Project Review

"Detecting blood manipulation from total hemoglobin mass using ^{15}-nitric oxide as tracer gas"

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The aim of all kinds of blood manipulation is to increase the total haemoglobin mass (tHb-mass), which is directly correlated to maximum aerobic power and hence performance. When using the current doping tests it is not yet possible to detect autologous blood transfusions or the application of all kinds of erythropoiesis boosting stimulants.

To minimize these illegal practices we recommend monitoring tHb-mass of endurances athletes over time. If the individual profile deviates substantially from the expected, the athlete has to undergo further follow-ups testing. Serial measurements of tHb-mass can also be used to demonstrate objectively that an athlete has or has not used blood doping practices.

Practical experience demonstrates that the recently developed method (optimized CO-rebreathing method) is valid, very reproducible and suitable to measure routinely an athlete's tHb-mass. The practicability and significance of the method was evaluated in two scientific projects by Prof. Schmidt (WADA 2006-2007 and 2008-2010).

Nevertheless, the acceptance of this method is low in federations and athletes due to the relatively high amount of CO applied during the CO-rebreathing test exceeding the internationally existing threshold limits. The use of another tracer instead of CO, i.e. the isotopically labelled nitric oxide, has several advantages compared to the established CO-rebreathing method. The amount of inhaled tracer gas can be 4000-fold reduced, avoiding a toxic load for the athlete. This reduction is a combination of the isotopic ratio of $^{15}NO/^{14}NO$ (300) and the highsensitivity of the used detection method (Faraday-Rotation-Spectroscopy) for the measurement of ^{15}NO.

Furthermore, NO has a 200-fold higher affinity to hemoglobin reducing the influence of possible confounding factors. We expect the NO-rebreathing technique using ^{15}NO as innovative tracer gas as an optimal method to determine tHb-mass.
As a consequence tHb-mass can be introduced as a key parameter into the athlete's biological blood pass.

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Result and Conclusions

The direct transfer of the determination of tHb-mass changing only the applied tracer gas from carbon monoxide to nitric oxide is not possible, even if the experimentally derived NO number for the saturation value is used instead of Hüfner's number. The formula assumes that the applied NO amount binds completely to HbNO. Reactions with other haemoglobin forms and different binding positions have to be quantified in order not to underestimate tHb-mass.

The estimated reaction of oxyHb with nitric oxide prevents high toxic concentrations of NO in the blood leading to metHb, which is converted back to haemoglobin with a certain time constant by the enzyme cytochrome b_5-reductase. Therefore for the routine tHb-mass measurement increasing the inhaled NO amount is not useful, since it only increases the metHb and the nitrate concentration in the blood.

As a consequence the use of nitric oxide instead of carbon monoxide has an additional physiological advantage. The endogenous concentration of the target haemoglobin form (HbNO) for the routine method of tHb-mass detection is extremely small. The interaction of NO with other haemoglobin forms prevents an increase of the HbNO concentration leading to a physiological range where the HbNO is relevant. Unfortunately with the laser breakdown after the first project year we were not able to measure neither the endogenous nor the increased or even saturated HbNO concentration since it was below our sensitivity limit of the chemiluminescence sensor and the Faraday-Rotation-Spectrometer (FRS). The only way is the improvement of the ^{15}NO sensitivity of the FRS.

Considering our measurements with NEM/EDTA, the HbNO concentrations should be measured directly after drawing the blood samples. Storing the samples at -80°C lead to a decrease in HbNO concentration compared to the native samples, even when stabilizing them with NEM measurements.