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Carbon Monoxide Rebreathing in Endurance Sport: From Diagnostic Total Haemoglobin Mass Assessment to Prohibited Non-Diagnostic Use - A Critical Narrative Review of Performance Effects, Safety, and Anti-Doping Regulation

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Abstract

Background: Diagnostic carbon monoxide (CO) rebreathing measures total haemoglobin mass (tHb-mass), whereas repeated non-diagnostic exposure has been proposed as an erythropoietic intervention in endurance sport.

Aim: To critically evaluate diagnostic validity, performance effects, safety, anti-doping detection, and current regulation.

Material and methods: A structured narrative review used a targeted PubMed update, citation chaining, and official WADA/UCI documents. Thirty-three full-text-accessible sources were selected purposively and synthesized thematically.

Results: Standardized CO rebreathing can provide reproducible tHb-mass estimates, but procedures are not automatically interchangeable. Acute CO impairs oxygen-dependent exercise. Repeated exposure increased tHb-mass in some small trials, yet effects on VO2max and sport performance were inconsistent; the 2026 crossover trial reported haematological expansion without performance improvement. Long-term safety is unquantified. WADA M1.4 prohibits non-diagnostic equipment-mediated CO delivery, while UCI rules impose additional cycling-specific conditions.

Conclusions: Diagnostic measurement and performance-oriented exposure must be separated. A tHb-mass increase is not proof of ergogenic benefit, safety evidence is inadequate, and non-diagnostic equipment-mediated use is prohibited.

Keywords: carbon monoxide; total haemoglobin mass; endurance performance; erythropoiesis; blood manipulation; anti-doping; narrative review

1. Introduction

Total haemoglobin mass (tHb-mass) is the circulating quantity of haemoglobin and is conceptually different from haemoglobin concentration, which varies with plasma volume. Across healthy and athletic populations, haemoglobin-related variables are strongly associated with maximal oxygen uptake (VO2max), while VO2max itself is an important but incomplete determinant of endurance performance and health [1,2]. Carbon monoxide rebreathing uses a controlled tracer exposure to estimate tHb-mass and derived red-cell, plasma, and blood volumes; contemporary methodological guidance describes its physiological assumptions, calculation, and quality-control requirements [3].

The expression 'CO rebreathing' can obscure two fundamentally different practices. Diagnostic use administers the smallest validated tracer exposure needed to answer a measurement question. Repeated non-diagnostic use administers CO to provoke erythropoietic or other adaptations. The apparatus may look similar, but purpose, frequency, expected benefit, acceptable risk, documentation, and regulatory status differ. Recent reviews therefore warn against both stigmatizing legitimate measurement and normalizing a hazardous performance-oriented intervention [4,5].

The ergogenic hypothesis is biologically plausible but not self-proving. Repeated CO exposure has increased tHb-mass in a controlled study of trained men, acute CO has impaired threshold-related exercise, an acute exposure can transiently alter erythropoietin signaling, and a 2026 crossover study found haematological expansion without improved performance [6-9]. These findings show why tHb-mass, VO2max, and sport performance must be treated as different outcome levels rather than interchangeable evidence of benefit.

Safety inference is equally difficult. Clinical guidance on CO poisoning establishes potential neurological, cardiovascular, and metabolic injury, but poisoning cohorts involve exposure conditions that differ substantially from a validated diagnostic procedure [10,11]. Conversely, the absence of major events in small, short exercise studies cannot establish the safety of repeated exposure in healthy athletes. A defensible review must therefore separate hazard identification from a quantitative estimate of risk, which is currently unavailable.

The regulatory boundary changed materially in 2025-2026. WADA added M1.4 to the 2026 Prohibited List and its explanatory material, while the UCI had already introduced medical rules and public guidance for cycling [12-15]. WADA prohibits the use of re-breathing systems or equipment to deliver CO unless it is a diagnostic procedure supervised by a medical professional. The UCI adds location, professional-supervision, interval, duplication, and documentation conditions for riders within its jurisdiction.

The controversy has also been framed through anti-doping science, exercise physiology, and ethics. A comparison with microdose recombinant erythropoietin emphasized the difficulty of

translating blood markers into performance claims, whereas recent commentaries highlighted the mismatch between uncertain benefit and potentially serious harm in healthy competitors [16-18]. These perspectives support a synthesis that integrates measurement validity, efficacy, safety, detection, and regulation rather than discussing any domain in isolation.

