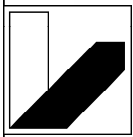




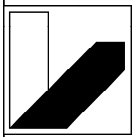
UNIVERSITÄT  
BAYREUTH

# Measurement of Hemoglobin Mass: Carbon Monoxide Method

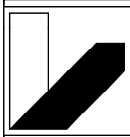
WORLD/JADA Symposium on Blood  
Manipulation and Doping in Sport  
7-8 November 2009 –Tokyo - Japan



This PPT presentation contains only a part of the slides presented at the WADA/JADA symposium in Tokyo. A great part of the data is not yet adequately published and can, therefore, not be presented here.

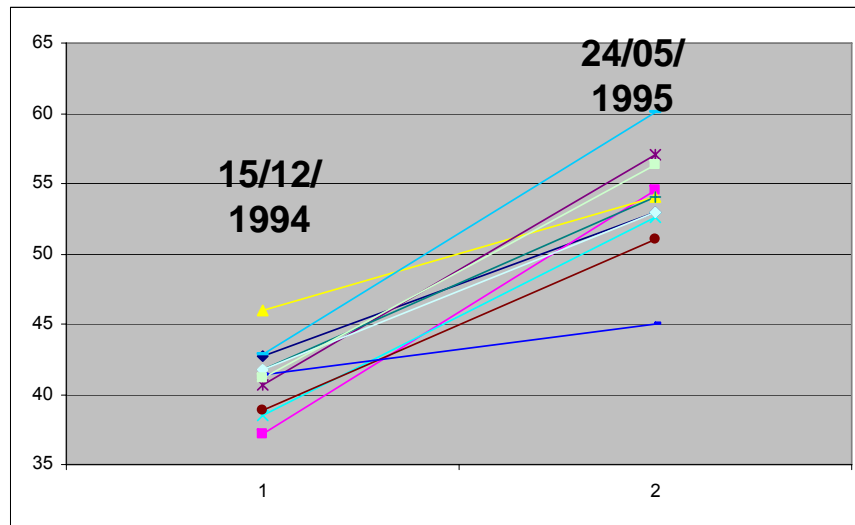


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2. The Method
3. Possible Limitation of the Method
4. tHb-mass in Athletes
5. Confounding Factors
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7. Alternative methods
8. Conclusion



## Magnitude of manipulation: Blood doping in the 90's

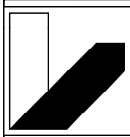
Data of a professional cycling team



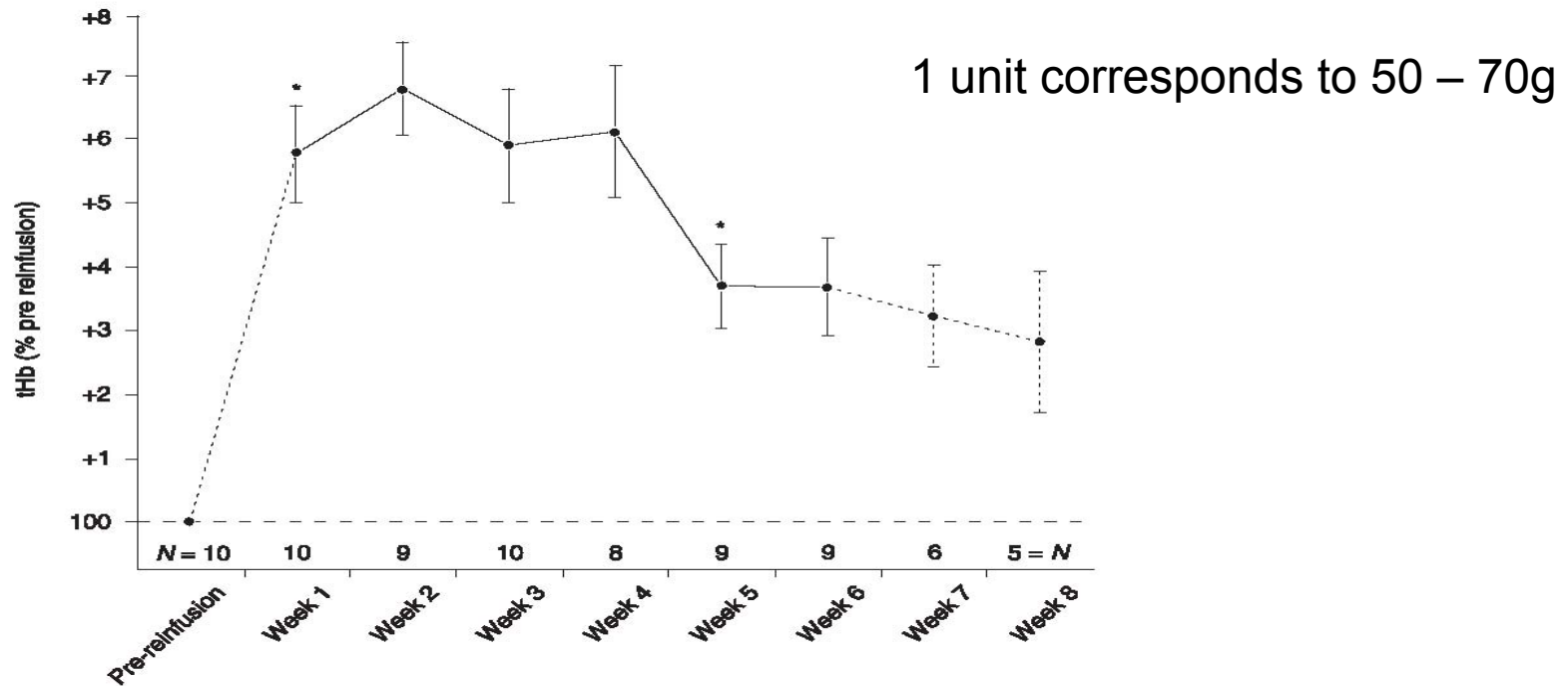
	15/12/ 1994	24/05/ 1995	Difference
<b>Hct (%)</b>	41,4 ±2,4	53,7 ±3,8	12,5 ±4,2
<b>Hb (g/dl)</b>	13,7	17,9	4,2
<b>tHb-mass (g)</b>	<b>1056</b>	<b>1377</b>	<b>321</b>



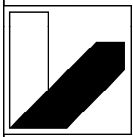
Equivalent to 4.5 blood bags



## Changes in tHb-mass (mean values) due to transfusion of blood – 1 unit

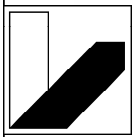


Pottgiesser, et al. *Vox Sang.* 96:119-27, 2009.



