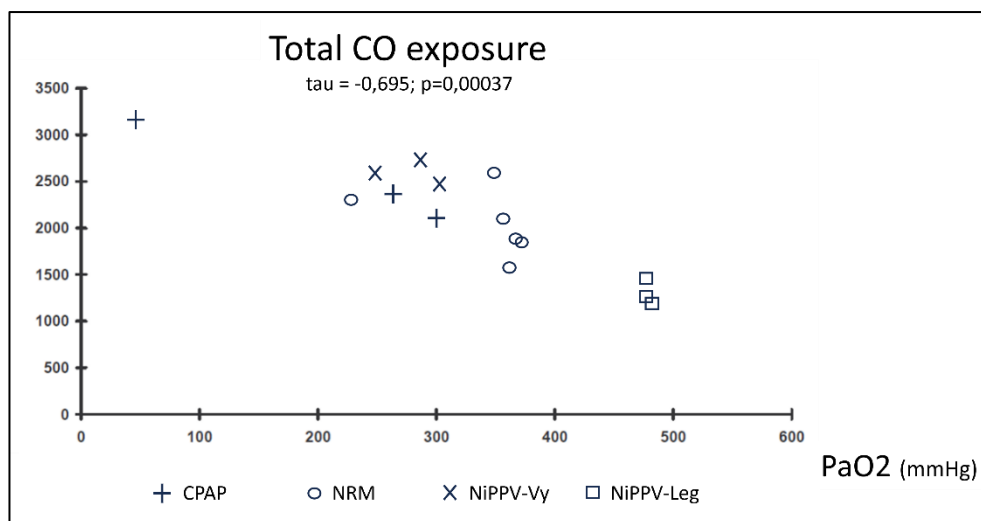


Fig. 7: In this graphic, pO_2 observed is related to the amount of total CO to which the pig is exposed (additional graphic not published). CPAP on extreme left is an outsider due to a technical problem during the procedure.

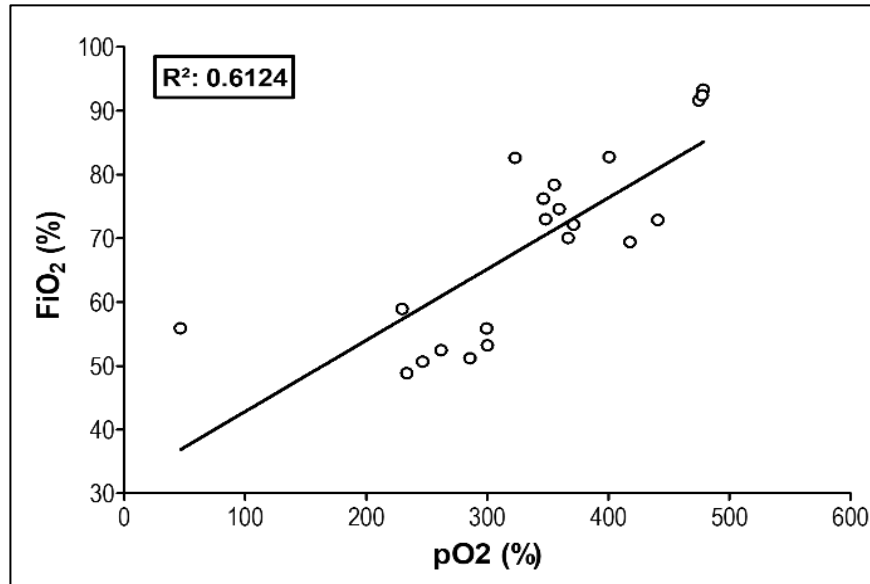


Fig. 8: P_{O2} seems to be correlated with effective FiO₂ delivered.

CPAP Boussignac and NiPPV on the specific model (NiPPV-Vy), being both open system and delivering 51% and 54% FiO₂ respectively, are to be abandoned for suspected or confirmed CO poisoning cases. Moreover, on CPAPb, clinically, the sedated pig's breathing seemed uncomfortable as is confirmed by the worst PaCO₂ observed at 47.8 mmHg (Table 1).

Second, as seen in Table 1bis, comparison of the results observed under NRM (no pressure delivered) and CPAPb (providing positive pressure) reveals that TRT time, COHbt_{1/2} and (VE) are relatively similar. However, delivered FiO₂ and resulting PaO₂ differed more significantly, suggesting that a positive effect on COHbt_{1/2} from positive pressure could compensate for the lower FiO₂ delivered by CPAPb compared with NRM.

	NRM (n=8)	CPAPb (n=5)	NiPPV-Leg (n=5)	NiPPV-Vy (n=3)
TRT time (min)	168 [102-228]	168 [90-210]	114 [102-120]	222 [210-228]
COHbt_{1/2} (min)	85 [46-116]	82 [40-94]	58 [52-79]	93 [92-113]
FiO₂ (%)	72 [59-83]	54 [49-73]	92 [76-93]	51 [51-53]
pCO₂ (mmHg)	43.3 [39.2-52.5]	47.8 [46.5-48.3]	44.6 [39.4-48.6]	40.6 [37.1-46.7]
pO₂ (mmHg)	369.4 [230.1-418.2]	281.0 [234.0-441.2]	475.0 [323.4-478.6]	286.2 [247.1-300.5]
VE (ml.min ⁻¹)	6539 [5587-10312]	7418 [6788-8460]	9726 [9377-11404]	8096 [6695-8602]

Table 1bis: Median [range] respiratory parameters on swine during different NBO treatments. TRT time: time of treatment; COHbt_{1/2}: elimination half-life carboxyhemoglobin; FiO₂: fraction of inspired oxygen; pCO₂, carbon dioxide partial pressure; pO₂: oxygen partial pressure; VE: minute ventilation; NRM: high concentration non rebreathing mask; CPAP: Continuous Positive Airway Pressure device; NiPPV-Leg: Non-Invasive Positive Pressure Ventilation device – AIROX®; NiPPV-Vy, Non-Invasive Positive Pressure Ventilation device – VYGON®.

Third, if the beneficial effect of NiPPV-Leg is only due to the improvement in FiO₂ provided by the hermetic mask, there should be a correlation between the total amount (litre) of oxygen delivered ($\Sigma (VE \times FiO_2)$) and their COHbt_{1/2} during phase III treatment by comparing each pig individually in the same treatment group. Analysing Tables 2, we note that this correlation does not exist.

For NRM, 'Babe' for example, who received above-average total O₂, had the worst COHbt_{1/2}. Or, 'Leo' received the highest total O₂ and was the best ventilated but only had an average COHbt_{1/2}. It cannot therefore be concluded that COHbt_{1/2} is directly correlated with total O₂ administered. For NiPPV, all the (VE) are increased

except for 'Bru' who surprisingly remains in the lead in terms of COHbt_{1/2}. 'Marty' had more O₂ and ventilated better under NiPPV but still had the same COHbt_{1/2} as under NRM. Experimental archives reported that he woke up at the end of phase III. We cannot rule out better results without this wake-up period. For the others, once again there was no clear correlation between the quantity of O₂ administered and COHbt_{1/2}. We therefore suggest that oxygenation is not the only determinant of COHbt_{1/2}. Furthermore, we note that a classification of performance on COHbt_{1/2} is consistent: pigs who are performing well on NRM are also performing well on NiPPV. This may also be due to individual compliance with sedation or to inter-individual specificities.

NRM (n=8)			
NRM (PHASE 3)	COHbt _{1/2} (min) NRM	Total O ₂ litres admin	VE ml/min
BRU	46	125122	10230 SD608
MARTY	52	102011	5880 SD1132
TIRE	78	124136	6300 SD794
TOTO	82	151173	7295 SD648
LEO	89	211503	11100 SD1233
DOMI	92	136204	6840 SD880
PEGGY	92	146846	5640 SD860
BABE	116	156897	5395 SD733
	85 SD23	141525 SD 32419	6840 SD2098
Σ (VE * FiO ₂) during NRM			
0,72 [0,59-0,83]			

NiPPV L (n=5)			
NiPPV L (PHASE 3)	COHbt _{1/2} (min) NiPPV L (n=5)	Total O ₂ litres admin	VE ml/min NiPPV L
BRU	52	133528	9800 SD242
MARTY	53	146506	8100 SD1006
TIRE	58	169942	11270 SD4355
PEGGY	58	200492	11900 SD 630
BABE	79	184278	10140 SD 711
	58 SD 11	169942 SD27245	10080 SD2440
Σ (VE * FiO ₂) during NiPPV			
0,92 [076-0,93]			

Tables 2: Individual COHbt_{1/2} values; Total O₂ administered and VE (ml.min⁻¹) for each individually named pig for the NRM and NiPPV-Leg groups. No clear correlation between the total O₂ administered and COHbt_{1/2} are observed.

D. The strength of the statistical analysis was limited by the small number of animals used in each intervention sub-group. After confirming bi-compartmental COHbt_{1/2} elimination on research questions 2 and 3, **are we able to develop new toxicokinetic strategies to address this ethical limitation and to strengthen our previous results?** We will address this question D in the following point 7.3.2 on research questions 2 and 3.

To end the discussion on research question 1, the final aim of CO poisoning treatment being to avoid DNS, we wanted to study the release of neuronal proteins into the blood. S100 β or NSE proteins and Copeptin are aspecific neuronal biomarkers that have been investigated to assess the level of severity of potential brain injury in cerebral trauma, stroke or CO poisoning. No study has reported convincing results regarding the evaluation of CO poisoning severity that may lead to recommendations. Our experimental protocol allowed the realization of more than 300 S100 β samples. Unfortunately, the preliminary analyses of these samples failed to detect S100 β . One of the reasons for this would be the moderate severity of our CO intoxication protocol administering 940 ppm for 110min. However, it could be that the antibody used was unsuitable, or because of the difference between animal and human S100 β ? Another avenue of investigation was to measure these same proteins in cephalorachidian fluids. However, lumbar puncture in pigs requires special expertise. We worked on the first test pig with the head of the anesthesia department to validate the technique, but the pig developed motor neurological disorders of the hind legs the following day. We have therefore abandoned these two lines of evaluation in our protocol comparing different NBO treatments.

7.3.2. On research questions 2 and 3

It is currently impossible to directly measure the CO content of muscle tissues non-invasively in human subjects (6). Our animal model allowed us to attempt an invasive evaluation with muscle biopsies taken throughout our procedure to estimate the relationship between COMb and COHb. We froze these biopsies for five years while waiting for the development of an adequate and affordable analytical technique, but unfortunately, COMb measurement in muscle biopsy is currently more the domain of forensic medicine. Indeed, if a victim is found burnt in a fire with no COHb or COMb detected, it is strongly suspected that the cause of death was something other than the fire or fire-smoke. Nevertheless, after consulting toxicologists or forensic physicians from Lausanne, Strasbourg and Liege on this issue, the size of the biopsies they analyse is much larger than ours in order to obtain a signal. In our protocol, we took around 40 samples a day (x 3-4 days for each i.e., 160 biopsy pieces each), and therefore requiring biopsies of the order of a few grams. Forensic scientists, in contrast, only need a few - but much bigger - samples. Another limitation was the excessive cost of analysis. We therefore abandoned this track after five years of freezing and destroyed the samples.

Anticipating the possibility of this analytical impasse, as Bruce has done with his mathematical model, we investigated two other potential non-invasive avenues, accessible in clinical practice, which could be a reflection of CO storage, such as late COHb rebound after cessation of treatment and the demonstration of a bi-compartmental modality of COHb removal, which are both the first steps that we hope to validate in order to motivate further studies in this direction of research. The ultimate hypothesis would be that the analysis of half-life times under bi-compartmental elimination modality could reflect a quantity of CO storage, which could itself be a CO-severity criterion.

Using the toxicokinetic of a xenobiotic to assess the unknown dose ingested and the severity of intoxication is already commonly proposed in cases of acetaminophen (Paracetamol) overdose, based on the Rumack-Matthew nomogram. This nomogram makes it possible to decide whether to start specific treatment with N-acetyl cysteine to prevent severe hepatic sequelae. The strategy we would like to propose is very similar.

Starting the thesis, we hypothesise that indirect total CO storage estimation (including COMb and not only COHb) could be allowed - firstly, to consolidate the results of our study into the possible benefits of one of the treatments, and, secondly, to test the relevance to future assessments of the correlation between $\text{COHbt}_{1/2}$, clinically accessible, and the extravascular CO storage. The aim is to challenge severe CO poisoning identification criteria in further studies which have to compare cohorts of the same CO poisoning severity. Very few studies have focused on $\text{COHbt}_{1/2}$ and/or rebound in relation to CO exposure conditions (and not only in relation with the treatment), while the pathophysiology of CO still remains incompletely understood. Nevertheless, our point of view and some elements in the literature converge in thinking that CO poisoning severity could be related to the CO exposure level, and especially the duration. A prolonged exposure to relatively low concentrations of CO may produce a large tissue-load of CO with low COHb, whereas a short exposure to a high concentration can produce a low tissue-load (less severe) and a high COHb (7). The $\text{COHbt}_{1/2}$, and not %COHb, being one of the reflections of the modality of this exposure, it could reflect the CO severity poisoning. Using data from our principal study, through rigorous sampling, we have first observed a slight rebound which remains to be confirmed clinically or by other experimental designs (as i.e., longer CO exposure duration). If it comes from CO storage, this rebound could argue in favour of a bi-compartmental distribution. Secondly, we demonstrated the first step required for future studies that will

explore the correlation between $\text{COHbt}_{1/2}$, CO storage and clinical impact: the bi-compartmental model is to be used! In contrast to our research question 1, the advantage of these two research answers is that sedation has no expected impact on the analysis or interpretation of our results. In addition, in the absence of conclusive studies on optimal NBO duration, these two toxicokinetic features are arguments for continuing NBO for at least 6h.