The present evidence base additionally includes small training studies of periodic CO exposure and altitude-plus-CO supplementation [19,20]; studies of procedure comparability and capillary versus venous sampling [21,22]; altitude, live-high train-low, oxygen-transport, and acute blood-loss evidence relevant to tHb-mass interpretation [23-27]; a sport-specific review of iron deficiency [28]; and anti-doping studies and syntheses addressing tHb-mass, blood withdrawal, the Athlete Biological Passport, and blood manipulation [29-33]. This breadth is necessary because the central question is not simply whether a biomarker can change, but whether the overall practice is valid, beneficial, safe, detectable, and permitted.

Table 1. Diagnostic and non-diagnostic carbon monoxide use in endurance sport

Dimension	**Diagnostic tHb-mass assessment**	**Repeated non-diagnostic use**
Purpose	Answer a defined measurement question	Induce erythropoiesis or another performance-related adaptation
Exposure logic	Controlled tracer exposure sufficient for measurement	Serial exposure intended to create biological change
Primary endpoint	tHb-mass/blood-volume estimate with stated uncertainty	Haematological, physiological, or performance response
Evidence	Established methodological guidance [3,21,22]	Small heterogeneous studies [6,9,19,20]
Risk-benefit	Direct diagnostic benefit with residual procedural risk	No therapeutic benefit; efficacy inconsistent; safety inadequately quantified
WADA 2026	Diagnostic exception under medical supervision [12,13]	Equipment-mediated delivery prohibited under M1.4
UCI	Permitted only under specified medical conditions [14,15]	All other inhalation protocols prohibited

Source: Own elaboration based on methodological, intervention, and regulatory sources [3,6,9,12–15,19–22].

Notes: The table distinguishes diagnostic tracer measurement performed to answer a defined measurement question from repeated CO exposure intended to induce adaptation. Recording tHb-mass does not by itself make repeated exposure diagnostic.

Research Objective

The objective of this critical narrative review was to distinguish diagnostic carbon monoxide rebreathing for total haemoglobin mass assessment from repeated non-diagnostic carbon monoxide exposure and to critically synthesize the evidence concerning measurement validity, haematological and performance effects, safety, anti-doping detection and attribution, and current WADA and UCI regulations.

Research Problems

The review addressed the following questions:

1. Under which conditions does diagnostic carbon monoxide rebreathing provide a credible estimate of total haemoglobin mass?
2. What acute physiological and exercise effects follow carbon monoxide exposure?
3. Does repeated exposure consistently increase total haemoglobin mass or improve VO2max, exercise economy, threshold-related outcomes, or sport performance?
4. What conclusions can be drawn regarding short- and long-term safety?
5. Can carbon-monoxide-related changes be specifically detected or attributed through anti-doping monitoring?
6. How do WADA M1.4 and UCI regulations distinguish permitted diagnostic use from prohibited non-diagnostic use?

Research Hypotheses

The review was guided by the following working hypotheses:

1. A standardized diagnostic carbon monoxide rebreathing procedure can provide reproducible total haemoglobin mass estimates, although different protocols and blood-sampling approaches are not automatically interchangeable.
2. Repeated non-diagnostic carbon monoxide exposure may increase total haemoglobin mass in some athletes but does not produce a consistent improvement in VO2max or sport performance.
3. The available studies are insufficient to establish the long-term safety of repeated exposure.
4. Currently available haematological biomarkers may indicate biological change but cannot

specifically attribute that change to carbon monoxide exposure.

2. Research materials and methods

2.1. Review design and objective

This article was designed as a structured critical narrative review. The topic crosses measurement science, exercise physiology, toxicology, ethics, anti-doping research, and regulation, and the available studies are too heterogeneous for a meaningful pooled effect. The objective was critical integration of the most direct and decision-relevant evidence rather than exhaustive systematic identification.

2.2. Information sources and source selection

PubMed was searched most recently on 14 July 2026. Search concepts combined 'carbon monoxide' with rebreathing, inhalation, supplementation, athlete, exercise, endurance, performance, haemoglobin/haemoglobin mass, blood manipulation, and biological passport terms. Reference lists and citing literature of pivotal method, intervention, safety, and anti-doping papers were examined. The 2026 WADA Prohibited List, its explanatory note, and the current UCI Medical Rules were checked directly on their official websites on the same date.