**In the upcoming years, there will be > 150 different blood-doping-substances available which can not be detected all.**

**Solution: To determine the biological effects of doping substances or doping methods and to calculate the likelihood of a manipulation.**



## Disadvantage of established markers (Hb, Hct, OFF-score)

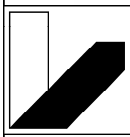
influenced by hemodilution and hemoconcentration  
due to

- acute exercise
- training periods
- exposure to altitude
- return from altitude
- fluid ingestion
- fluid loss (sweating, diarrhea)
- infusion of plasma expanders

## Advantage of total hemoglobin mass

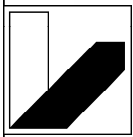
target of all blood manipulations  
not influenced by hemodilution and hemoconcentration

 Hemoglobin mass has to be considered in anti-doping fight

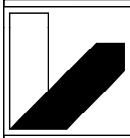


## Pre-conditions for Implementing tHb-Mass for Anti-Doping Purposes

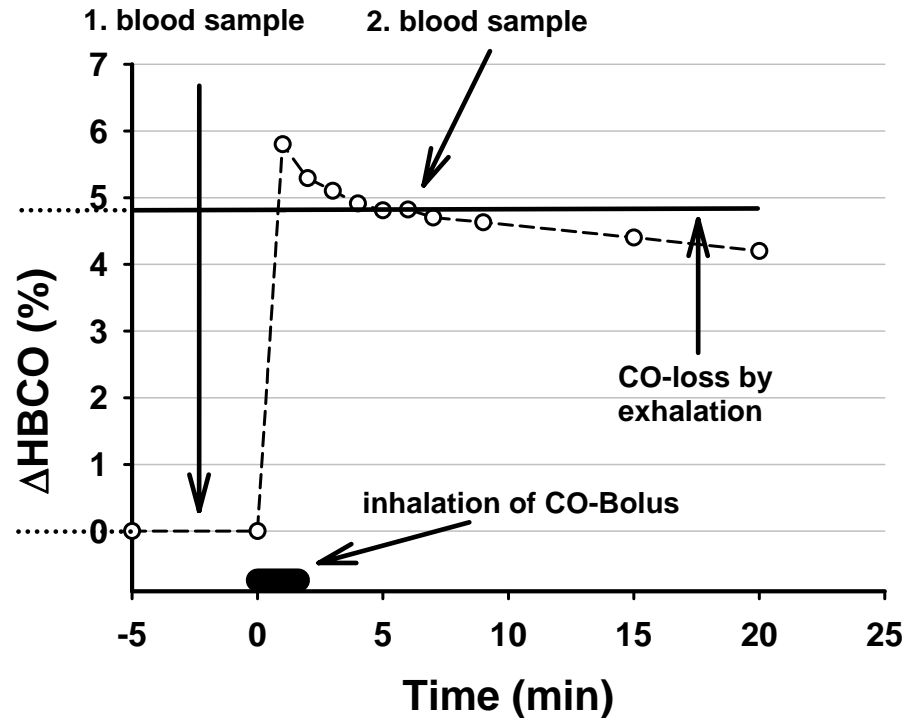
1. Existence of an accurate and reliable method for its determination
2. The method has to be easy performed, non-invasive, harmless, and not influencing health, and performance of the athlete
3. tHb-mass has to stable over long time periods
4. Effects of confounding factors have to be known
5. All oscillations of unknown origen must be considerably lower than the effects of doping



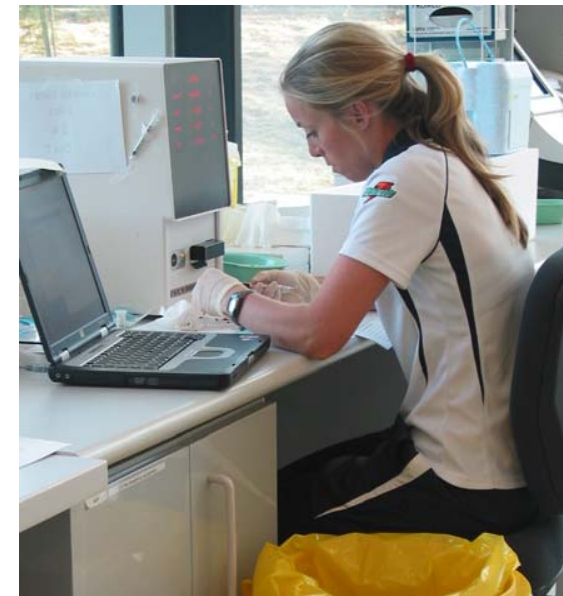
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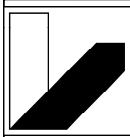