For this, once again, we focused on the most specific biomarker confirming CO poisoning: the COHb. While an isolated value does not predict the CO poisoning severity, we explored its kinetics. It is now possible to analyse the relationship between $\text{COHbt}_{1/2}$ and the CO severity poisoning without delay if early venous sampling are considered prospectively in future studies, from out-of-hospital initial care and during emergency room management - i.e. during the first two hours of care - in sufficient numbers.

It should be noted that we looked at the CO elimination bi-compartmental analysis in a second stage of the thesis. The first analysis published in the swine principal paper used a mono-compartmental analysis of $\text{COHbt}_{1/2}$ (by calculating the slopes of the semi-log decay phases to obtain the terminal elimination constant) which highlight the $\text{COHbt}_{1/2}$ discrepancies between mono- and bi-compartmental assessment using the same data (Table 3; Fig. 9).

Mono- or Bi-compartmental COHb_{t1/2} toxicokinetic

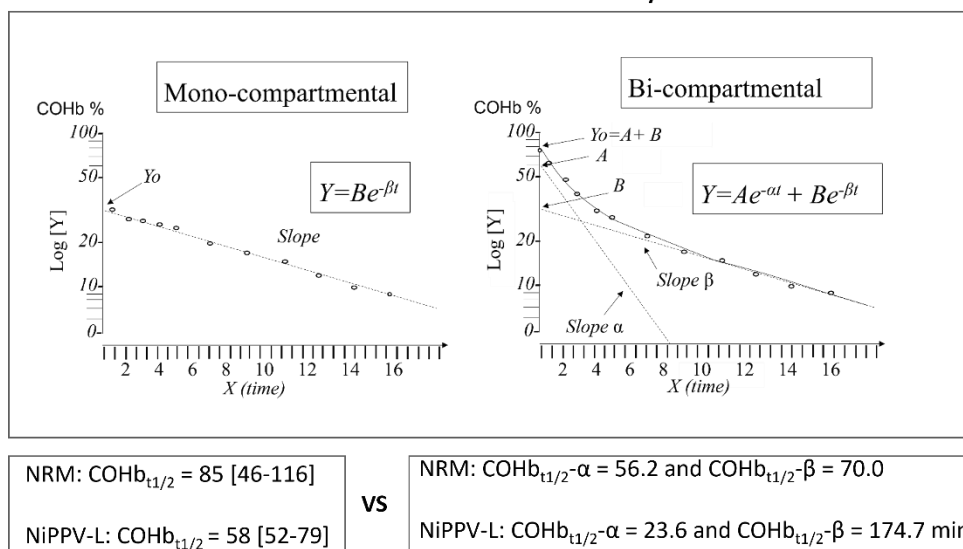


Fig. 9: Comparison of the COHb_{t1/2} calculated from our experimental data, considering a mono- or bi-compartmental toxicokinetic model.

To illustrate the methodological origin of the controversy between the mono- and bi-compartmental model or the COHb_{t1/2} discrepancies we mentioned, we present an example based on our own data. By calculating the COHb_{t1/2} using a mono-compartmental model and using all the samples (COHb_{t1/2}=0.693/Slope) for our AA control group (n=4) we obtained the following results: 281min; 228min; 273min and, surprisingly, 130min for one of them. If we examine the details of the procedure corresponding to the 130min COHb_{t1/2}, we can realise that for logistical reasons we had to stop the experiment after 90min COHb washout, whereas the other three experiments lasted from 6 to 10 hours. For this COHb_{t1/2} at 130 min, we therefore probably only collected early samples corresponding to the α- phase, ignoring the slower elimination β-phase, which explains this discrepancy (Fig. 10).

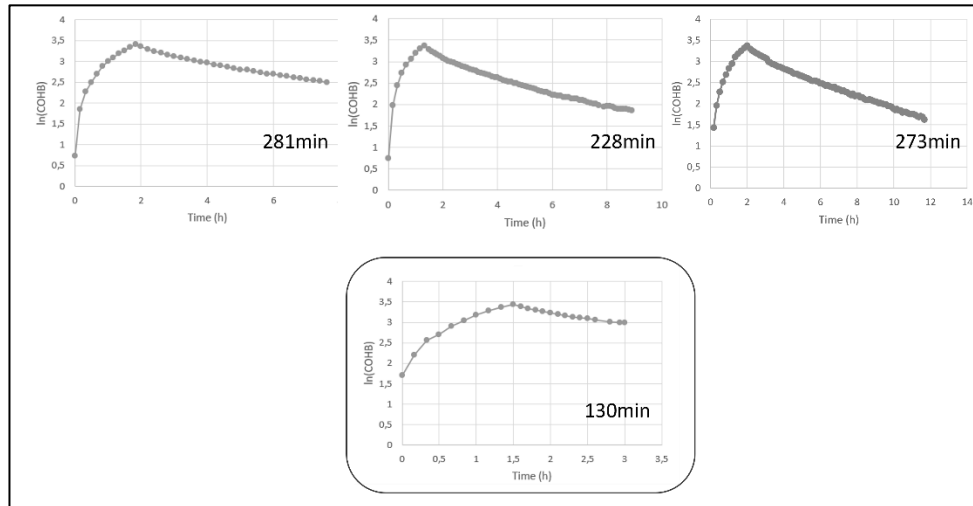


Fig. 10: COHbt_{1/2} at 250min (SD69) obtained from our experimental data on the ambient air control group (n=4). Using a mono-compartmental model with samples taken every 6 min.

Therefore, new detailed analysis performed using the bi-compartmental model allowed us to compare separately individual parameters for all α -phase (first rapid elimination) and all β -phase (second slow elimination) on each treatment with different toxicokinetic approaches, trying to confirm results suggesting NiPPV-L benefit on COHbt_{1/2} (2). This hypothesis is suggested, for example, in a study comparing COHbt_{1/2} NBO-treated on NRM or on High Flow Nasal Cannula (HFNC), a difference was observed to be only statistically significant at 60 min, i.e., in the early phase (8). It seems to be relevant to split phases analyses. So we tested several toxicokinetic approaches (Fig. 9; Table 3) as COHbt_{1/2}- α (min) and COHbt_{1/2}- β (min); their α -cst and β -cst respectively; 'A' and 'B' (the y-axis intercept for the α -phase or β -phase) corresponding to the decay (in % of initial total COHb) of COHb during the first or secondary phase respectively; AUC_{0-t} (Area Under the Curve) which reflect the total exposure to COHb during time to reach 5%; ratio α -cst/ β -cst or 'A'/'B' expressing the relative importance of α -phase over β -phase; time-to-reach 5% as

the extrapolated time (min) to reach COHb backed accurately at 5% using slopes (Table 3). We compare all this data on multivariate analyses with canonical graph. At the end we apply a Tukey-Kramer HSD test to avoid increasing α mistake.

	NRM		CPAP	NiPPV Vy		NiPPV Leg	AA	All	P-value
COHb_{t1/2 from} (*)	85 [46-116]	82 [40-94]	93 [92-113]	58 [52-79]	251 [130-273]				0.18
COHb_{t1/2}-α	56.2 ±16.2	34.03 ±13.5	26.9 ±5.0	23.6 ±3.9	41.6 ±5.7			39.4 ±6.2	0.39
COHb_{t1/2}-β	70.0 ±22.1	99.6 ±17.3	120.6 ±10.0	174.7 ±63.8	310.5 ±23.5			141.8 ±22.4	0.0004
α-cst	0.022 ±0.0055	0.0032 ±0.0084	0.028 ±0.0047	0.0032 ±0.0042	0.018 ±0.0032			0.026 ±0.0027	0.4524
β-cst	0.026 ±0.0079	0.0081 ±0.0021	0.0058 ±0.00053	0.0053 ±0.0013	0.0023 ±0.00015			0.012 ±0.0034	0.06
A	15.7 ±0.8	16.0 ±3.4	12.3 ±1.8	21.6 ±2.0	6.6 ±0.4			15.1 ±1.2	0.001
B	12.7 ±1.0	15.1 ±1.4	17.5 ±1.7	8.7 ±1.2	21.2 ±1.1			14.5 ±1.0	0.0001
A/B ratio	1.33 ±0.18	0.90 ±0.20	0.73 ±0.15	2.56 ±0.64	0.32 ±0.036			1.21 ±0.19	0.0013
AUC_{0-∞}	2614.8 ±231.2	2716.3 ±368.0	3505.0 ±173.0	2263.6 ±340.4	9946.5 ±1032.6			3844.8 ±579.1	<0.0001
AUC_{0-t}	1849.1 ±169.3	2041.9 ±312.6	2661.2 ±144.5	1292.7 ±42.0	6574.5 ±525.4			2629.9 ±376.0	<0.0001
Time to reach 5%	163.2 ±15.3	168.7 ±22.4	217.1 ±5.9	110.15 ±4.1	647.9 ±64.0			237.7 ±38.7	0.0001

Table 3: Comparison between different toxicokinetic approaches on different O₂ therapy modalities compared to AA. (*) median terminal COHb_{t1/2} (min) using non-compartmental analysis in our previous study in NiPPV-L favour (58min, n=5) and NRM (85min, n=8) but with a lack of statistical power (p=0.18) (2).

No significant difference was observed for $\text{COHbt}_{1/2-\alpha}$, suggesting that distribution of CO in extra vascular compartment seems to occur at the same place whatever the treatment - even if, once again, there is a positive trend in favour of the NiPPV-L. In addition, the phenomenon of CO distribution in the initial α -phase (Table 3) seems to be more dominant than elimination by exhalation, regardless of ventilation and/or FiO_2 provided. One might think that this α -phase depends mainly on exposure characteristics and not on the treatments administered, but we cannot demonstrate this on the homogeneous CO poisoning study design we have used. Nevertheless, by focusing on the associated A-constant reflecting the y-axis intercept for the α -phase that NiPPV-L, CPAPb- and NRM-treated swine, it was significantly higher when compared to AA-treated, with COHb decreasing of 21.6%, 16%, 15.7% respectively during α -phase compared to 6.6% for AA ($p=0.0004$; 0.03 and 0.02 respectively).

As expected, $\text{COHbt}_{1/2-\beta}$ was higher in the AA-treated group when compared to every other treatment group, while $\text{COHbt}_{1/2-\beta}$ for NiPPV-L is higher compared to other but probably to washout a lesser residual %COHb level. The corollary is the B-associated constant significantly lower in the NiPPV-L treated group when compared to AA, NiPPV-V and CPAP ($p<0.0001$; 0.003; 0.02 respectively) but this difference was not significant when compared to NRM treated group ($p=0.13$). This observation reflects a smaller contribution of the β -phase when compared to the α -phase for %COHb decrease in this NiPPV-L group of swine (8.7% vs 21.6%, $p=0.02$). For other groups of treatment, the differences between A- and B-constants are not significant except for AA-treated pigs for which B-constant is significantly higher than A-constant (21.2% vs 6.6%, $p=0.002$). So, to highlight this relevant toxicokinetic point of view we computed the ratio of A/B to reflect this relative importance of both elimination phases (Fig. 11). As a reminder, just-inhaled

free CO has a relatively low association constant with Hb (K_a), compared with its dissociation constant (K_d). This property could mean that a specific treatment could provide more benefit in one phase than in another. In our case, it could be suggested that NiPPV-L may be recommended in further studies as soon as possible from start on the scene and for $\pm$ one hour along the α -phase. Afterwards, the benefit could be non-significant and NRM could be provided. Nevertheless, to date, no clinical or case report on humans treated with NiPPV has been published to confirm this hypothesis. In the future, each phase may indeed deserve a different treatment.

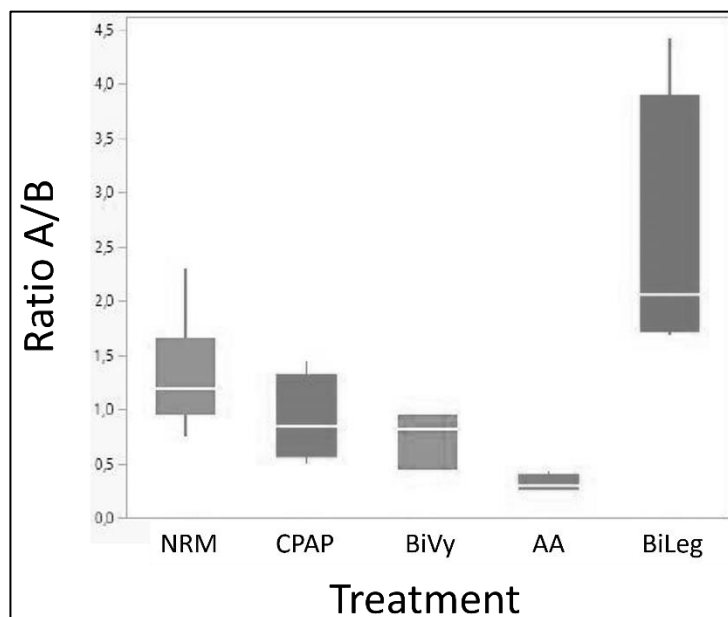


Fig. 11: Ratio A/B for each treatment. The post-hoc analysis revealed that NiPPV-L-treated pigs had a significantly higher ratio than every other group, corroborating the observation that the α -phase contributes more to elimination of COHb in NiPPV-L-treated swine than in every other groups.