Sources were selected purposively for direct relevance, methodological importance, recency, and balance of positive, null, and adverse findings. Priority was given to human CO-exposure studies; guidance or validation studies for tHb-mass measurement; evidence linking haemoglobin mass with oxygen transport; clinically relevant CO toxicology; anti-doping research; recent critical syntheses; and official regulatory documents. Animal-only work, therapeutic CO-releasing materials, pulmonary diffusion testing unrelated to tHb-mass, and studies in which CO rebreathing was only an incidental outcome measurement were not used as core evidence.

2.3. Critical appraisal and synthesis

Direct exposure studies were examined for design, population, sex, comparator, exposure purpose, duration, tHb-mass, erythropoietin, iron indices, VO2max or VO2peak, threshold, economy, sport performance, adverse-event reporting, and major limitations. Measurement studies were examined for protocol comparability, sampling, co-oximetry, mixing, leakage, repeatability, and field implementation. Anti-doping sources were assessed for biological sensitivity, specificity, confounding, and attribution limits. Regulatory statements were based on official texts rather than secondary reporting.

No formal risk-of-bias instrument was applied across this mixed corpus, and no meta-analysis was attempted. Findings were synthesized thematically. Confidence descriptors are narrative rather than GRADE ratings: greater confidence reflects consistent direct human evidence with an appropriate comparator and relevant endpoint; lower confidence reflects small samples, indirect outcomes, short follow-up, inadequate blinding, or sparse safety ascertainment.

3. State of knowledge

3.1. Diagnostic assessment of total haemoglobin mass

CO rebreathing estimates tHb-mass from the change in carboxyhaemoglobin after administration of a known tracer quantity, with corrections for CO not incorporated into circulating blood. The calculation assumes accurate gas delivery, adequate intravascular

mixing, valid pre- and post-exposure sampling, reliable co-oximetry, and control of loss from the circuit or vascular compartment. The CORP guidance provides the clearest integrated account of these assumptions and of the controls required for a defensible measurement [3].

A result is procedure specific. Steiner and Wehrlin showed that CO-based rebreathing procedures and calculations cannot simply be treated as interchangeable [21]. A laboratory changing its device, dose calculation, rebreathing duration, correction factor, or analyzer may create an apparent biological change. Longitudinal monitoring should therefore use one validated protocol and report its local typical error. Duplicate determinations can improve confidence when the expected biological change is close to measurement uncertainty, but repetition must remain clinically and regulatorily justified.

Sampling is another controllable source of error. Royal et al. found comparable mean tHb-mass estimates from capillary and venous blood, but test-retest typical error was substantially larger for capillary sampling (5.5%) than for venous sampling (2.1%) [22]. The two approaches therefore should not be described as equally reliable. The sample site, warming or arterialization procedure, timing, analyzer, calibration, and handling should remain constant and be documented; sample sites or time points should not be mixed within an athlete series.

A diagnostic report should include the indication, protocol version, device, analyzer, sample site and times, administered and recovered gas, leak check, operator, recent exercise and altitude exposure, quality-control result, individual duplicate values when applicable, and laboratory-specific error. A gram value without this context is insufficient for longitudinal interpretation. It also becomes difficult to distinguish a genuine diagnostic service from repeated exposure intended to induce adaptation.

Table 2. Main validity conditions for diagnostic CO-rebreathing assessment

Component	Potential distortion	Control
Gas delivery	Incorrect tracer quantity or circuit loss	Calibrated system; leak check; record administered and recovered gas [3]
Mixing/timing	Sample taken before adequate circulation	Use protocol-specific timing and standardized posture/activity [3]
Procedure	Apparent change caused by a different method or calculation	Do not assume interchangeability; validate locally [21]
Sample site	Site or handling difference mistaken for biology	Use one validated site and fixed schedule; venous TE 2.1% vs capillary TE 5.5% in Royal et al. [22]

Component	Potential distortion	Control
Co-oximetry	Biased pre-to-post COHb difference	Same calibrated analyzer and quality-control process [3]
Repeatability	Technical variation mistaken for adaptation	Report local typical error and duplicate values when justified [3,21]

Source: Own elaboration based on methodological and validation studies [3,21,22].