## Optimized CO-Rebreathing Method

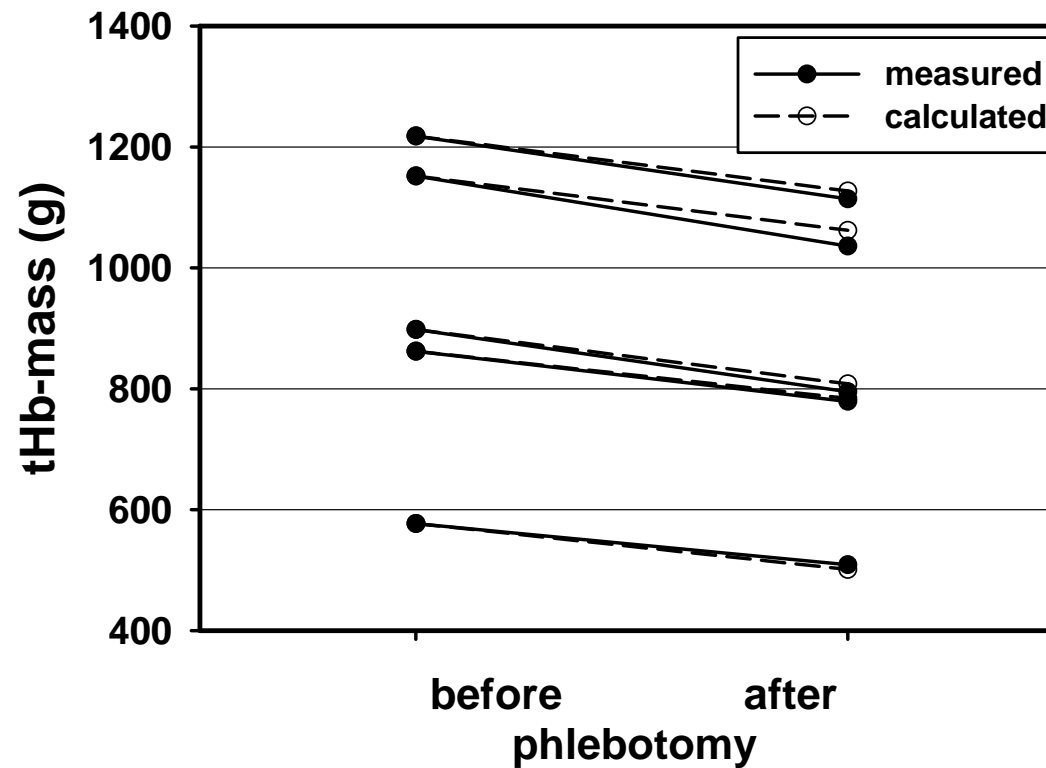


Equipment:      Spirometer  
                     Hemoximeter (OSM-3, Radiometer)  
                     CO-Tester





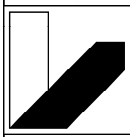
## Accuracy tHb-Mass and Phlebotomy (550ml)



Mean difference between  
measured and calculated  
values: 9 +/-12g

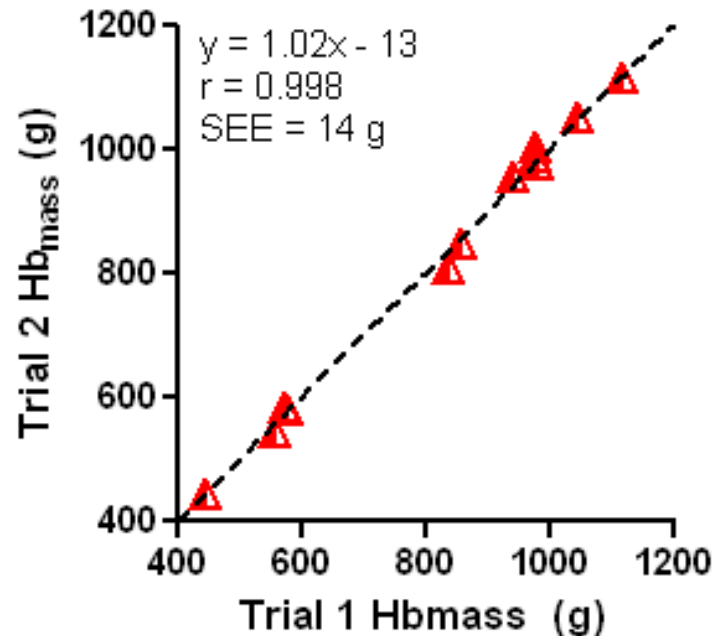
$$9/900g = 1\%$$

Schmidt & Prommer *Eur.J.Appl.Physiol* 95 :486-95, 2005.



## Reliability: Test - Retest

### Test retest reliability (n = 12)

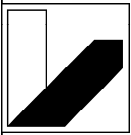


Typical Error: 1.2%  
90% Confidence Limits: 0.9-1.9%

Typical Error (within subject SD)  
 $TE = SD \text{ of difference scores} \div \sqrt{2}$   
(use log transformed data and  
back transform to express as CV)

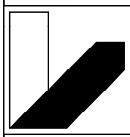
<http://sportsci.org/resource/stats/xrely.xls>

Gore et al *Med Sci Sports Exerc.* 38(6):1187-93, 2006



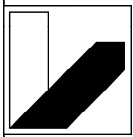
- Certification if Typical Error  $\leq 2\%$  for tHb-Mass
- 1.7% Schmidt & Prommer *Eur.J.Appl.Physiol* 95 :486-95, 2005
- 1.1% Gore et al. *Med.Sci.Sports Exerc.* 38:1187-93, 2006
- 2.1% Eastwood et al. *J.Appl.Physiol* . 104 (4):982-85, 2008
- 1.4% Prommer& Schmidt *Med.Sci.Sports Exerc.*, 40:2112-18,2008
- 1.2% Saunders *J Sci.Med.Sport* 12 (1):67-72, 2009
- 1.0% Pottgiesser, et al. *Vox Sang.* 96:119-27, 2009
- 1.9% Clark et al. *Eur.J.Appl.Physiol* . On line First March 18, 2009
- 1.3% Garvican, et al. *ECSS Conference Oslo July 2009, Abstract Book p 216*
- 2.0% Robertson et al. *Med.Sci.Sports Exerc.* 42: In Press, Feb 2010