This observation in favour of NiPPV efficiency is supported by the fact that the ‘time to reach 5%’ COHb was the shortest in NiPPV-Leg-treated [110.15min], despite not reaching significant difference with other treatment groups on NRM [163.2min] ($p=0.55$); on CPAP [168.7min] ($p=0.55$) and on NiPPV-Vy [217.1min] ($p=0.15$), except with the AA group which had significantly longer time values than every other groups of treatment (Table 3). This could be due to the high variability in data.

In addition, supporting these differences, once again, on the second analysis we also observed similar differences when comparing AUC_{0-t} values which reflect the total exposure to COHb during time to reach 5% (Fig. 12).

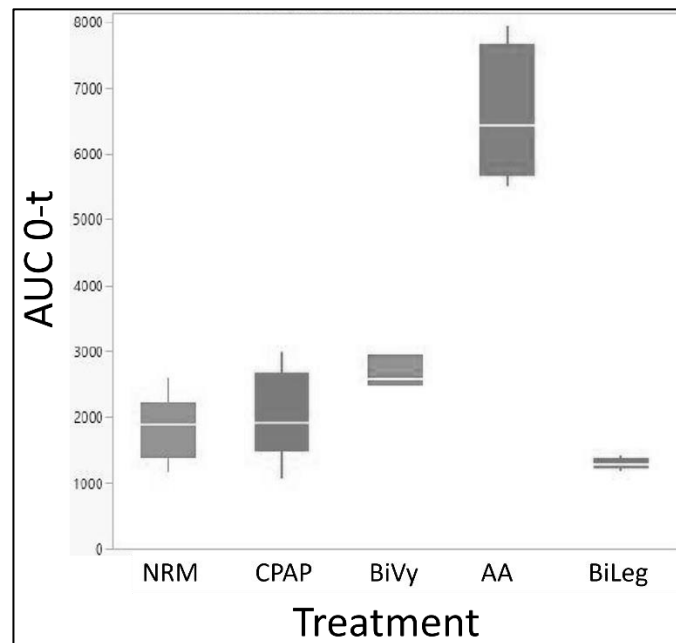


Fig. 12: ‘ AUC_{0-t} ’ with ‘t’ corresponding to the last sample reaching COHb at 5% for each treatment.

And lastly, to combine all information from Table 3, we performed a clustering discriminant analysis pooling all parameters (Fig. 13).

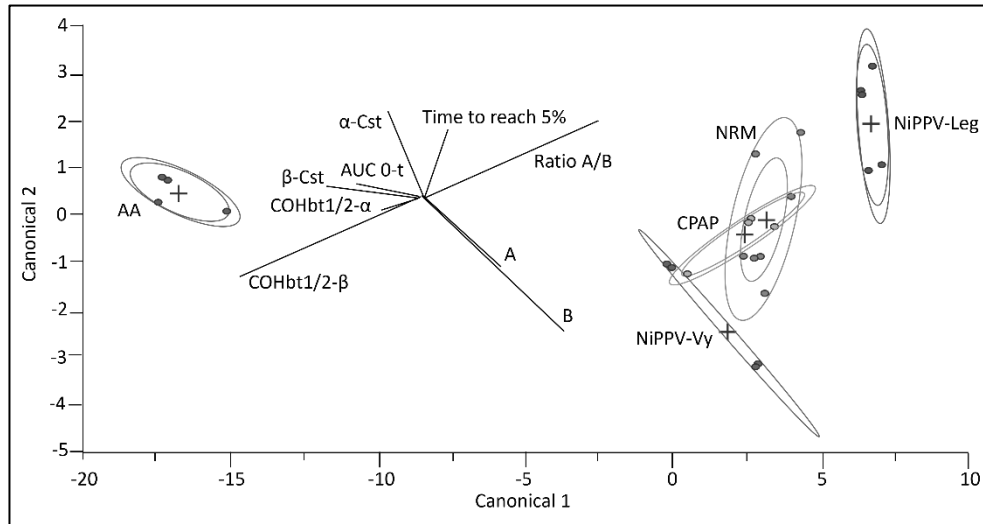


Fig. 13: Clustering discriminant analysis pooling all parameters from *Table 3* using canonical graph. It clearly appears that AA treated group is well discriminated. On the same line, the NiPPV-Leg group was also apart (rightmost) and showed a relatively extraordinary profile when compared to NRM, CPAPb and NiPPV-Vy. AUC_{0-t} is the most discriminative parameter followed by the A- and B-constant. Other parameters were less discriminant.

Although several publications suggest poor correlations between the blood COHb level and the severity of CO poisoning (9), it is well established in clinical practice that COHb should be eliminated as expediently as possible and, according to certain criteria, on HBO (10). Shimazu suggested, for example, that toxicity of CO would be better represented by the area under the COHb elimination curve ($\sum (\text{COHb} \times \text{duration})$) (13).

Limitations to the use of HBO are the availability of the HBO chambers, the controversy surrounding HBO effectiveness in avoiding delayed neurological syndromes, and the possible induction of noxious reactive O₂ species stress (11, 12). Therefore, we investigated other ways of clearing COHb as NiPPV (2) using different ventilatory devices and a new toxicokinetic approach that should be challenged as a reflection of the severity of intoxication. As we observed, a bi-compartmental model could allow to highlight treatment efficiency compared to others, not only COHbt_{1/2} α- or β-, but also other toxicokinetic parameters (Table 3) which could provide another perspective. We confirmed, with the new assessment provided by our swine study, our suggestion that clinicians use NiPPV to increase COHb elimination compared to NRM or CPAPb (Fig. 1 to 3). Multivariate analyses with canonical graph (Fig. 13 from Table 3) show that, whatever the toxicokinetic approach, NiPPV-L is the best treatment. However, we are not able to show significance due to too much variability and insufficient experimenting to decrease this variability (unpublished analyses).

7.3.3. On research questions 4 and 3

We have spent a lot of time developing the two Haldane constant M-value reviews, with the certainty that these values would be essential to the interpretation of our results. Little literature is devoted specifically to the M-value and the values reported are often secondary elements drowned in the studies' discussions. We reviewed 120 years of literature. This research was very valuable in allowing us to deepen our toxicokinetic understanding. In practice, it made a contribution to (a) our study design, (b) the discussion on COHbt_{1/2} observed, (c) the interpretation of COHb rebound, (d) the impact of pH and temperature in our experiments. Nevertheless, this M review has contributed little to the extrapolation of our results to humans, which was why we had begun its systematic review.

(a) and (b) Swine M-value at 37 °C is very little studied (only one M-value found) and assumed to be 130, whereas the median M-value in humans is around 218 (2). We therefore expected slower absorption compared to human, with a safety margin up to 150 min. Surprisingly, our absorption curves on our porcine model showed a reach at COHb% 30% in ±110min, very comparable to the CFK human model reporting a COHb of 30-40% under 1000ppm in ±100 min (See above, Fig. 5) (2, 14). It seems that the elimination phase duration is also similar to human since we get a COHbt_{1/2} at 251min on AA-treated swine (control group); 85min on NRM-treated and 58min on NiPPV-treated group (Table 3) compared to human with 240-300min AA-treated (14); 74min on NRM (15) reported and no data published on NiPPV.

The choice of the swine model was partly motivated by this M-value inferiority to the human one (130 compared to 218) to be sure being able to carry out all the experimental phases of the same experiment (phases I to IV) during the working hours of the laboratory. Due to experimental constraint, it should be remembered

that the swine conditioning including its sedation and the setting up of all the vascular accesses already absorbed two hours in the morning before starting the first intoxication phase (Phase I).

Due to this M-value at 130, we expected to observe a slower phase of intoxication and a faster phase of elimination, but this was not the case. This could be explained by increased minute ventilation during CO absorption or a potential hypoventilation during treatment phase, explaining a faster absorption and slower elimination than expected but, as we saw above, that doesn't seem to be the case either. However, this swine M-value, too little studied, is perhaps erroneous, or variable between swine breeds. As we have reported, the murine world presents a great deal of inter-species variability. We met researchers from INSERM in Creteil (France) who worked on the CO hemoglobin affinity 20 years ago, but a M-value estimation on our own pigs was not in their current research program. On the other hand, positively, the absorption curves and COHbt_{1/2} similarities of swine AA- and NRM-treated when compared to humans that we observed allows us to extrapolate to humans in a coherent way from swine NiPPV-L-treated.

(c) In-depth examination of the meaning of the M as a combination of constant association and dissociation has enabled us to better interpret the meaning of the rebound or the α - and β -phases. Even if the rebound is low, we have *a priori* ruled out the hypothesis of endogenous CO or the recapture of free CO on cessation of treatment. Ventilation is perfectly capable of eliminating this free CO, present in small quantities under AA compared with the quantity released by COHb under NBO.

Moreover, in blood, 1ml CO = $\pm 44.6 \mu\text{mol CO} = \pm 5\% \text{ COHb}$. The basic physiological level of endogenous COHb is estimated at between ± 0.4 and $\pm 0.8\%$. Its continuous production is $\pm 0.4\text{ml/h}$ (i.e., 10ml per day) with constant elimination estimated at ± 0.1 to $0.2\%/h$. A rebound systematically observed in all our experiments,

sometimes up to 1.2%, as is the case with NiPPV-Leg (Fig. 14), cannot therefore be explained by exclusive endogenous CO production.

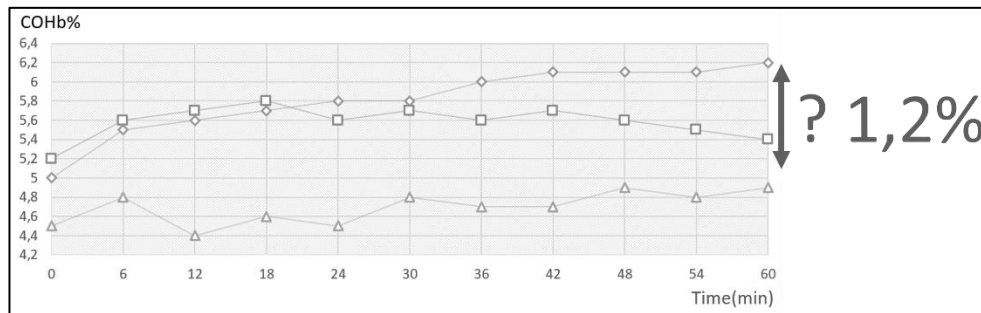


Fig. 14: %COHb AA-treated after NBO on one of the NiPPV-Leg experiments.

The fact that the rebound was most significant in the NiPPV-Leg group ($p < 0.0001$), which eliminated the COHb the fastest (Ch.3; Fig. 5), could also suggest a late release of a quantity of extravascular CO which did not have time to be eliminated in such a short time.

Concerning COHb rebound, we have therefore found no other explanation than release from extravascular tissues such as COMb. We shared this hypothesis through several exchanges with Eugene Bruce (Emeritus Professor in the Department of Biomedical Engineering, University of Kentucky) and our discussions ended as follows:

"... I accept your insights. The question is whether the time scale of your rebound is too fast to be affected by kinetics that seem to be slower than my model assumes. Based on your comments, I am now convinced that might be the case. It seems to me that your discussion in your email could be the basis for a similar discussion in your manuscript. It would say, essentially, that there might be a concern about uptake by Hb of CO already in the blood, but you have reasons to think it would be a small or negligible effect."

Our model fits quite well data from very short CO exposures; however, it is quite possible that the time constants of response that we estimated by fitting our model to data are influenced by an effect of slow kinetics of CO and Hb binding that we could not distinguish.”

“Regarding endogenous CO production, it is always present and contributing to blood CO level. I don't know any reason for it to change transiently. As I think you are saying in your manuscript, it is so small that it probably does not account for your observation.”

Of course, as we mentioned in our submitted article, further rebound observations under other study designs, notably with longer exposure times, will have to confirm this hypothesis.

(d) Reviewing physiological conditions influencing M-value, one of the only certainties that our systematic reviews reported is the impact of temperature to which we have paid particular attention. There is a negative correlation between temperature and M: when temperature increases by one degree, M decreases by 4.5 units. In addition, as the sweat glands are not developed on swine, the excess heat is eliminated by mean of an evaporation system called thermal polypnea. We had to avoid this polypnea to avoid impacting CO absorption or COHbt_{1/2}. In addition, temperature variability, like pH variability, shifts the O₂Hb dissociation curve. For these three reasons, we have checked the rectal temperature each 6 to 10 min. Transient moderate hyperthermia was observed during 6 of the 25 experiments (3 in CPAP-treated (n=5) and only one in AA-treated (n=3), one in NiPPV-V-treated (n=3) and one in NRM-treated (n=8)). No hyperthermia was identified for NiPPV-L-treated. We adjusted swine temperature by uncovering the pig or by exposing it to a heat lamp.