Notes: COHb, carboxyhaemoglobin; TE, typical error; tHb-mass, total haemoglobin mass. Capillary and venous sampling produced comparable mean tHb-mass estimates, but capillary sampling showed greater test–retest error than venous sampling (5.5% vs 2.1%).

3.2. Total haemoglobin mass, oxygen transport, and performance

The relationship between haemoglobin and VO2max is strong enough to make tHb-mass a biologically relevant marker, but it is not deterministic [1]. Oxygen uptake reflects the product of cardiac output and the arterial-venous oxygen-content difference. Increasing circulating haemoglobin may increase potential arterial oxygen content, yet pulmonary diffusion, maximal cardiac output, vascular conductance, tissue extraction, mitochondrial capacity, economy, pacing, and fatigue resistance can remain limiting [2,25].

Altitude evidence illustrates both responsiveness and variability. Meta-analysis of optimized CO-rebreathing measurements showed a mean increase in haemoglobin mass after altitude training but substantial between-athlete variation [23]. After normobaric live-high train-low exposure, tHb-mass contributed to VO2max change without explaining the entire response [24]. These findings make altitude a useful physiological comparator, not proof that an artificial CO protocol reproduces all central and peripheral adaptations of hypoxic residence.

Acute blood loss supplies a complementary causal model. In the study by Skattebo et al., removal of 150 mL did not significantly reduce VO2max, whereas 450 mL reduced VO2max by approximately 7% in a small male sample [26]. The graded response supports the role of circulating haemoglobin while showing that the functional effect depends on magnitude, compensatory plasma changes, iron availability, and timing.

Outcome scaling also matters. Absolute tHb-mass and absolute VO2max describe whole-body oxygen transport, while ratios to body mass or lean mass answer different questions. A ratio can change because its denominator changes. Trials should prespecify the expression relevant to the sport and report both biomarker and performance outcomes rather than selecting the most favorable scale after analysis.

3.3. Acute CO exposure and erythropoietic signaling

CO binds haemoglobin with high affinity, reduces available oxygen-binding capacity, and shifts the remaining oxyhaemoglobin dissociation relationship. Direct intravascular and

intracellular observations during exercise showed impaired skeletal-muscle oxygenation even with mild carboxyhaemoglobin exposure [27]. This mechanism predicts an acute performance cost when exercise begins before sufficient clearance.

Kontro et al. provided modern controlled evidence. After CO exposure, VO2peak was 4.2% lower, peak power 3.3% lower, and the respiratory compensation point 6.3% lower; nine of 16 participants could not maintain the workload previously identified as maximal lactate steady state [7]. The response varied with training status, but the central observation was impairment rather than an acute ergogenic effect.

An acute endocrine response should not be confused with a durable haematological adaptation. DiMarco et al. found that CO, hot-water immersion, and their combination transiently increased erythropoietin, with an average response in women but not men and no clear additive effect of combined stimuli [8]. The study was mechanistic: it did not establish red-cell expansion or improved performance. Exposure-to-test timing and pre-exercise carboxyhaemoglobin therefore need explicit reporting in every efficacy study.

3.4. Repeated non-diagnostic exposure and efficacy

The small intervention literature gives a mixed signal. Schmidt et al. studied 22 moderately trained men for three weeks. tHb-mass increased from 919 +/- 69 to 962 +/- 78 g in the CO group, approximately 4.8%, and remained elevated during follow-up. Ferritin fell from 106 +/- 37 to 72 +/- 37 ng/mL. VO2max showed a positive trend and correlated with individual tHb-mass change, but the group-level performance evidence was not definitive and no sport-specific competition endpoint was included [6].

Wang et al. randomized 12 male collegiate soccer players to four weeks of treadmill training with or without periodic CO exposure. Within the CO group, tHb-mass increased by 3.7%, VO2max by 2.7%, and submaximal oxygen uptake decreased by about 4%, interpreted as improved running economy [19]. Only six participants were assigned to each arm, and the report emphasized within-group significance. A change from baseline in one group is not by itself proof that the between-group treatment effect is real.