Gore 2009

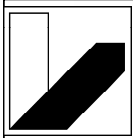


## Interim conclusion

1. The CO-rebreathing method takes in total 15 min
2. The method is highly specific and reliable (TE < 2.0%)
3. The accuracy is not influenced by diffusion of CO from Hb to myoglobin
4. The accuracy is not influenced when using different OSM3 analyzers
5. The blood samples can be stored at -70 C° for 6 months without losing accuracy

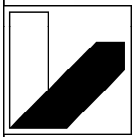


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2. The Method
3. Implementation Issues
4. tHb-mass in Athletes
5. Confounding Factors
6. Application for Anti-Doping Purposes
7. Alternative methods
8. Conclusion



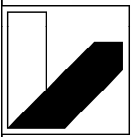
## CO-effects on the human body

1. CO dosis of the test = 50 -100 ml. Maximum effect on COHb = 5%
2. Physiological CO-production = up to 30ml/day
3. Acute Effects of Carbon Monoxide on health and well-being
  - COHb < 5%      no effects
  - COHb > 5%      in rare cases head ache
  - COHb > 10%     head ache, dizziness, weakness
  - COHb > 20%     decrease of cognitive ability
  - COHb > 30%     beginning unconsciousness
  - COHb > 40%     collaps
  - COHb > 60%     muscle cramps, coma, respiratory depression, dead

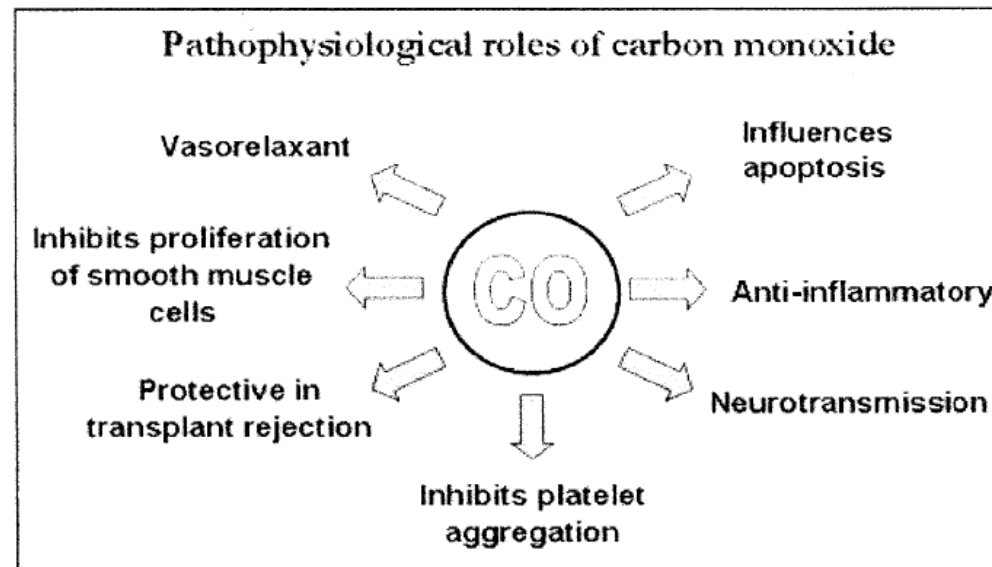


## CO-effects on the human body

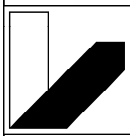
4. Positive experiences from > 10.000 CO-rebreathing tests (questionnaire):
  - no accidents
  - no circulatory disturbances
  - in very rare cases light head ache (some minutes)
  
5. Positive effects on performance 1 day after the test (examples)
  1. place (yellow jersey) at Tour de France
  - European record (swimming)
  1. place ironman (time < 8h!)
  - World best time in running (1500m)