Moreover, M-value on human doesn't change in the relative affinity between pH 6.8 and 8.8 (16). And, the available data on the animal world are insufficient to show whether the pH influences M. The pH was monitored throughout the procedure and clearly maintained values close to physiological values (See Appendix Table 1; Fig. 1 to 4).

7.3.4. On research question 5

Noting that the dramatic prognosis of CA CO-induced with almost 100% mortality reported in the literature and that the hope of seeing prospective studies with enough patients included in this specific case seems utopian, we decided to observe COHbt_{1/2} during resuscitations on CA CO-induced in order to see if we could find reasons for these fatal outcomes in the toxicokinetic field. Referring to general toxicokinetic, Shimazu points out that it is interesting to question whether what is considered to be a compartment is an anatomical or physiological entity: either an intra-vascular compartment and an extra-vascular compartment, or well-perfused and poorly perfused intravascular compartment (17). In a cardiac arrest, it is possible that the peripheral extra-vascular poorly perfused compartment serves as the second or a third compartment, with consequent extravascular CO storage which could explain in part the catastrophic mortality rate.

We had only one opportunity to observe these data. We did not find any toxicokinetic reasons for these dramatic situations except the rescue-response time but, on the contrary, the unexpected COHbt_{1/2} at 40.3min observed encouraged us to publish this in case report form. This observation, compatible with the time devoted to a standard resuscitation time, should encourage the clinician to continue these efforts during resuscitation, in particular by prolonging resuscitation. This was already suggested in the ERC recommendations concerning toxic cardiac arrest but without putting forward any objective argument concerning CO, apart from the potential secondary organ donation. In this case report, as often observed in retrospective clinical studies, we lacked samples in the first extra-hospital hour. Therefore, we did not discuss the bi-compartmental model in this article. In our article, we discuss at length the hypotheses that might explain this

short $\text{COHbt}_{1/2}$, which of course needs to be confirmed by additional clinical investigations.

7.4. FURTHER PERSPECTIVES

Recurring reminders in this thesis of the limits of knowledge in relation to HBO efficiency for CO poisoning should not be read as a desire to invalidate this technique, but rather to recall the absence of sufficient evidence to encourage the systematic use of HBO, which continues to justify the search for alternative therapies (21).

The objective is to propose a non-invasive alternative, easily and quickly accessible, avoiding potentially damaging hyperoxygenation while waiting for more convincing studies on the CO adequate treatment. We can see, for example, that isocapnic hypercapnia, which seems to be an effective treatment for COHb elimination rate, is not clinically accepted or used at the moment, probably because it requires additional, specific equipment in already over-equipped ambulances and emergency departments. That is the advantage of trying to use equipment that is already available and already mastered by healthcare teams, as we propose with NiPPV.

Using the Haldane equation from Chapter 1, we see that the therapeutic avenues targeting COHb elimination are: (a) increasing FiO_2 or PaO_2 (NBO and/or HBO); (b) promoting alveolar recruitment; (c) increasing ventilation to remove free CO; (d) interfering with M with potent chelators in the early stages of development; (e) promoting the in vivo oxidation of CO to CO_2 . While we are the only ones to investigate NiPPV to improve at the same time (a),(b),(c) and specific CPAPb during this thesis, others tried to enhance ventilation or oxygen supply with high flow nasal canula oxygen therapy (8, 22, 23), CPAP (19, 20, 24) or to catalyse CO in CO_2 (25) or to develop chelators (25, 26) (Fig. 15).

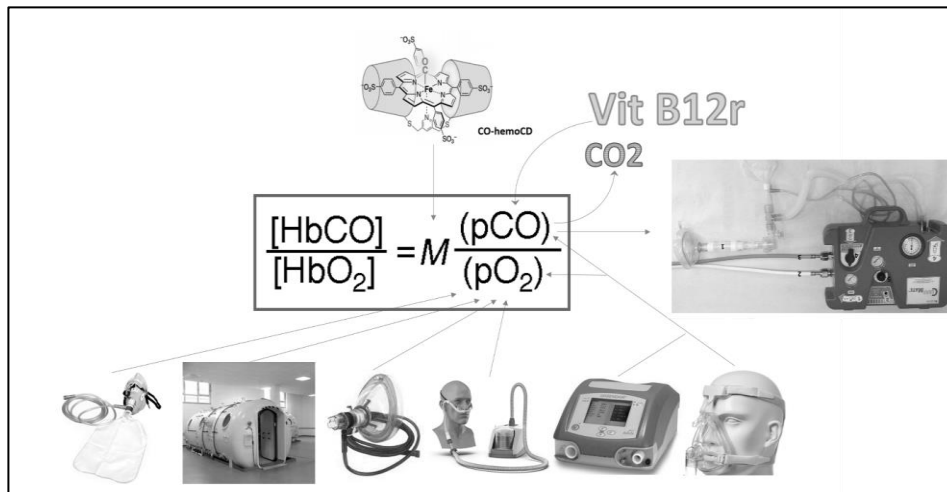


Fig. 15: Treatments still under investigation in the last 20 years and their expected action in relation to the Haldane equation. Anticlockwise: NRM; HBO; CPAP; HFNC; NIPPV, Isocapnic Hyperventilation, VitB12r and powerful chelators.

Our study certainly raises the question of good O₂ practice. The FiO₂ delivered at 15 l.min⁻¹ using conventional NRM is expected to reach up to 100%. However, due to unavoidable leaks around the mask and rebreathing, a maximum concentration of 85% is usually reached. In our study, where we mimicked the same leaks, the mean FiO₂ obtained was 72% (2). This illustrates that, apart from our consideration for new treatment alternatives, NRM does not deliver one-fifth of the expected dose. In our opinion, the universally-used conventional NRM is not suitable for the management of CO poisoning. Interestingly, as far back as 60 years ago, special attention was being paid to the fact that immediate administration of O₂ required a specific hermetic mask. However, the wide availability of the inexpensive NRM in most emergency departments makes it the non-optimal device of choice (18).

Since we published our swine study investigating NIV-CPAP on CO poisoning in 2018, the first prospective clinical study of humans assessing CPAP (5cmH₂O) for

mild CO poisoning with similar %COHb to ours from 20 to 35% appeared (19, 20). In their discussion, they cite our 'favourable' experimental results from 2018 to justify in part their clinical study. We guess the researchers were focusing on our NiPPV and the discussion surrounding a hermetic mask, as our negative results with our CPAPb seemed to be ignored. In this human study, in the absence of any sedation, CPAP lowered COHbt_{1/2} faster than NRM at 45min[30-120] compared to 105min[70-90], respectively (19). The more significant improvement they showed could again be related to the FiO₂ because their CPAP is a classical CPAP, with hermetic mask, contrary to our CPAPb, open system including the virtual valve as described in Chapter 1 (sub-chapter 4.3.), permitting the inhalation of atmospheric room air in case of high inspiratory flow. Surprisingly, they do not discuss other physiological reasons than FiO₂ to explain their results, such as improved alveolar recruitment or increasing slightly ventilation, even if theoretically, CPAP does not have a direct impact on ventilation.

So, the initial low statistical significance has been strengthened by other toxicokinetic approaches. This animal pilot study appears to highlight the benefit of NiPPV on several COHb toxicokinetic perspectives, and demonstrated the absence of side-effects (pH, lactate, blood pressure) and especially the absence of NiPPV-induced hypocapnia (44.6mmHg on NiPPV-treated, Table 1 and Appendix Fig.3 and 4). To better isolate the precise origin of this benefit and to confirm the value of NiPPV in this indication, only a clinical study comparing Weaver's reference COHbt_{1/2} at 74min will be able to address this question. We encourage a prospective study with NiPPV, including early COHb blood samples (Fig. 16). Nevertheless, a pressure support increasing ventilation during NiPPV on a human without respiratory distress could be uncomfortable for the patient and/or induces hypocapnia. To resolve both elements, a classical NiPPV device could be supplied without an O₂ cylinder but with 95% O₂ and 5% CO₂, which is already available

commercially at low cost. Inhaling CO₂ supply can result in reflex hyperventilation (3-to-4-fold MV for 5%CO₂) but the inspiratory pressure should support the potential hyperventilation discomfort. This is, of course, speculative but would allow the very rapid elimination of COHb while limiting the risks of hyperoxygenation. This could first be investigated by an experimental human study in the absence of CO poisoning.

Isocapnic hyperventilation has shown very good results in reducing COHbt_{1/2} and avoiding hyperoxygenation, based on the same goals as ours. Unfortunately, the developers don't seem to have published any clinical case series or significant prospective studies. They have limited themselves to experimental studies. What's more, a specific material is required, which discourages its application for a method which has not been shown to be especially effective in preventing DNS. This is why, in the case of a positive study, our NiPPV could gain wider acceptance, as the material has already been acquired by healthcare teams.

Moreover, despite the certainty that O₂ is the antidote for CO poisoning, it is worth remembering that no rigorous study has compared AA to NBO, and the absence of O₂ toxicity is not demonstrated. If we take into account Bruce's mathematical model, physiological ventilation would, initially, not have time to evacuate the CO released in the blood by COHb due to the massive O₂ supply at the start of treatment. This free CO would therefore be available to bind to myoglobin and extravascular tissue as cytochromes (33, 34). In the future, in the spirit of '*primum non nocere*', in the absence of cardiac or neurological failure, it must be relevant to investigate NiPPV's capacity for forcing the elimination of this free CO (pCO) and thus decreasing the risk of cardiomyoglobin or the cytochromes CO upload. This strategy could be consistent only in the early COHb elimination phase (α -phase). This would require investigating another NiPPV group on FiO₂ 0.21 (PS 10-12; PEP4) to our proposal (Fig. 16) for a future study.

If investigators are able in the future to demonstrate new CO chelators or VitB12r clinical efficacy on humans, it could supersede all other therapies employed for 150 years. However, the use of a specific and effective antidote also depends on its price, which sometimes limits its availability, as is the case for the antidote against digitalis, which costs several thousand euros, or the antidote against cyanide in fire smokes which, despite its effectiveness, cannot be widely distributed to all emergency medical vehicles or in all countries, especially in developing countries where the prevalence of CO poisoning is highest.

Moreover, VitB12r have shown toxicokinetic efficacy in eliminating CO in a pre-clinical study on human in vitro blood and on in vivo. B12r, the Vitamin B12 (Hydroxocobalamin) reduced by Vit C (ascorbic acid) catalyses a CO to CO₂ conversion. This resulted in a clinically-significant off-gassing of CO₂ at levels five to eight times greater than controls on a given period of time (25). In case of initial CO₂ overproduction, the respiratory support of the NiPPV could promote its elimination.

The question of the M seemed to be an old story that had been resolved, but the M animal value remains a hot topic. Hopper in 2021 still underlines the importance of knowing the animal values for future studies in analysing the therapeutic effects of low dose CO. Furthermore, the principle of action of the new treatments with potent CO chelators is based on the strength of the affinity of Hb compared to the chelator for CO and for O₂. It is very likely that they will become part of the therapeutic arsenal of the future as with hydroxocobalamin for cyanide poisoning, for which we have had the opportunity to develop European recommendations in 2013 (35). These new treatments are evaluated on animal models and the protocols must consider the specificities of the animal model to extrapolate their results to humans.

In view of all the experimental results demonstrated in this thesis, we would propose this clinical study design to confirm our hypotheses (Fig. 16).

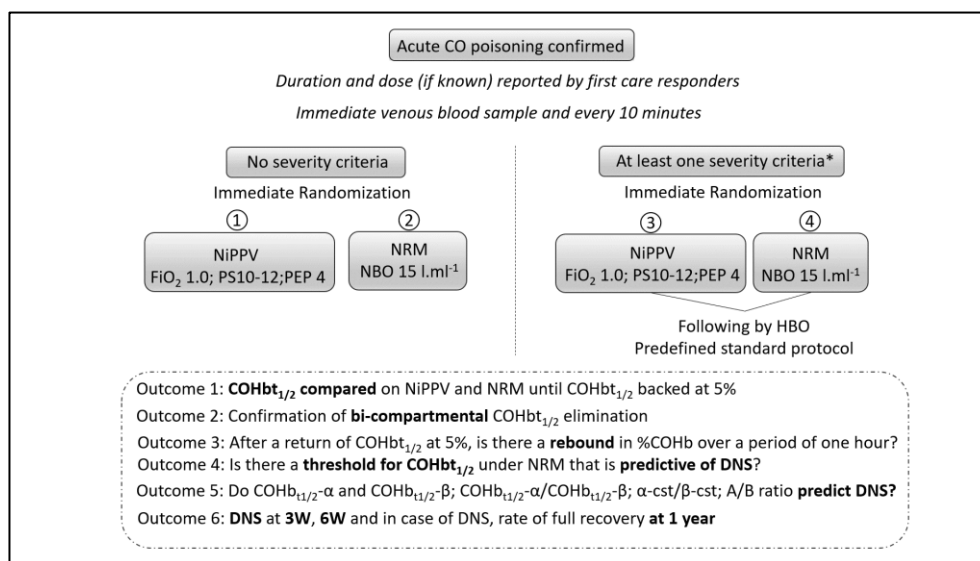


Fig. 16: Design of a further clinical study needed to confirm our hypotheses. (*)Evidence of cardiac involvement, severe acidosis, transient or prolonged unconsciousness, neurological impairment, myocardial ischaemia/arrhythmia diagnosed by ECG or the patient is ≥ 36 years old (30).