Urianstad et al. compared altitude training supplemented with CO, altitude training alone, and sea-level training in 31 male cyclists. The combined condition produced a larger tHb-mass response, but maximal and submaximal performance measures did not demonstrate clear superiority over altitude alone [20]. For athletes who already use an altitude camp, the scientifically relevant effect is the increment over altitude, not merely the difference from sea-level training.

The 2026 randomized crossover study by Villanova et al. is informative because it separated haematological and functional outcomes in the same trained swimmers. Eight adults, four women and four men, entered four-week CO and air conditions, although some endpoint analyses included only six participants. CO increased tHb-mass and intravascular volumes, but muscle oxidative capacity and swimming outcomes did not improve [9]. Interpretation must remain narrow: oral iron (25 or 100 mg daily according to ferritin) was recommended, VO2max was not measured, the main maximal test was a 200 m swim, and no dedicated taper preceded post-testing. The study challenges an automatic biomarker-to-performance inference but cannot exclude an effect in a different or adequately powered endurance test.

The reviews and commentaries reach a correspondingly cautious interpretation. Biological plausibility and some positive biomarker findings coexist with null functional outcomes, heterogeneous exposure patterns, male-dominated samples, incomplete blinding, and underpowered performance tests [4,5,16-18]. The most defensible hierarchy is therefore: repeated CO can stimulate erythropoiesis under some conditions; it may increase tHb-mass in some athletes; evidence for a consistent VO2max benefit is weak; and evidence for a meaningful sport-performance benefit is weaker still.

Iron status is a potential mediator and cost rather than a minor covariate. The ferritin decline in the Schmidt trial is consistent with increased iron use for erythropoiesis [6]. Endurance athletes already experience iron deficiency through dietary, gastrointestinal, haemolytic, sweat, and menstrual pathways [28]. Future studies would need prespecified deficiency criteria, stable supplementation, menstrual and dietary characterization, and follow-up of recovery. A transient tHb-mass gain accompanied by depleted iron stores may not represent a net performance advantage.

Individual-response claims also require caution. An apparent 'responder' must first exceed analytical error and normal within-person variation. A verified tHb-mass response must then be distinguished from a VO2max response and a sport-performance response. Present sample sizes cannot support reliable selection rules based on sex, baseline fitness, iron status, or prior altitude responsiveness.

Table 3. Human studies directly informing CO exposure, haematology, and exercise outcomes

Study	Design/population	Main signal	Key limitation
Wang et al., 2019 [19]	Randomized training study; 12 male soccer players	CO arm: tHb-mass +3.7%, VO2max +2.7%, lower submaximal oxygen cost	Six per group; within-group emphasis; no competition endpoint
Schmidt et al., 2020 [6]	Controlled study; 22 trained men	tHb-mass +4.8%; ferritin decreased; VO2max trend	Men only; small; no sport-performance endpoint
Kontro et al., 2022 [7]	Randomized crossover; 20 ramp tests, 16 MLSS tests	VO2peak -4.2%; peak power -3.3%; 9/16 failed prior MLSS workload	Acute impairment, not long-term adaptation
DiMarco et al.,	Randomized crossover;	Transient EPO response,	Mechanistic

Study	Design/population	Main signal	Key limitation
2024 [8]	16 adults	larger average signal in women; no additive combined effect	endpoint; no red-cell or performance outcome
Urianstad et al., 2024 [20]	Three-group trial; 31 male cyclists	Altitude plus CO increased tHb-mass; no clear performance advantage over altitude	Co-intervention; men only; modest groups
Villanova et al., 2026 [9]	Randomized crossover; 8 trained swimmers; smaller endpoint-specific analyses	tHb-mass and volumes increased; muscle oxidative capacity and swimming outcomes unchanged	Iron supplementation; no VO2max; short swim test; no dedicated taper

Source: Own elaboration based on direct human studies [6–9,19,20].

Notes: CO, carbon monoxide; EPO, erythropoietin; MLSS, maximal lactate steady state; tHb-mass, total haemoglobin mass; VO2max/VO2peak, maximal/peak oxygen uptake. Findings are summarized descriptively because the protocols and endpoints were heterogeneous; no pooled effect estimate was calculated. Operational dosing instructions are intentionally omitted.