## Pathophysiological and protective effects of CO

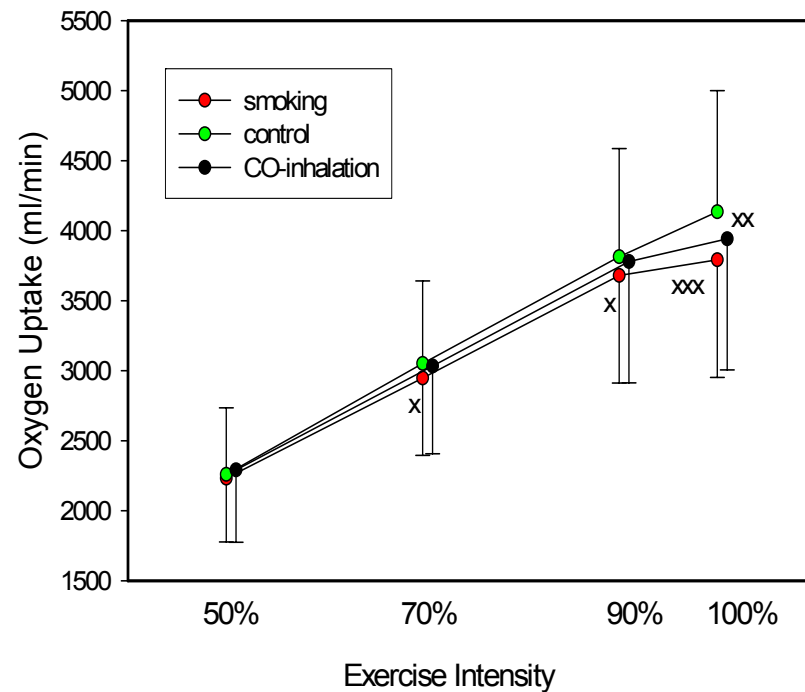


CO is currently tested for its application as a medical drug

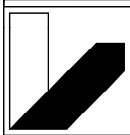


## Acute effects on performance Comparison of CO-inhalation and smoking

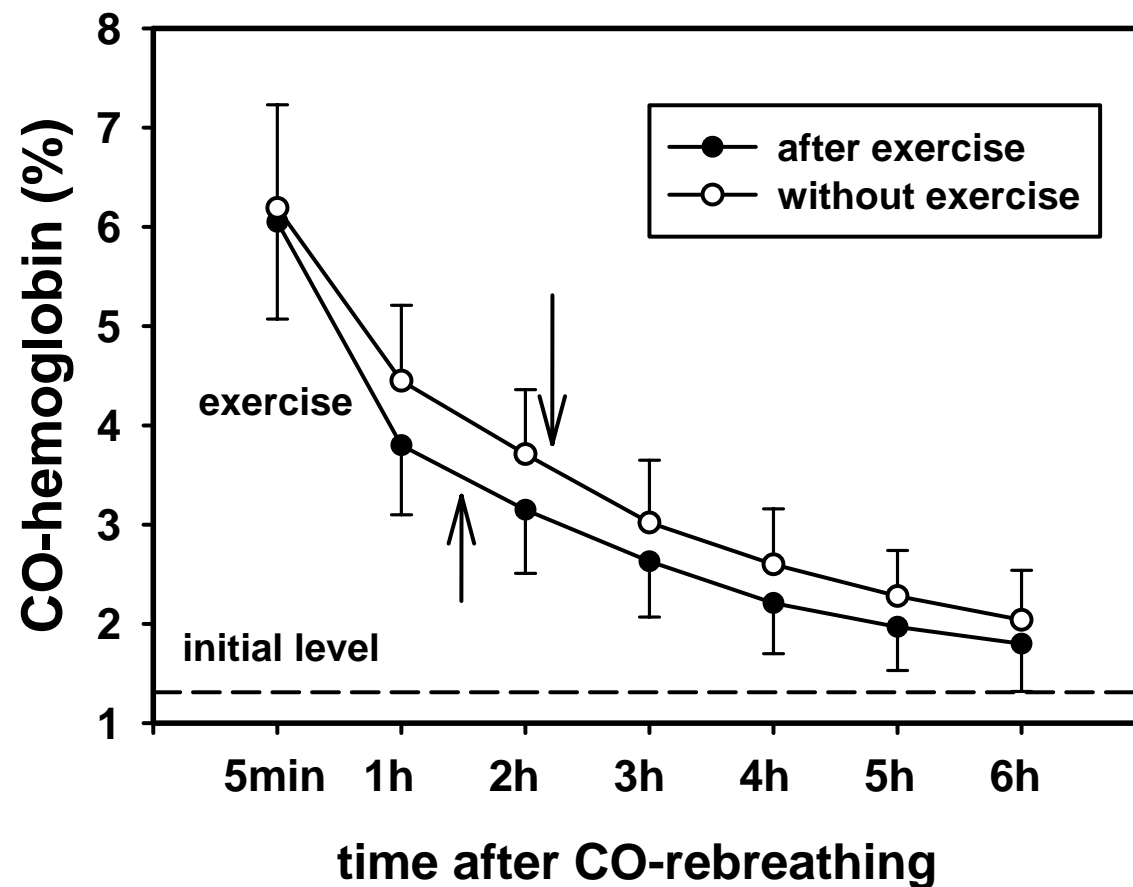
VO<sub>2</sub> peak decreases  
after CO-inhalation           -3-4%  
after smoking                   -6%



Schmidt et al. 2007



## CO-Hb half-time

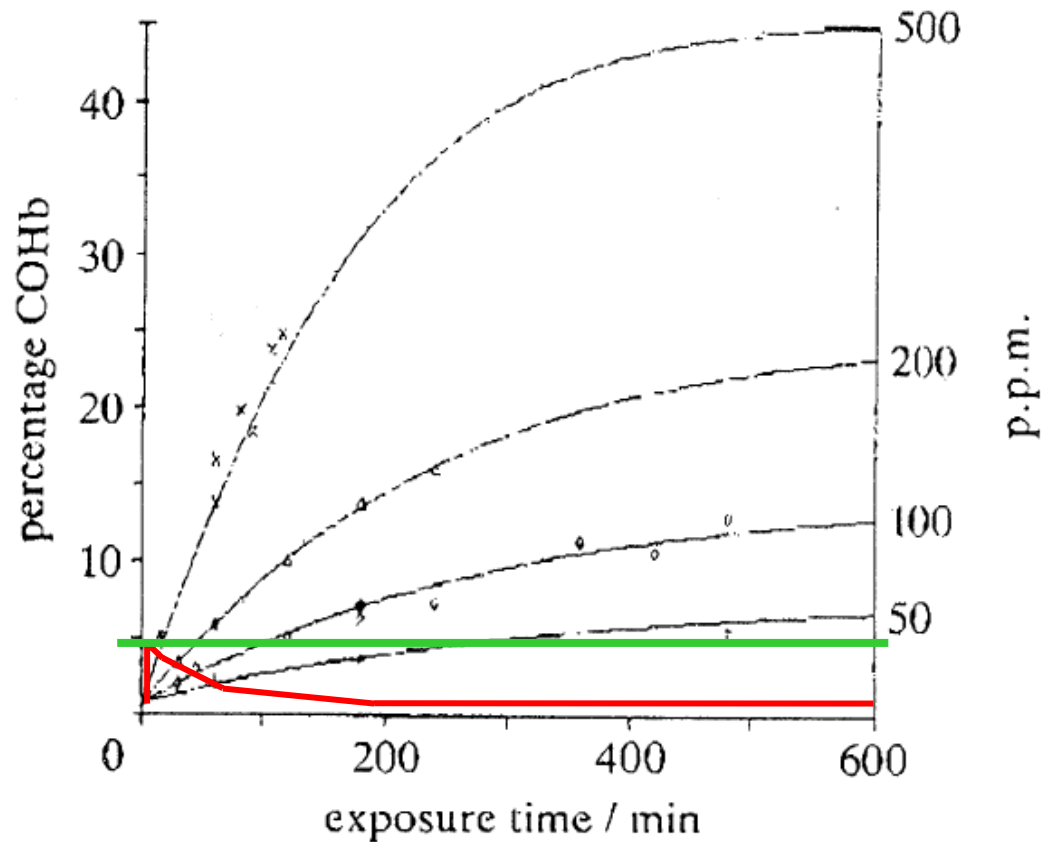


n = 13	Mean (min)	SD (min)
without exercise	132	27
with exercise	89	23

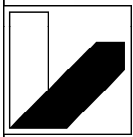
Schmidt and Prommer 2005



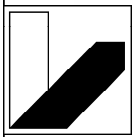
## Short-term effect of CO-rebreathing vs. long-term effect of CO-exposure