In relation to the following-up of patients after CO poisoning, in France, there is no protocol or recommendation consensus, although some anti-poisoning centres provide follow-up at one month. More than 50% of general practitioners (GP) are unaware of the DNS, which appears in 5 to 40% of cases within 3 weeks of CO poisoning. The symptoms of DNS are many and varied, varying in severity and depending on the location of the white matter: neurological, neuropsychiatric, behavioural or memory disorders. Diagnosis is considered as clinical for 70% of GP and paraclinical (MRI) for 30%. In addition to a neurological examination, a

neuropsychiatric assessment can be carried out using various cognitive tests such as the Mini Mental State Examination (MMSE), Hamilton Depression Scale, Clinical Demential Rate (CDR)... DNS appears to have a good prognosis, with a total recovery rate of 50 to 75%, which may also explain why its prevalence is underestimated. However, the symptomatology of DNS may persist for several months, with permanent sequelae in some cases. If there is any doubt on the part of the GP, the DNS can be confirmed by MRI to look for lesions of the white matter, and a consultation with a neurologist should be carried out systematically to assess the disorder in more detail and consider re-education or specific drug treatment for depressive disorders or extra-pyramidal syndrome following CO poisoning (36).

The emergency physician should therefore systematically warn the patient and his family of the possibility of the occurrence of DNS and send a letter to the GP to ensure follow-up at 3-6 weeks with a maximum reported period of appearance of 240 days.

Identifying the risk factors predictive of the onset of DNS after moderate CO poisoning is still very difficult, which is why we are proposing to evaluate this new potential severity criterion, toxicokinetic, in order to refer to a specific treatment and/or to screen patients who would be systematically referred to a neurology consultation at 3 weeks for in-depth neurological assessment.

7.5. TAKE HOME MESSAGE

For all further study or clinical assessment, we encourage a systematic evaluation of this $\text{COHbt}_{1/2}$ from several COHb venous samples during treatment, including very early samples from front-line ambulances.

Bi-compartmental elimination must always be considered and evaluated. To simplify the evaluation of $\text{COHbt}_{1/2}$ on mono- and bi-compartmental models, we suggest the development of a downloadable phone app that can be used to obtain them by simply entering the sampling times and COHb%.

COHb rebound must be assessed in clinical practice or under other experimental procedures.

Human M-value at 37°C is 218 $\pm$ 8 at any pH and should become the reference used in CO studies.

Our analysis did not provide evidence for the existence of a species-specific Hb that would approximate human M-value sufficiently to suggest it as a replacement for human blood in future studies.

✓ **Recommendation for any further study evaluating a treatment for CO poisoning:**

COHb being bi-compartmental, we show that it is possible to assess the two elimination phases separately - but also their $\text{COHbt}_{1/2-\alpha}/\text{COHbt}_{1/2-\beta}$; $\alpha\text{-cst}/\beta\text{-cst}$; A/B ratio (Fig. 9 and Table 3). Distinguish $\text{COHbt}_{1/2-\alpha}$ and $\text{COHbt}_{1/2-\beta}$ provides toxicokinetic data which could be helping to differentiate treatment efficiency, NBO duration or oxygen therapy modalities.

✓ **Recommendation for any further study evaluating the CO poisoning severity:**

The correlation between the CO poisoning severity and COHbt_{1/2} on mono- or bi-compartmental models and/or the presence of a rebound at the end of treatment must be systematically investigated.

✓ **Recommendations for clinicians to implement immediately:**

Before future studies clinically confirm the benefits of NiPPV for CO poisoning on COHbt_{1/2} compared to NRM, we recommend applying a mask with 15l.min⁻¹ of O₂ as tightly as possible throughout the NBO treatment.

COHbt_{1/2} can be assessed at 4 hours using a mono-compartmental model to be compared to the current reference at 74min. A COHbt_{1/2} >74min could be considered as a severity criterion with a systematic request for advice from the hyperbaric physician to consider the recourse to HBO.

While awaiting future studies, treatment with NBO for at least 6 hours is necessary. Samples taken during the next hour without treatment could be taken to verify the absence of COHb rebound, especially for CO exposures considered to be long. Identification of any COHb rebound > 1% would require a systematic request for advice from the hyperbaric physician to consider the recourse to HBO.

In case of COHb rebound or a COHbt_{1/2} estimated to be longer than the current reference at 74 min and no indication for HBO from the hyperbaric physician, it might be wise to continue NBO up to 12h.

The emergency physician should therefore systematically warn the patient and his family of the possibility of the occurrence of DNS and send a letter to the GP to ensure follow-up at 3-6 weeks. If there is any doubt on the part of the GP, the DNS can be confirmed by MRI, and a consultation with a neurologist must be scheduled.

✓ **Recommendations for the management of cardiac arrest on CO poisoning:**

We encourage the clinician to continue these efforts during resuscitation, in particular by prolonging resuscitation.

Calculating COHbt_{1/2} during CPR may help in decision-making regarding the optimal duration of resuscitation efforts and further therapy with HBO or ECLS.

7.6. GENERAL CONCLUSION

This thesis discussed the carboxyhemoglobin toxicokinetic after acute carbon monoxide poisoning. Despite 130 years of CO studies, the understanding of its toxicity mechanisms, and the treatment that depends, on it remains unclear. Because the treatment's validation seems to be at a methodological dead-end, we have explored an alternative way of treatment. And, because the CO poisoning evaluation and the CO storage are at an analytical dead-end, we have explored CO toxicokinetic tracks to circumvent this obstacle.

At the beginning of this thesis, we posed the question of how to improve acute care management of carbon monoxide poisoning. As clinical studies fail to agree on management concerns, we decided to investigate a toxicokinetic point of view. We have endeavoured to provide an answer to the **five research questions** that were formulated:

- 1. What is the COHbt_{1/2} on NIV (NiPPV or CPAP) compared to NRM on NBO?** In our pilot experimental swine study, we have determined that the NiPPV (closed NiPPV-Leg system) gave the shortest COHbt_{1/2} (58 vs. 85 min with NRM) and the lowest AUC for COHb (63 vs. 79 min with NRM). In contrast, we didn't find any benefits for CPAP Boussignac assessed. This suggests that NiPPV can be more efficient than NRM at eliminating CO. Nevertheless, the benefit of optimising FiO₂ could be greater than the benefit on ventilation itself. Our results support the design of a clinical study to avoid sedation bias and confirm the NiPPV benefit.

2. Does COHb toxicokinetic fit with a mono- or a bi-compartmental model?

Using data from our swine study, we confirm that the COHb half-life is biphasic and thus follows a bi-compartmental model. Our assessments reinforced by our discussion resolves the historical controversy. The bi-compartmental elimination model is in favour of a CO storage. Future clinical studies should consider this model when estimating their COHbt_{1/2} which could be accurately assessed as a new CO severity criterion when treatments are compared.

3. Is there a %COHb rebound after COHb backed at 5% and NBO cessation?

Continuing our swine experimental protocol beyond cessation of treatment, we observed for the first time a slight COHb rebound when COHb is returned to 5% and O₂ therapy is stopped. After the bi-compartmental elimination model, this is a second argument compatible with a CO storage. We argued that a treatment of less than 6h does not seem to be adapted for any mild CO poisoning. We suggested future clinical studies should consider occurrence of a COHb rebound to be confirmed.

4. Which are the accurate M-values for human and animals? Which are the impacts of species and various physiological conditions on M?

Our meta-analysis and systematic review provide up-to-date data for understanding and carrying out further studies on CO metabolism. M-value at 37°C is confirmed at 218 ±8 without certainty of the pH influence. There is a negative correlation between temperature and M: when temperature increases by one degree, M seems to decrease by 4.5 units. We published the largest review of M-values collecting data. In the animal world, Haldane's constant at 218 has proved to be inaccurate for most species. In the different mammals explored, M at 37°C is between 109 and 215 under physiological conditions. There is also some intraspecies variability, especially among mice. Our analysis did not provide

evidence for the existence of a species-specific Hb that would approximate sufficiently to human M-value to propose it as a replacement for human blood in relevant or future studies. Only T° provides evidence of influencing M, but no general T° correlation is applicable for all species. In the future, animal investigations on CO need to evaluate M at 37°C under physiological conditions to better fit the extrapolation of data obtained from animals to humans. Further studies are also required to clarify the pH influence on the M-value for human and animal. Other factors like 2,3-DPG concentration in red cells, atmospheric pressure, pregnant state, methemoglobin, and sickle Hb do not seem to influence M. However, there is insufficient data to perform a meta-analysis.

5. What is the COHbt_{1/2} during CPR on CO-induced CA?

Over the period of crafting this thesis, we reported a human COHbt_{1/2} case during prolonged CPR after cardiac arrest on CO poisoning. This CPR included controlled mechanical ventilation at a FiO₂ of 1.0. For the first time, we published a COHbt_{1/2} much lower than expected at 40.3min compared to 74min observed on mild CO poisoning spontaneously breathing. This result suggests that prolonged CPR may lead to a COHb level which could be compatible with successful ROSC. Calculating COHbt_{1/2} during CPR may help in decision-making regarding the optimal duration of resuscitation efforts. Further evidence-based data are needed to confirm this result.

We hope to have helped the clinician better understand the complexity of CO poisoning and its challenges, and to generate interest in developing new clinical-prospective studies based on the results and discussion of our experimental investigations. Obviously, CO poisoning treatment cannot be reduced to "giving oxygen".

Key contribution of this thesis

First, our animal model has allowed us to demonstrate the feasibility and relevance of designing an active clinical study for evaluating COHbt_{1/2} under NiPPV after CO poisoning - and from the first stages of care management.

Secondly, our toxicokinetic analysis confirms a biphasic COHb decay in favour of a bi-compartmental COHbt_{1/2} model. This tilts the balance of the argument in this controversial issue. The demonstration of this model comes down in favour of a CO storage, allowing us to estimate the COHbt_{1/2} with more accuracy.

Thirdly, COHb rebound or stagnation at the end of treatment had never been demonstrated before. This could also be an argument in favour of CO storage and would confirm the necessity of an O₂ therapy of at least 6h.

Fourthly, we confirm the human M value at 218 which should become the reference in CO literature.

Fifthly, we published the largest review of M-values in the animal world. Animal M value varies according to species, being for mammals explored under physiological conditions between 109 and 215. Swine M value is only very slightly studied and estimated at 130.

Sixth, we observed an unexpected COHbt_{1/2} during human CPR on CO-induced cardiac arrest. This COHbt_{1/2} at 40.3min could allow us to reach COHb considered as infra-toxic (COHb<10%) in a time compatible with a reanimation period. This suggested continuing CPR beyond standard 30min in case of CO-induced cardiac arrest.

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APPENDIX

SUPPLEMENTAL DATA

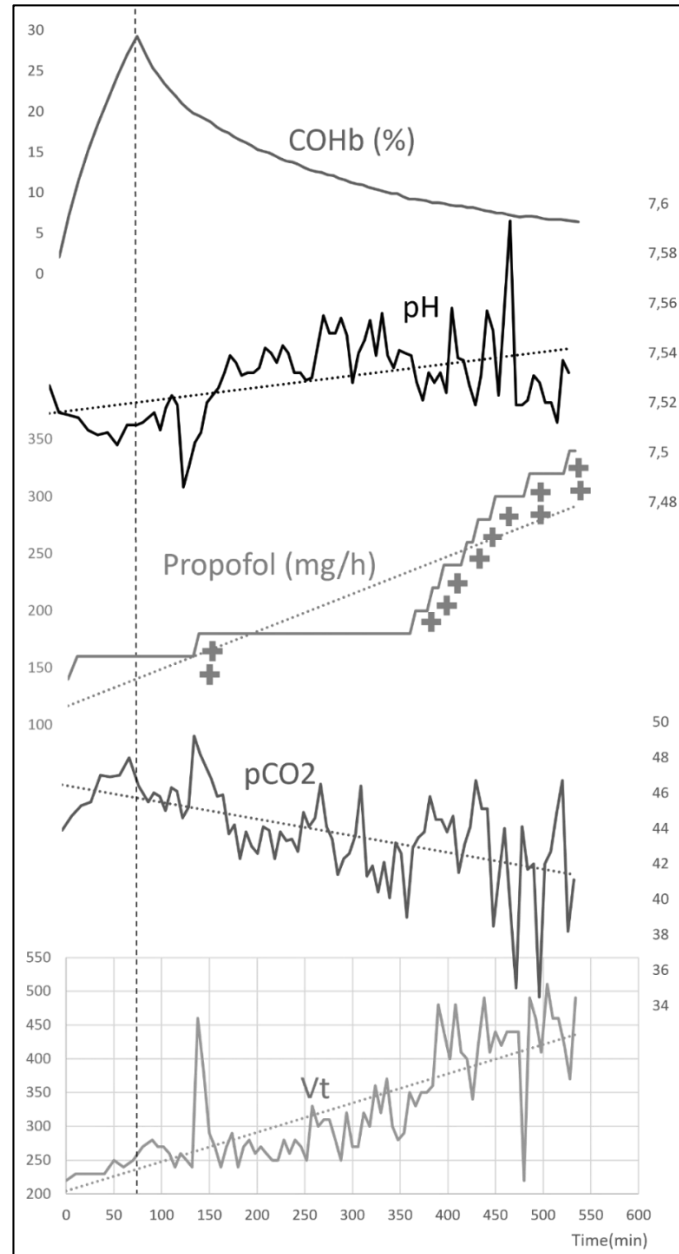


Fig. 1: Respiratory parameters evolution for one swine during the three phases I to III on AA-treated (control group). Vt:Tidal Volume (ml); pCO₂: Carbon dioxide partial pressure (mmHg); Propofol for sedation (mg/h); '+' = Propofol bolus 20 mg; arterial pH ; COHb : Carboxyhemoglobin evolution on 940 ppm CO exposure (%).