3.5. Safety

The intervention studies were designed primarily to detect physiological effects, not uncommon harm. Samples ranged from eight to 31 participants, follow-up was short, and adverse-event definitions were not standardized. No severe event in such cohorts cannot exclude arrhythmia, syncope, neurocognitive injury, technical failure, cumulative exposure, or harm to operators and bystanders. Repetition also multiplies opportunities for leakage, inadequate ventilation, incorrect gas delivery, and exercise before CO clearance.

Clinical CO poisoning can involve headache, nausea, confusion, seizures, myocardial injury, arrhythmia, loss of consciousness, and delayed neurological sequelae [10,11]. Symptoms and outcome are not determined by a single carboxyhaemoglobin value. These sources establish severity and vulnerable organ systems, but their exposures differ from controlled diagnostic

testing. They should inform hazard controls without being used to assign a numerical event probability to a validated diagnostic procedure.

The reverse inference is invalid as well. Methodological guidance for diagnostic CO rebreathing presumes calibrated equipment, trained supervision, ventilation, sampling, co-oximetry, and predefined exclusion or stopping criteria [3]. Tolerability under those conditions does not validate repeated performance-oriented exposure in an altitude camp, hotel room, vehicle, or improvised setting.

Safety evaluation should distinguish event probability, consequence severity, and reliability of the exposure system. Standard reporting should include person-exposures, symptoms, withdrawals, near misses, equipment faults, ambient CO, pre- and post-exposure carboxyhaemoglobin, emergency actions, and delayed follow-up. Pulse oximetry cannot quantify carboxyhaemoglobin and should not be treated as the principal safeguard.

Potential susceptibility modifiers include anemia, iron deficiency, pregnancy, cardiovascular or respiratory disease, medication, altitude, dehydration, and recent exercise. Iron depletion deserves particular attention because it may both reduce performance and represent an adverse consequence of stimulated erythropoiesis [6,28]. The current evidence is insufficient to define a safe repeated-exposure protocol, a long-term risk estimate, or a subgroup in whom performance-oriented use is medically acceptable.

3.6. Anti-doping detection and attribution

tHb-mass has been evaluated as an indicator of changes in the circulating haemoglobin pool. Pottgiesser et al. examined its combination with biological-passport information for detection of autologous blood doping [29]. Krumm et al. showed that even low-volume blood withdrawal can alter haematological passport biomarkers [30]. Together, these studies demonstrate sensitivity to blood-volume manipulation but also the importance of timing and magnitude.

The Athlete Biological Passport interprets longitudinal patterns rather than treating a single value as proof. Training load, altitude, plasma-volume shifts, illness, iron status, analytical variation, and legitimate medical procedures may confound haematological variables [31]. Reviews of blood manipulation and detection likewise emphasize that physiological plausibility, an atypical profile, and attribution to a specific prohibited method are different evidentiary steps [32,33].

A validated CO-specific detection signature is not yet established. Carboxyhaemoglobin has a limited time window and may reflect smoking, environmental, or occupational exposure. Erythropoietin, reticulocytes, ferritin, tHb-mass, plasma volume, and passport variables change on different time scales and are not individually specific. Controlled longitudinal research is needed to distinguish exposure, adaptation, and alternative explanations.

Detectability and prohibition are separate. A method can be prohibited even if every instance is not analytically identifiable, and a high tHb-mass does not prove CO use. Any attribution would require validated analysis, appropriate timing, chain of custody, exposure history, expert interpretation, and application of the rule in force on the relevant date.

3.7. Anti-doping and federation regulation

WADA M1.4 took effect on 1 January 2026 within the section on manipulation of blood and blood components. It prohibits 'the use of re-breathing systems or equipment to deliver carbon monoxide' unless the use is a diagnostic procedure under the supervision of a medical professional [12,13]. The wording targets equipment-mediated delivery and preserves a narrow diagnostic exception; it should not be paraphrased as a general ban on every environmental CO exposure.

The UCI introduced a health-based restriction on 10 February 2025 and incorporated the current relationship with WADA M1.4 into Part 13 of its Medical Rules [14,15]. Article 13.3.066 states the in- and out-of-competition prohibition on systems or equipment allowing CO inhalation. Article 13.3.067 defines the diagnostic exception: use in a medical facility, supervision by a qualified healthcare professional experienced with CO, duplicate tHb-mass measurement, a two-week interval before a second measure, and medical-file documentation for specified professional-team riders. All other CO inhalation protocols are prohibited.