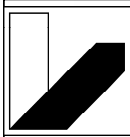
The CO-rebreathing test produces only temporarily the effect which are chronically allowed for workers



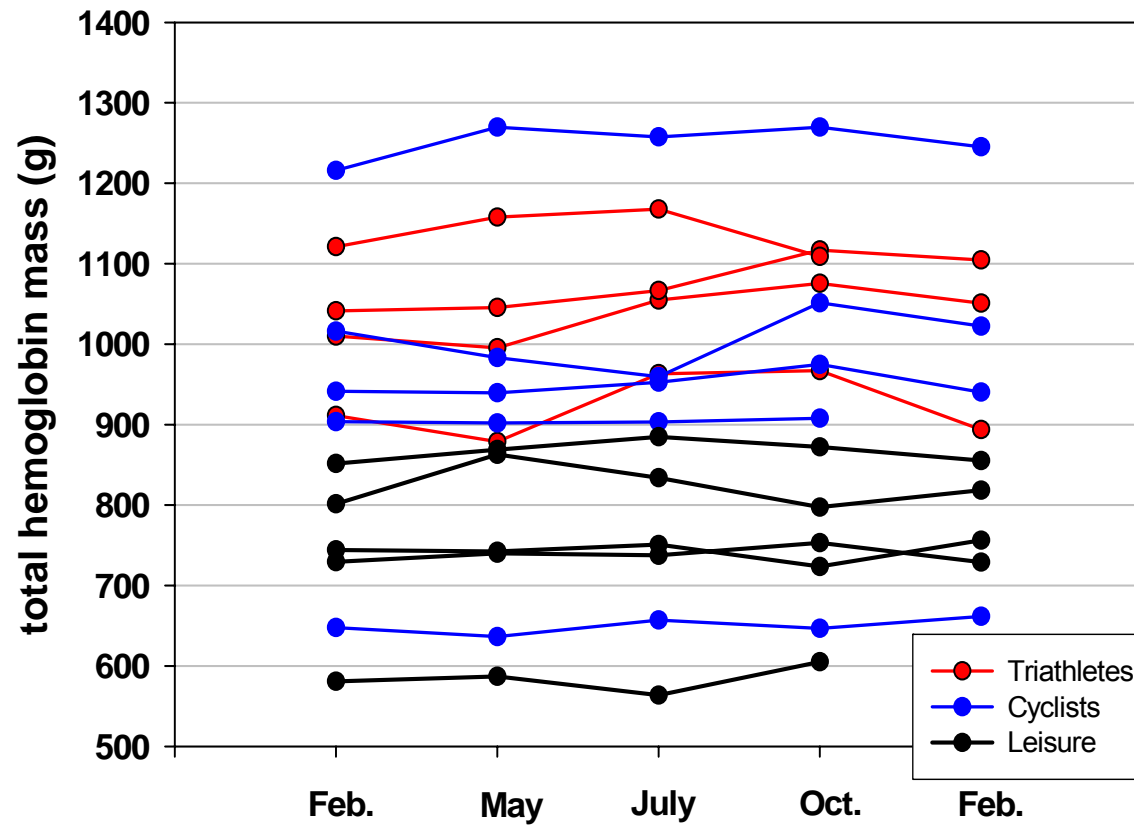
1. CO-rebreathing test transiently reduces  $VO_{2max}$  by 3-4%
2.  $t_{1/2}$  of COHb is ~ 2h
3. Baseline COHb is reached within 8 – 10h after the test
4. Max. COHb values allowed for 8h per day for workers are not exceeded when using the test
5. One day after the test athletes frequently showed best performances
6. Test can be applied 12h before a competition and any time thereafter or during training periods



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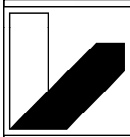
## Changes in tHb-mass due to training