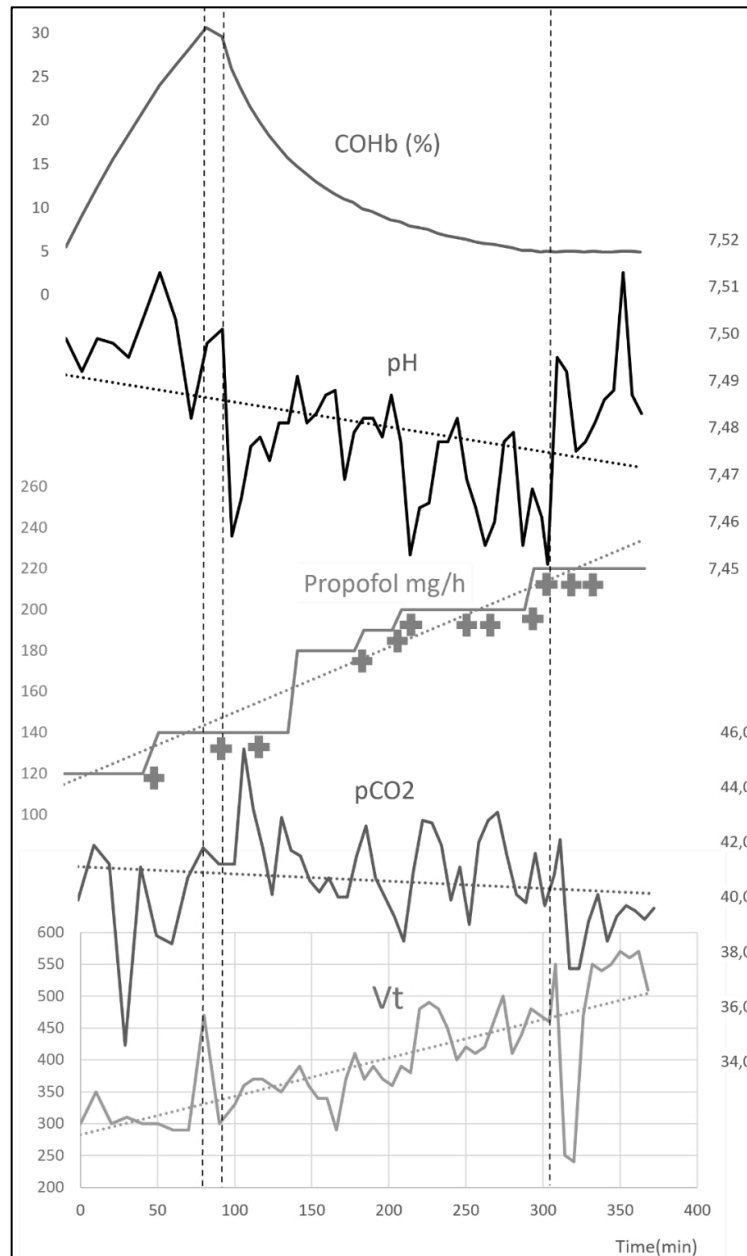
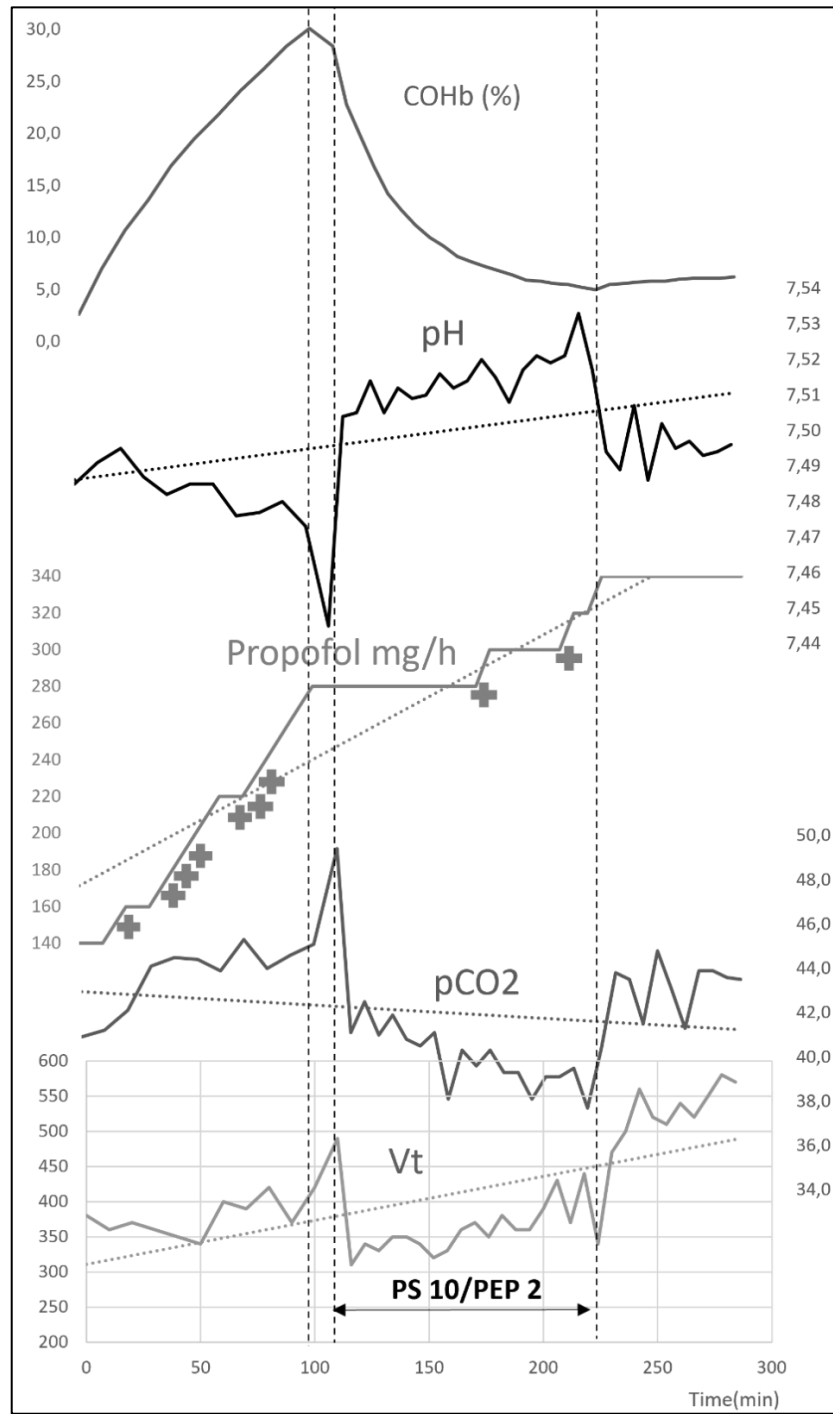


Fig. 2: Respiratory parameters evolution for one swine during the four phases I to IV on NBO NRM-treated. Vt:Tidal Volume (ml); pCO₂: Carbon dioxide partial pressure (mmHg); Propofol for sedation (mg/h); '+' = Propofol bolus 20 mg; arterial pH; COHb : Carboxyhemoglobin evolution on 940 ppm CO exposure (%).



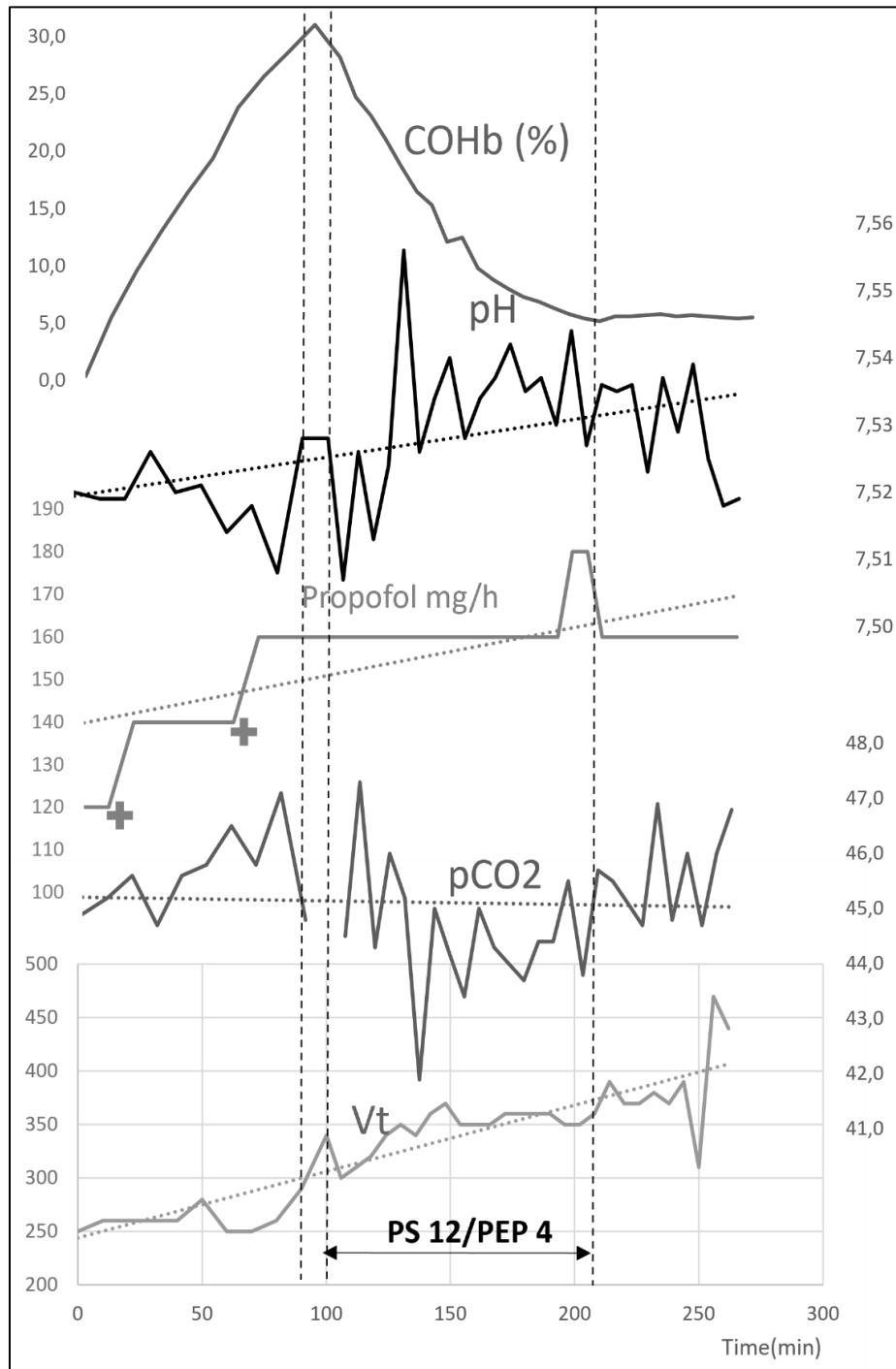


Fig. 3 and 4: Respiratory parameters evolution for two swine during the four phases I to IV on NBO NiPPV-Leg-treated. Vt:Tidal Volume (ml); pCO₂: Carbon dioxide partial pressure (mmHg); Propofol for sedation (mg/h) ; '+' = Propofol bolus 20 mg; arterial pH ; COHb : Carboxyhemoglobin evolution on 940 ppm CO exposure (%); Pressure support 10mmH₂O (adjusted from PaCO₂) and Positive Expiratory Pressure 4cmH₂O (adjusted from swine respiratory comfort). Protocol's target were fixed at 12 and 4 respectively.