Purpose is not established by the device or by writing down a tHb-mass result. A single validated assessment ordered to answer a genuine measurement question may meet the diagnostic exception. Serial exposures scheduled to provoke erythropoiesis remain non-diagnostic even if a measurement is recorded after each exposure. Repetition after technical failure or for another clinical reason must be documented and checked against the exact current rule.

Cyclists may therefore face both the WADA framework and federation-specific medical requirements. Other sports may add their own rules. Because prohibited lists and federation regulations change, the applicable document is the version effective on the procedure date. Clinicians and teams should retain a contemporaneous copy and seek written advice from the relevant anti-doping organization or federation when the classification is uncertain.

Table 4. Regulatory boundary current to 14 July 2026

Instrument	Prohibited conduct	Diagnostic exception/conditions	Practical implication
WADA 2026 Prohibited List, M1.4 [12,13]	Re-breathing systems or equipment used to deliver CO	Diagnostic procedure supervised by a medical professional	Non-diagnostic equipment-mediated delivery is prohibited at all times
UCI Medical Rules 13.3.066-13.3.067, E0226	All CO inhalation protocols outside authorized conditions	Medical facility; experienced qualified professional; duplicate	Cyclists must comply with UCI medical

Instrument	Prohibited conduct	Diagnostic exception/conditions	Practical implication
[14,15]		measurement; specified interval and documentation	rules in addition to WADA

Source: Own elaboration based on official WADA and UCI documents [12–15].

Notes: WADA M1.4 provides a narrow exception for medically supervised diagnostic procedures. UCI Articles 13.3.066–13.3.067 impose additional cycling-specific conditions. The applicable rules should be rechecked immediately before manuscript submission and before any procedure.

4. Discussion

4.1. Principal interpretation

The evidence is asymmetric. Diagnostic CO rebreathing has a defined measurement benefit and an established framework of technical controls. Acute CO exposure reduces effective oxygen transport and can impair high-intensity steady-state exercise. Repeated exposure can increase tHb-mass in some protocols, but the response is not universal and has not translated consistently into VO2max or sport-performance improvement. Long-term safety remains unknown because the studies able to detect a biomarker change are not large or long enough to detect uncommon or delayed harm. Regulation is more definitive than efficacy: non-diagnostic equipment-mediated delivery is now prohibited under WADA M1.4, with additional UCI requirements in cycling.

The central error in the public debate is outcome substitution. tHb-mass is a mechanistically relevant biomarker, VO2max is an integrated laboratory outcome, and sport performance adds economy, technique, fatigue resistance, pacing, environment, and decision-making. A statistically significant change in the first cannot be presented as proof of a meaningful change in the third. The Villanova crossover study is important precisely because the haematological and performance conclusions diverged [9].

A narrative confidence hierarchy follows. Confidence is moderate to high that a standardized diagnostic procedure can yield reproducible tHb-mass estimates within its validated setting [3,21,22]. Confidence is moderate that acute CO impairs oxygen-dependent exercise [7,27]. Confidence is low to moderate that repeated exposure increases tHb-mass consistently and low that it improves sport performance [4-6,9,16,19,20]. Confidence about long-term safety is very low because adequate surveillance does not exist; very low confidence means uncertainty, not reassurance.

4.2. Interpretation of efficacy

Study design explains much of the disagreement. Protocols differ in exposure pattern, baseline fitness, sex, iron status, training, altitude, timing of post-testing, and comparator.

Training-plus-CO requires a between-group treatment contrast, not separate within-group P values. Altitude-plus-CO should be judged against altitude alone. A crossover trial requires adequate washout, period analysis, complete data, and outcome blinding. Small samples and multiple endpoints produce unstable estimates and increase the chance of selective emphasis.

Timing can reverse the apparent effect. Testing while carboxyhaemoglobin remains elevated measures acute impairment. Testing after clearance may capture erythropoietic adaptation, and later testing measures persistence. Training quality during the exposure phase may be reduced even if final tHb-mass increases. Future reports should place exposure, carboxyhaemoglobin, training load, iron status, tHb-mass, VO2max, and performance on a common time axis.

Sex-specific conclusions are premature. Most repeated-exposure participants were men. DiMarco et al. observed an average acute erythropoietin response in women but not men [8], while the 2026 crossover study included only four participants of each sex [9]. Menstrual status, hormonal contraception, pregnancy exclusion, baseline iron, body-size scaling, and sex-by-treatment interaction should be specified prospectively rather than interpreted from tiny subgroups.