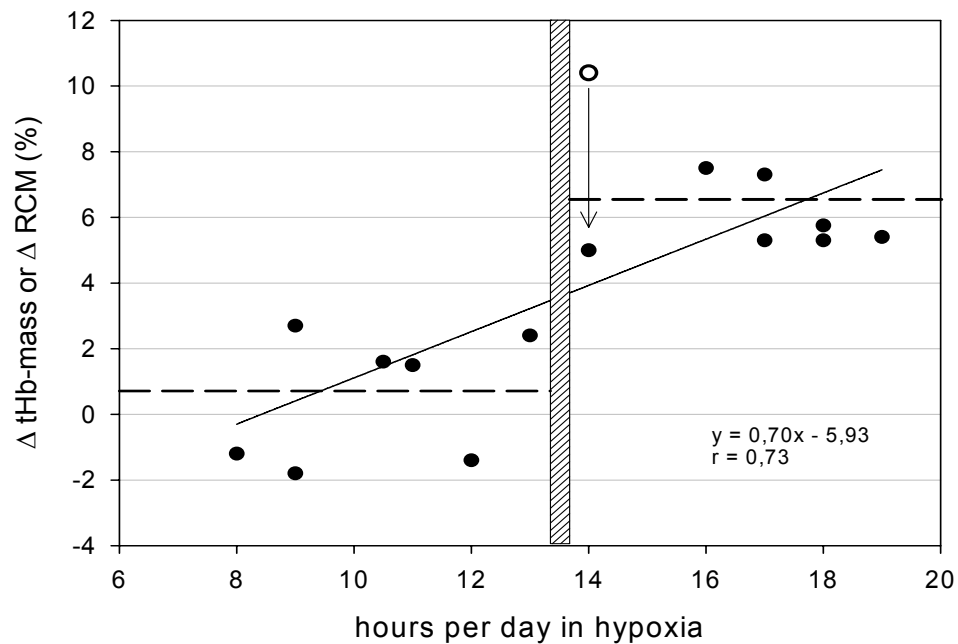
Oscillation:  $1.8 \pm 3.2\%$

TEM: 2.2%

Prommer, et al. *Med.Sci.Sports Exerc.* 40:2112-18, 2008

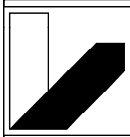


## Changes in tHb-mass due to LHTL (analysis of 14 studies)

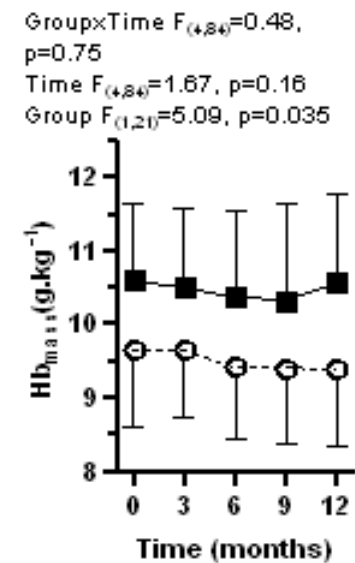
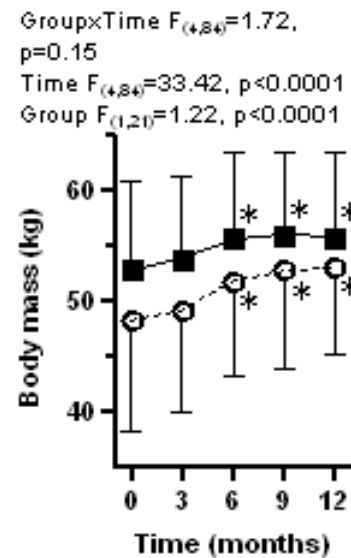
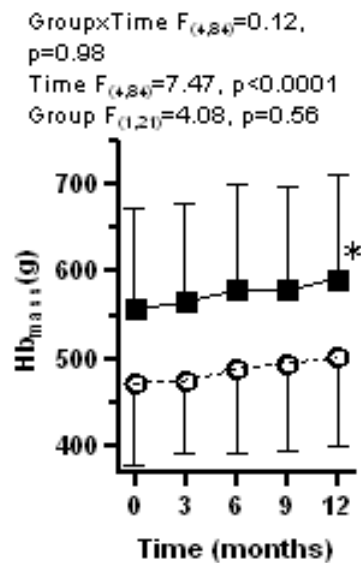


CV		♂	♀	all
	without altitude	2.27	3.33	2.81
LHTL < 10h /day	with altitude	2.44	3.14	2.77

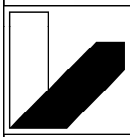
Exposure to altitude less than 12h/day has no effect on tHb-mass!



## Changes in tHb-mass (mean values) due to maturation 13/14 – 14/15 years of age

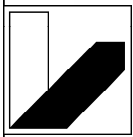


Eastwood, et al *Eur.J.Appl.Physiol.* 105:715-21, 2009.

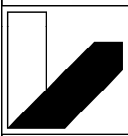


## Variation in tHb-mass

Error of the method	±1-2%
tHb-mass is stable over several years	
normal oscillation	± 2-3%
Effects of: training	± 2-3%
stage racing	± 2-3%
hypoxic training (LHTL)	± 2-3%
However, some confounding factors exist	
Effects of: natural altitude training	+4-8%
illness	-0-30%
injury	-0-10%
maturation	+0-10%
Blood manipulation	
blood infusion (1 unit)	+5-10%
EPO (Hct from 44% to 49%)	+10-12%

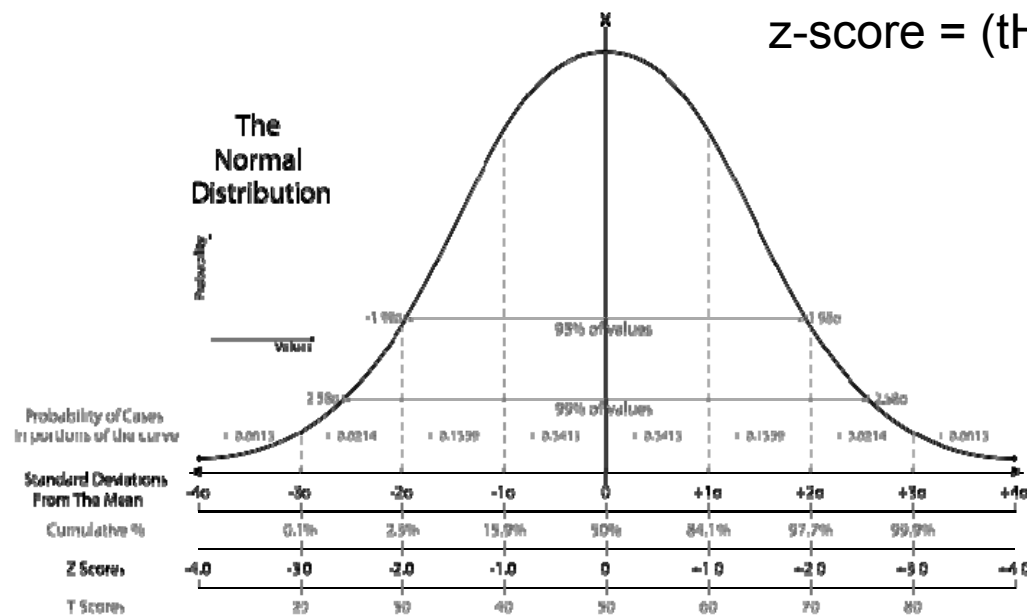


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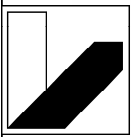