	Phase I START					Phase I END					Phase III START					Phase III END				
	NRM	CPAP	PSV- Leg	PSV- Vy	AA	NRM	CPAP	PSV- Leg	PSV- Vy	AA	NRM	CPAP	PSV- Leg	PSV- Vy	AA	NRM	CPAP	PSV- Leg	PSV- Vy	AA
Elapsed time (min)	0	0	0	0	0	120	110	100	120	100	130	120	110	133	108	297	308	220	362	495
HR (min-1)	77 [49-94]	77 [78-104]	75 [62-79]	64 [55-103]	69 [55-84]	74 [55-94]	70 [64-95]	78 [56-92]	61 [47-89]	73 [57-90]	68 [51-89]	66 [65-95]	74 [55-90]	63 [53-93]	73 [56-90]	65 [54-83]	63 [51-92]	61 [51-92]	57 [50-62]	86 [82-89]
SBP (mm Hg)	109 [83-143]	99 [90-129]	128 [80-145]	109 [103-113]	117 [116-133]	107 [96-137]	114 [83-125]	117 [93-134]	118 [99-127]	114 [105-124]	114 [99-136]	110 [83-120]	114 [94-154]	116 [95-124]	111 [103-121]	106 [94-119]	113 [88-134]	114 [103-141]	106 [98-128]	116 [107-151]
DBP (mm Hg)	70 [52-94]	70 [45-84]	63 [52-89]	70 [70-75]	71 [58-79]	75 [54-92]	79 [59-88]	74 [53-89]	82 [70-87]	63 [45-71]	75 [66-88]	73 [57-78]	81 [51-86]	82 [62-88]	69 [45-77]	670 [52-76]	70 [56-83]	65 [56-77]	59 [52-71]	67 [66-99]
COHb (%)	2.3 [0.2-2.6]	2.1 [0.0-2.3]	2.3 [0.4-2.6]	2.6 [2.4-2.8]	2.1 [0.1-5.5]	30.1 [29.8-30.7]	30.0 [29.4-30.3]	30.4 [29.4-31.0]	30.3 [29.9-31.4]	29.8 [29.2-31.0]	28.4 [27.6-29.6]	27.8 [27-28.4]	28.4 [28.2-28.7]	28.9 [28.1-29.3]	27.9 [26.8-29.7]	4.9 [4.8-5.1]	5.0 [4.8-5.1]	5.0 [4.5-5.2]	5.0 [4.9-5.0]	9.3 [5.1-20.0]
Hb (g.dl ⁻¹)	10.2 [9.0-11.7]	10.2 [9.3-11.0]	8.6 [7.8-10.3]	10.9 [9.0-11.2]	8.4 [7.9-9.9]	9.6 [8.2-11.3]	9.8 [8.4-10.3]	8.0 [7.6-9.2]	10.7 [8.8-11.2]	8.8 [7.4-9.5]	9.6 [8.3-11.3]	9.9 [8.4-10.3]	8.0 [7.5-9.7]	10.6 [8.9-11.1]	8.6 [7.4-9.5]	10.0 [8.3-11.4]	9.9 [8.9-10.7]	8.3 [7.8-10.3]	10.3 [8.7-10.9]	8.2 [7.2-9.7]
pH	7.50 [7.47-7.53]	7.51 [7.45-7.52]	7.52 [7.46-7.52]	7.49 [7.48-7.50]	7.49 [7.47-7.53]	7.48 [7.46-7.50]	7.47 [7.42-7.48]	7.50 [7.46-7.53]	7.47 [7.46-7.47]	7.49 [7.45-7.51]	7.48 [7.46-7.50]	7.49 [7.44-7.50]	7.49 [7.44-7.53]	7.47 [7.46-7.48]	7.50 [7.4-7.51]	7.51 [7.39-7.54]	7.50 [7.44-7.53]	7.53 [7.40-7.58]	7.50 [7.48-7.53]	7.50 [7.50]
Lactate (mmol.l ⁻¹)	0.8 [0.6-1.2]	1.3 [1.2-1.8]	1.0 [0.7-1.3]	0.8 [0.5-1.1]	0.7 [0.5-1.4]	0.8 [0.5-1.3]	1.3 [1.2-1.2]	1.0 [0.5-1.4]	0.5 [0.4-1.2]	1.1 [0.6-1.7]	0.8 [0.5-1.3]	1.2 [0.8-2.0]	1.0 [0.5-1.7]	0.5 [0.4-1.2]	1.0 [0.6-1.7]	0.5 [0.4-1.3]	1.0 [0.8-1.2]	0.6 [0.3-2.1]	0.4 [0.4-0.7]	0.7 [0.8]
T° (°C)	36.8 [35.9-38.2]	36.3 [35.1-37.1]	37.6 [37.1-38.6]	37.0 [34.9-37.1]	36.4 [36-37.5]	36.8 [35.3-37.6]	37.5 [34.5-37.8]	37.2 [36.5-37.8]	36.3 [35.5-36.4]	36.2 [35.5-37.7]	36.7 [35.2-37.6]	37.5 [34.5-37.8]	37.2 [36.4-37.8]	36.3 [35.5-36.3]	36.2 [35.5-37.7]	37.0 [36.4-38.1]	37.1 [34.3-38.7]	36.5 [36.1-37.4]	35.8 [35.3-37.2]	36.8 [35.4-38.2]

Table 1: Electronic supplemental material published (2). HR: heart frequency; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; COHb: Carboxyhemoglobin; Hb: Hemoglobin; pH: Lactate; Temperature. PSV = NIPPV.

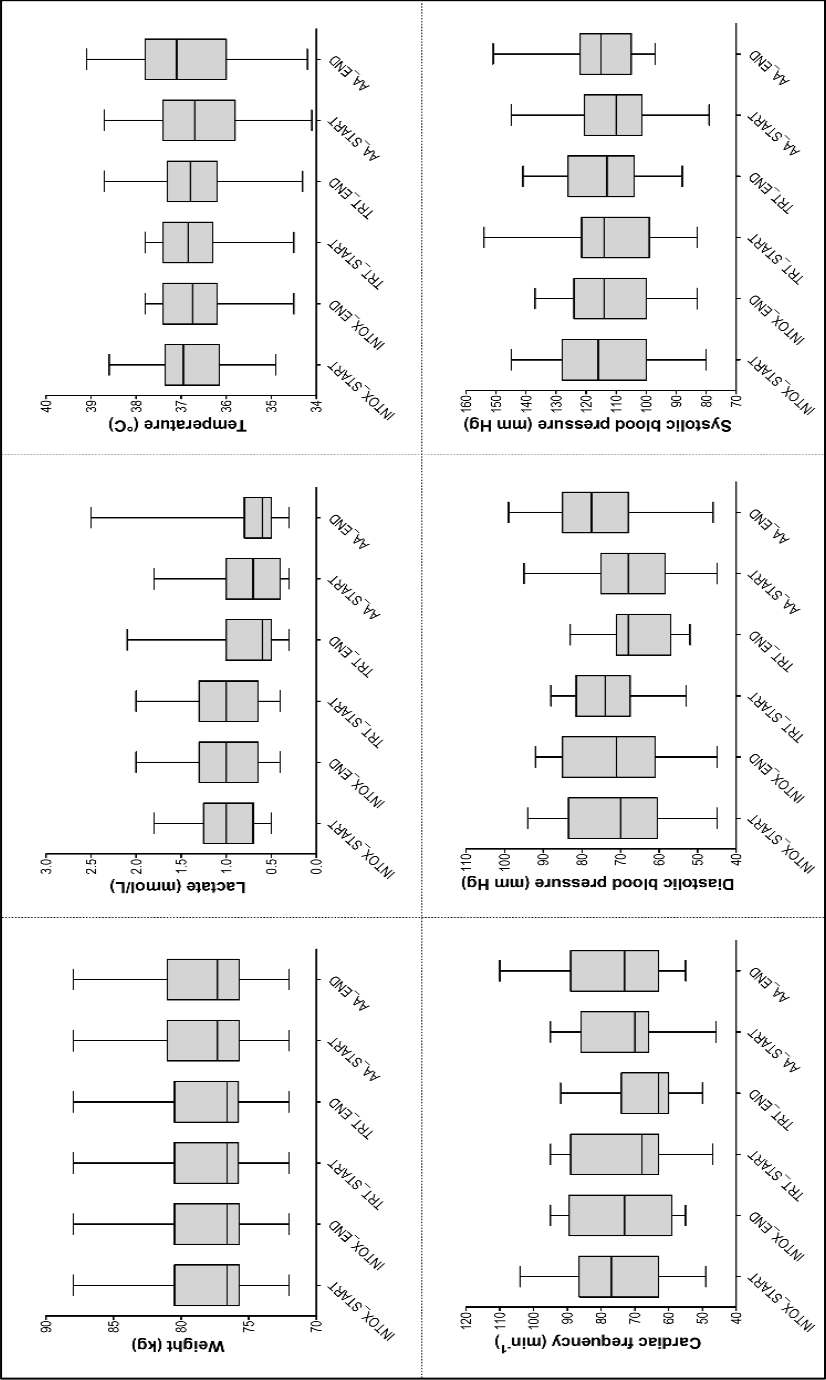


Fig. 5: Several parameters monitored starting and ending each protocol phase (unpublished data). Arterial blood pressures were also monitored and never revealed hypotension.

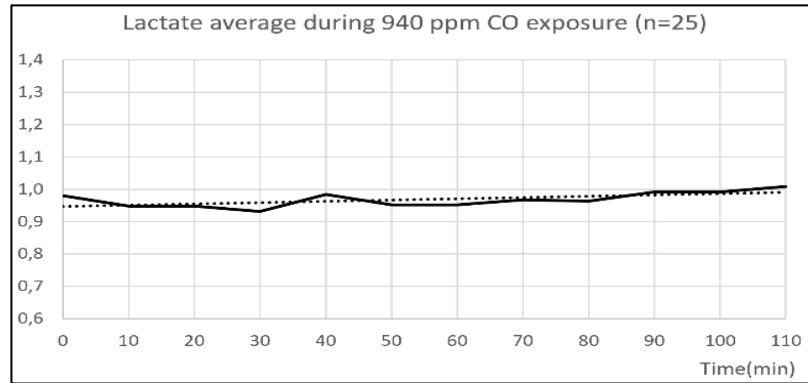


Fig 6: Minor lactate increase is observed during the CO exposure but will be progressively normalized when the CO exposure is stopped whatever the treatment and even without intervention under AA (See Table 1) (unpublished data). On the other hand, the pH rather exceeded the upper limits before time '0' as CO exposure starting with a value at 7.5 ± 0.023 regardless of the intervention or regardless of the treatment phase (see Table 1) (unpublished data).

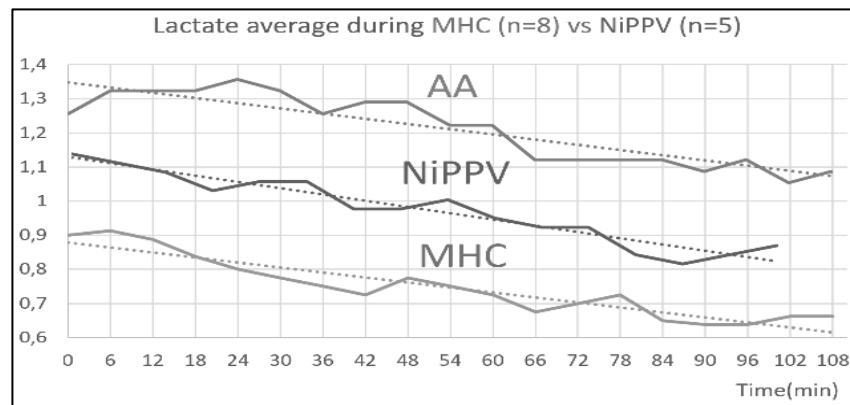


Fig. 7: Mean lactate levels during phase I CO exposure (t0) and the following phase III treatment comparing NiPPV (n=5) vs. NRM (n=8) and AA (n=3) (as control group). The lactate clearance kinetics appeared similar whatever intervention. While lactates were slightly increased during CO exposure, no significant elevations that would reflect respiratory acidosis resulting from elevated PaCO_2 were observed during treatment (no published data).