4.3. Safety, ethics, and professional responsibility

For a healthy athlete, the benefit-risk threshold should be demanding. There is no therapeutic need for repeated performance-oriented CO exposure, the functional benefit is uncertain, and the agent can cause severe neurological and cardiovascular harm [10,11,17,18]. The burden of evidence therefore rests with claims of safety and meaningful benefit. A consent form cannot neutralize occupational risk, team coercion, inadequate emergency capacity, or a prohibited-method rule.

Physicians have a defensible role in diagnostic assessment when there is a defined indication, validated protocol, trained staff, informed consent, contraindication screening, environmental controls, documentation, and compliance with current rules. They should not allow a diagnostic service to become a serial adaptation program. The same professional boundary protects athlete health and the legitimacy of tHb-mass measurement.

4.4. Research priorities

Diagnostic research should prioritize harmonization between laboratories, analyzer comparability, lower-exposure validation, standardized reporting, and field quality control. Safety research should use predefined adverse-event categories, ambient monitoring, person-exposure denominators, neurocognitive follow-up, and independent oversight. Anti-doping research should characterize the joint time course of carboxyhaemoglobin, erythropoietin, reticulocytes, ferritin, tHb-mass, plasma volume, and passport markers.

Research designed primarily to optimize a prohibited performance-manipulation protocol is difficult to justify. If future human exposure studies address a legitimate public-interest question, they require prospective registration, concealed allocation, blinded outcome assessment, an active comparator, a single primary performance endpoint, sex-balanced recruitment, prespecified iron management, independent safety monitoring, stopping criteria, and public reporting of technical faults and adverse events.

Table 5. Descriptive confidence in the evidence and research priorities

Question	Current signal	Narrative confidence	Main gap
Diagnostic reliability	Good when one standardized protocol is used	Moderate-high	Inter-laboratory harmonization and field quality control
Acute exercise effect	Impairment, not benefit	Moderate	Sport-specific time course after exposure
Repeated tHb-mass response	Increase in some protocols	Low-moderate	Heterogeneity, small samples, iron and sex effects
Endurance performance	Inconsistent; 2026 crossover result negative	Low	Blinded, adequately powered sport-performance trials
Long-term safety	Unknown with plausible severe harm	Very low	Surveillance, person-exposures, delayed follow-up
Detection/attribution	Longitudinal markers may inform but are nonspecific	Low	Validated CO-specific time-course evidence

Source: Own elaboration based on the evidence synthesized in this review.

Notes: Confidence categories are narrative descriptors rather than formal GRADE ratings. They reflect study design, consistency, directness, sample size, and the clinical or performance relevance of the evaluated endpoint.

5. Conclusions

Diagnostic CO rebreathing and repeated non-diagnostic CO delivery are scientifically, ethically, and regulatorily distinct. A standardized diagnostic procedure can provide credible tHb-mass and blood-volume information when gas delivery, mixing, sampling, co-oximetry, protocol comparability, and local error are controlled.

Repeated exposure can stimulate erythropoiesis and increase tHb-mass in some small studies, but non-response occurs and haematological gains have not reliably improved VO2max or sport performance. Acute CO can impair oxygen-dependent exercise, iron stores may be consumed, and existing trials cannot exclude uncommon, cumulative, or delayed harm.

The current evidence does not establish a favorable efficacy-safety balance for performance-oriented use. Under the 2026 WADA List, equipment-mediated CO delivery is prohibited except as a medically supervised diagnostic procedure; current UCI rules impose additional cycling-specific conditions. Future work should improve diagnostic quality, safety surveillance, and anti-doping interpretation rather than optimize a prohibited manipulation method.

Practical implications

- Use CO rebreathing only for a defined diagnostic measurement question under trained medical supervision and current-rule compliance.
- Document the protocol, analyzer, sample site and timing, quality control, local error, recent altitude and exercise, and any adverse event.
- Do not interpret a tHb-mass increase as proof of improved performance or as evidence of a unique cause.
- Do not relabel serial adaptation-oriented exposure as diagnostic testing.
- Recheck the WADA List and applicable federation rules on the date of every proposed procedure.

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