A third generation approach to detect  
Erythropoietin  
abuse in athletes

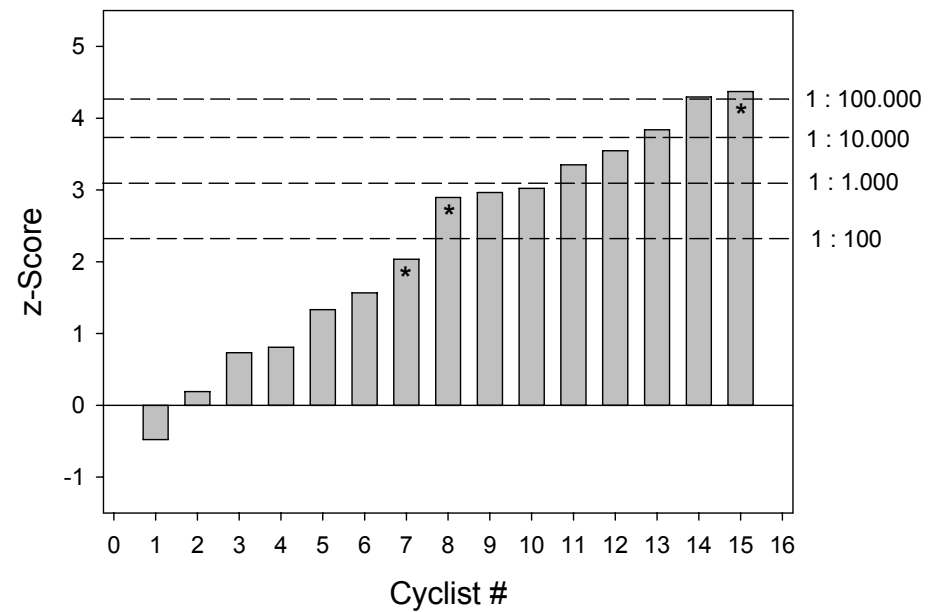
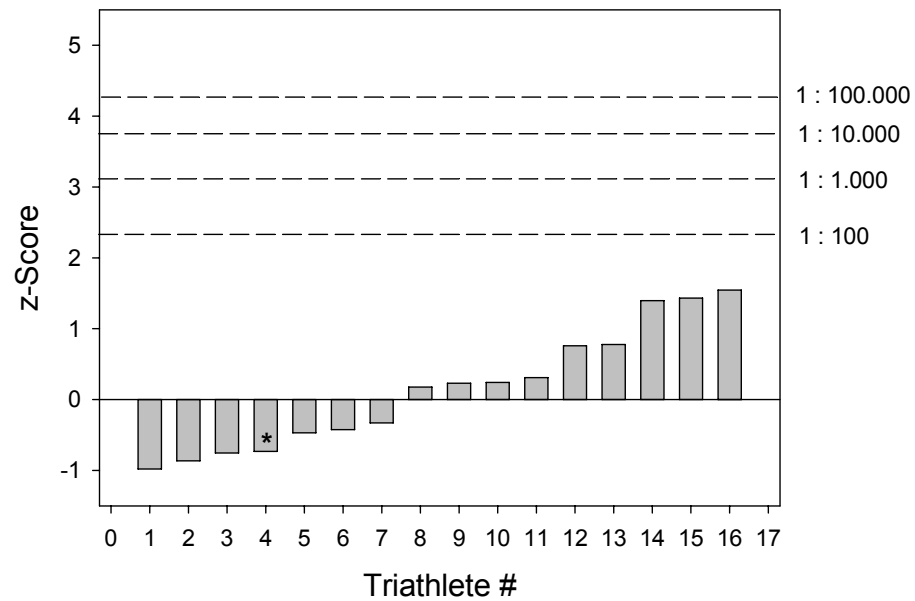
$$z\text{-score} = (\text{tHb}_{\text{current}} - \text{tHb}_{\text{mean}}) / \sqrt{(\sigma^2 (1 + 1/n))}$$



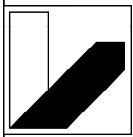
Ken Sharpe, Michael J. Ashenden, and Yorck O. Schumacher: Haematologica 2006; 91:356-363



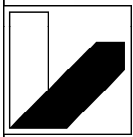
## Doping-prevalence in triathletes and cyclists in 1998/99



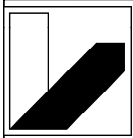
Schmidt and Heinicke 2008



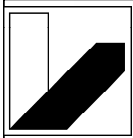
1. tHb-mass can be implemented into the models used for the biological passport
2. The sensitivity of tHb-mass can be increased in combination with reticulocytes
3. tHb-mass is the only parameter detecting blood manipulation immediately after re-transfusion
4. tHb-mass detects 2 units of blood
5. tHb-mass (in combination with reticulocytes) detects 1 unit of blood with a sensitivity of 40% for about 4 weeks



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- CO-rebreathing for tHb-mass is accurate and reliable (TE < 2%)
- COHb does not exceed critical values
- In elite athletes, variability of tHb-mass during training and racing is low (~2-3%)
- tHb- mass is influenced by confounding factors (natural altitude training, illness/injury, and maturation)
- Using tHb-mass the sensitivity to detect blood manipulations (99.9%) is > 70% for 2 units of blood and 40% for 1 unit
- Combination of tHb-mass with reticulocytes increases the sensitivity



tHb-mass is the only parameter showing the effects of all kinds of blood manipulation

Today, the CO-rebreathing method is the only method to determine tHb-mass accurately



Hemoglobin mass has to be considered in anti-doping fight

1. As a screening method
2. To exclude cheating athletes