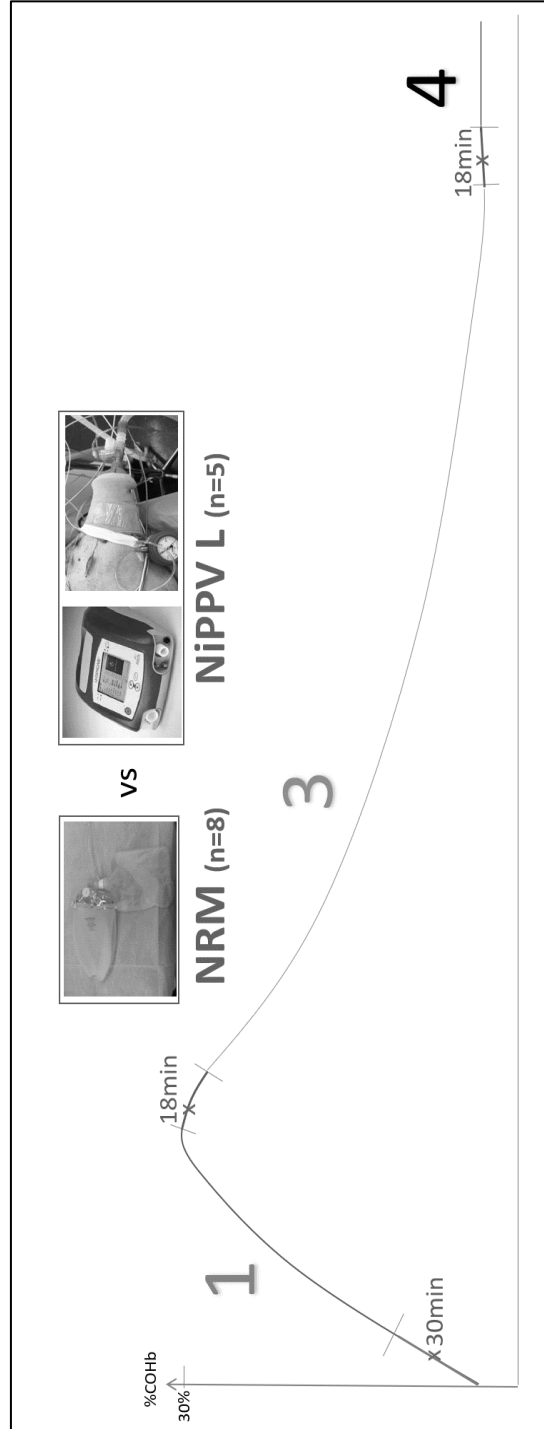


Fig. 8: Experimental procedure on NRM and NiPPV-Leg corresponding to the individual respiratory data pooled in Table 2 and Fig. 9 for NRM and, Table 3 and Fig. 10 for NiPPV-Leg (2).

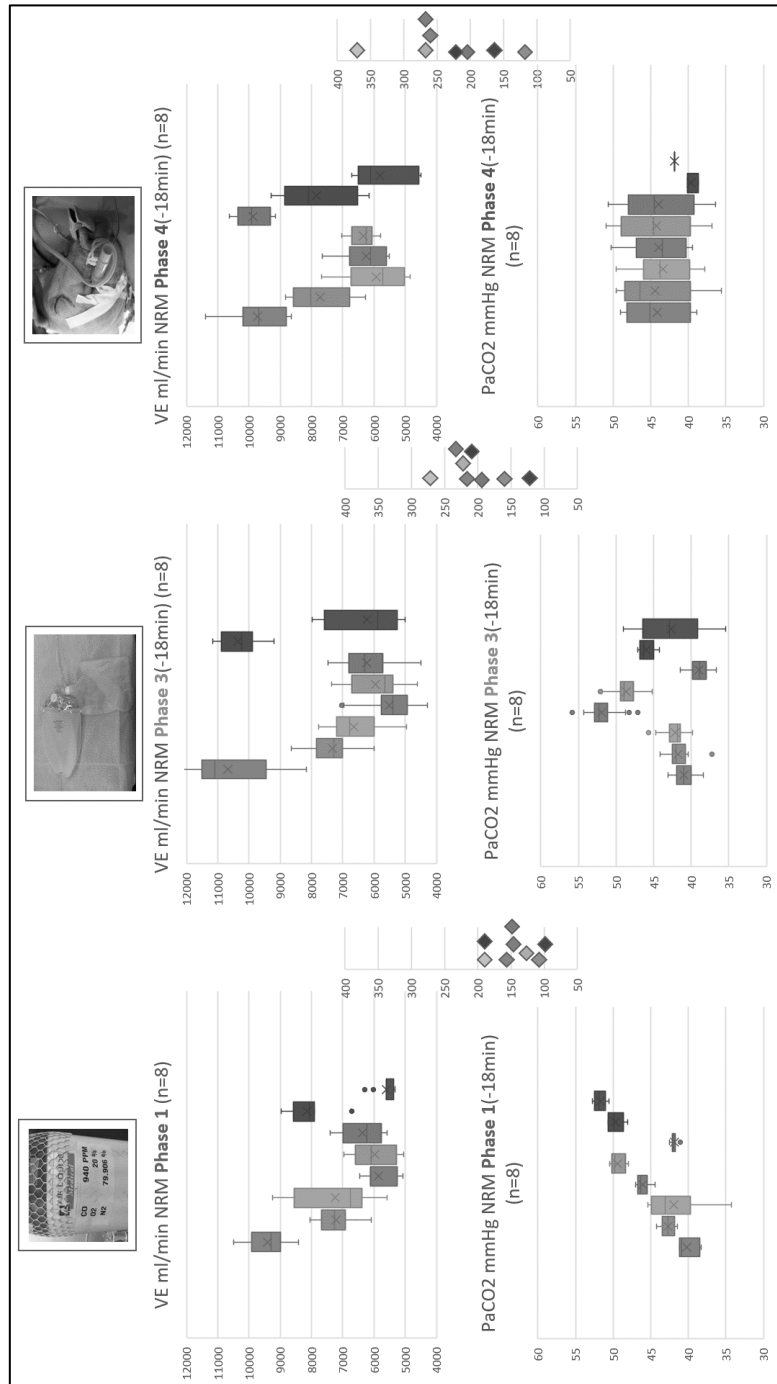


Fig. 9: Box plots for individual respiratory data on NRM. On the right of each column (from 50 to 400 mg.h⁻¹), the median amount of Propofol administered is indicated for each pig. From left to right: LE, TO, DO, BA, PE, TI, BR and MA (2).

NRM		LE NRM	TO NRM	DO NRM	BA NRM	PE NRM	TI NRM	BR NRM	MA NRM
Phase 1 (all)	VE	9300 SD636	7260 SD578	6760 SD1245	6120 SD517	6080 SD678	6230 SD676	8060 SD638	5595 SD288
Phase 4 (-18 1 st min)	VE	9690 SD943	8025 SD951	5280 SD976	6100 SD806	6260 SD427	9915 SD564	8105 SD1219	6100 SD904
Phase 3 (-18 1 st min)	VE	11100 SD1233	7295 SD648	6840 SD880	5395 SD733	5640 SD860	6300 SD794	10230 SD608	5880 SD1132
Phase 1 (-18 1 st min)	PaCO2	40,9 SD1,4	42,4 SD1,0	42,1 SD3,9	46,0 SD0,9	49,9 SD1,0	41,7 SD0,4	49,6 SD1,1	51,9 SD0,8
Propofol mg/h 151 [101-198];SD30,4		161	113	131	149	198	152	180	101
Phase 4 (-18 1 st min)	PaCO2	39,3 SD0,5	41,7 SD0,8	44,7 SD1,7	50,0 SD0,7	48,1 SD1,1	37,4 SD1,5	46,0 SD1,0	43,5 SD3,2
Propofol mg/h 240 [122-370];SD75,3		260	122	260	260	370	203	220	160
Phase 3 (-18 1 st min)	PaCO2	41,0 SD1,3	42,0 SD1,5	41,8 SD1,4	51,7 SD1,8	48,9 SD1,9	38,9 SD1,3	46,4 SD1,0	42,9 SD4,5
Propofol mg/h 214 [124-271];SD46,4		217	166	238	241	271	194	211	124
All Phase 1 n=8	VE	6500 SD1381 on Propofol 151 mg/h [101-198]							
Phase 1(-18 1 st min)	PaCO2	45,4 SD4,4							
Phase 4 (-18 1 st min)	VE	6710 SD1790 on Propofol 240 mg/h [122-370]							
Phase 4 (-18 1 st min)	PaCO2	43,7 SD4,2							
Phase 3 (-18 1 st min)	VE	6840 SD2098 on Propofol 214 mg/h [124-271]							
Phase 3 (-18 1 st min)	PaCO2	42,8 SD4,9							
									Median ± SD

Table 2: Medians (±SD) for individual respiratory data on NRM. LE; TO; DO; BA; PE; TI; BR and MA are nicknames to identify each pig (2).

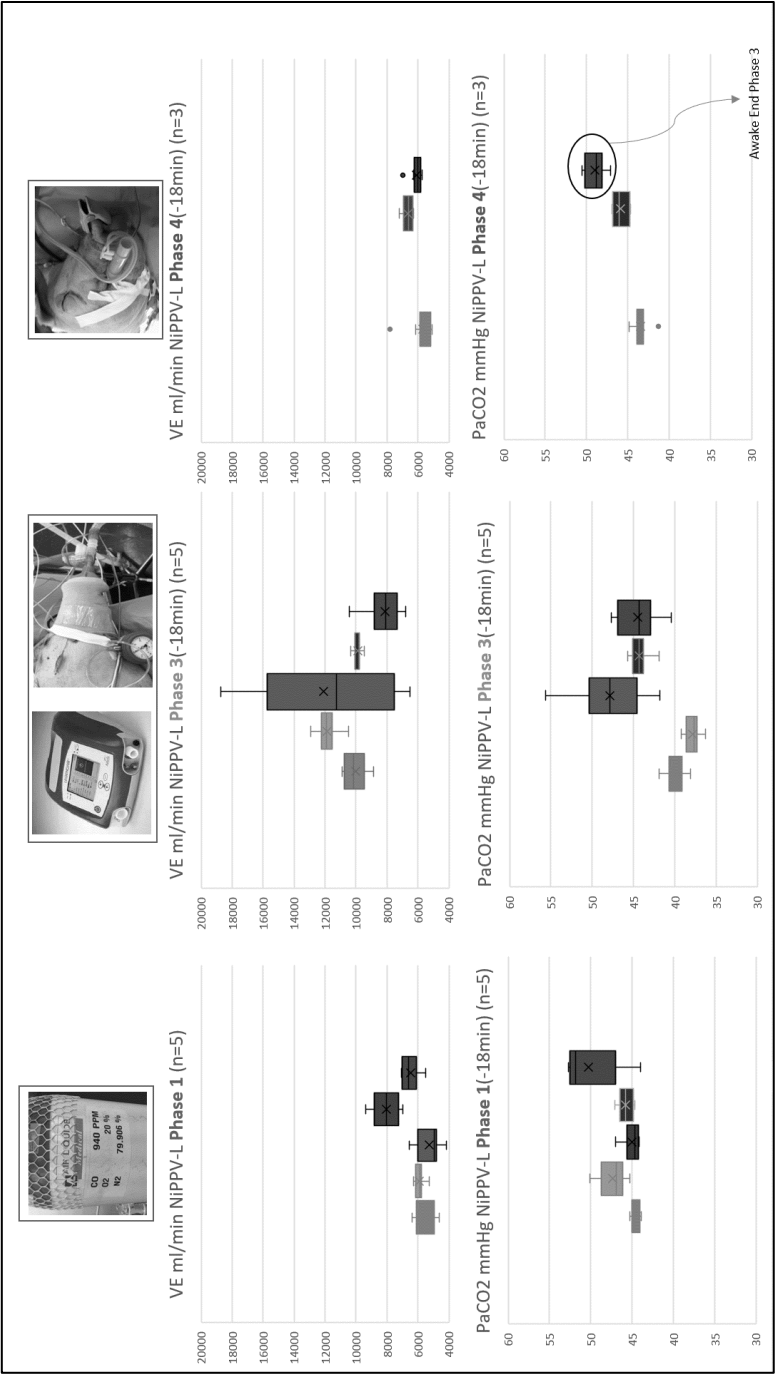


Fig. 10: Box plots for individual respiratory data on NiPPV-Leg. From left to right: BA, PE, Tl, BR and MA (2).

NIPPV-Leg		BA NIPPV-L 81kg	PE NIPPV-L 74kg	TI NIPPV-L 76kg	BR NIPPV-L 76kg	MA NIPPV-L 78kg
Phase 1 (all)	VE	5760 SD629	5880 SD293	4950 SD768	8030 SD839	6630 SD552
Phase 4 (-18 1 st min)	VE	5400 SD956			6460 SD159	6175 SD426
Phase 3 (-18 1 st min)	VE	10140 SD711	11900 SD630	11270 SD4355	9800 SD242	8100 SD1006
Phase 1 (-18 1 st min)	PaCO ₂	44,6 SD0,5	46,9 SD1,7	44,7 SD1,1	45,8 SD0,9	51,8 SD3,4
Propofol mg/h 169 [100-275]; SD63		275	185	169	165	100
Phase 4 (-18 1 st min)	PaCO ₂	43,6 SD1,1			46,0 SD1,1	48,9 SD1,2
Propofol mg/h 160 [100-340];SD104		340			160	100
Phase 3 (-18 1 st min)	PaCO ₂	39,5 SD1,1	37,7 SD0,9	47,9 SD3,9	44,4 SD1,0	44,3 SD2,3
Propofol mg/h 160 [100-301];SD75		301	202	160	160	100
All Phase 1 n=5	VE	6120 SD1162 on Propofol 169mg/h [100-275];SD63				
Phase 1(-18 1 st min)	PaCO ₂	45,6 SD2,8				
Phase 4 (-18 1 st min)	VE	6175 SD697 on Propofol 160 mg/h [100-340]; SD104				
Phase 4 (-18 1 st min)	PaCO ₂	45,4 SD2,6				
Phase 3 (-18 1 st min)	VE	10080 SD2440 on Propofol 160mg/h [100-301];SD75				
Phase 3 (-18 1 st min)	PaCO ₂	42,85 SD4,2				
						Median ± SD

Table 3: Medians (±SD) for individual respiratory data on NIPPV-Leg. BA; PE; TI; BR and MA are nicknames to identify each pig (2).

