“Boosting the 15-nitric oxide sensitivity for total hemoglobin mass measurements using an optical cavity”

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PROJECT REVIEW

The aim of all kinds of blood manipulation is to increase the total hemoglobin mass (tHb-mass), which is directly correlated to maximum aerobic power and hence performance.

To minimize these illegal practices we recommend monitoring tHb-mass of endurances athletes over time Serial measurements of tHb-mass can also be used to demonstrate objectively that an athlete has or has not used blood doping practices.

The practicability of a 15NO-rebreathing method in analogy to the established optimized CO-rebreathing method was evaluated in a scientific project by Prof. Schmidt, Prof. Bloch and Dr. Gäbler and funded by WADA (2010-2012). 15NO has several advantages compared to CO, which could lead to a broad acceptance of the method in federations and athletes.

The amount of tracer gas which has to be inhaled can be 4000-fold reduced, avoiding a toxic load for the athlete. Furthermore, NO has a 200-fold higher affinity to hemoglobin reducing the influence of possible confounding factors. We expect the NO-rebreathing technique using 15NO as innovative tracer gas as an optimal method to determine tHb-mass.

The practicability of the new 15NO-method is still limited by the sensitivity of the detector. Physiological loss mechanisms and handling of the blood samples as test routine method require a higher sensitivity than commercially available. With the further improvement of the 15NO-sensor, setup in the first project, we expect to meet the demanding detection limit for a tHb-mass routine test. As a consequence tHb-mass can be introduced as a key parameter into the athlete’s biological blood pass.

Results and Conclusions :

We are developing a new detection method for the determination of total hemoglobin mass (tHb-mass) using isotopically labelled nitric oxide (15NO) replacing the tracer gas carbon monoxide in the established CO rebreathing method. Using isotopes allows the reduction of the inhaled amounts of toxic tracer gases below international maximum allowable concentrations. In a first project we did not achieve the required sensitivity to determine the basal Hb15NO concentration. Therefore we improved in the actual follow-up

project the sensitivity of our laser spectrometer integrating an optical resonator into our spectrometer setup.
We were able to improve the sensitivity of our detector by a factor of 67.8 compared to the previous sensitivity. The achieved sensitivity limit corresponds to the application of 1 ml 15-nitrite solution with a concentration of 1.5 nM.

With the obtained sensitivity we were able to detect the basal Hb15NO concentrations of n=6 volunteers. The mean value of the basal Hb15NO concentration was 1.63 nM (± 0.16 nM). We tried to determine the tHb-mass of the volunteers applying 15 ppmV of 15NO in a 3 l anesthetic bag following the CO-rebreathing protocol. Additionally we applied 10 ppmV of 15NO continuously over a period of 60 minutes.

Following the inhalation process we observed a transient Hb15NO peak with a fast rise during the rebreathing process. Stopping the rebreathing protocol after two minutes leads to a decrease of the measured Hb15NO concentration. Ten minutes after the start of the inhalation process the Hb15NO values have reached again basal levels.

During the continuous 15NO inhalation we also observed a fast rise of the Hb15NO level followed by a slow decrease over the complete inhalation time.
In both approaches the calculated tHb-masses were too high by several orders of magnitude. In literature several interactions of nitric oxide with different hemoglobin states or forms are described which would explain the overestimated tHb-masses. In order to achieve correct tHb-masses all the relevant pathways have to be considered and the different losses of the administered tracer gas (conversion to 15-nitrate and 15-nitrite etc.) have to be quantified.