Post-diving measurement of urine parameters of oxidative stress using quadrupole time-of-flight mass spectrometry (QToF-MS)

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Introduction

The aim of this study was to evaluate a feasibility-study for measuring oxidative stress markers from different radical pathways in human urine with only one preparation step. For the detection and quantification a high-resolution quadrupole- time of flight mass spectrometry system (QToF-MS), which was linked to an UHPLC-system was used. Three different radical pathways were selected: the lipid metabolism (8-iso-prostaglandin-F_{2α}); the DNA damaging (8-oxo-2-deoxyguanosine); and the observation of the hydroxyl radicals (dihydroxylated benzoates, DHBs). For this feasibility-study, we used human urine samples from a cohort of six German Navy-Divers, who had performed a total of 21 dives with a closed-circuit oxygen-rebreather.

Experimental:

- Urine samples from six trained divers (n=6, all males)
- Age was between 20-24 years (mean 23)
- A closed-circuit oxygen rebreather were used (pO2 between 1.2 - 1.6 bar)
- The duration of the dives varied between 60-90 min
- Samples: before, after and 6h after the dive
- Additional: QC samples for validation

Sample Analysis:

UHPLC Method:
- Sphinx column (1.0*100 mm, 1.7 µm particles)
- Eluent A: water + 0.1 % FA
- B: ACN + 0.1 % FA
- Column temperature: 20 °C
- Gradient with a total runtime of 15 minutes

Results:

- Median for the samples with n=63, shown in µmol/L/ mg creatinine
- Used and validated detection parameter for the quantification
  - Signal-to-noise ratio (S/N) was for all marker compounds greater than 3
  - Minimum intensity was 75 counts (threshold)
  - At least seven spectra were necessary for the peak confirmation

Discussion:

- The highest levels of the oxidative stress markers were observed directly after the dives
- This seems to be attributable to a combination of increased pO2 and the physical work, which caused a generation of ROS (due to aerobic phosphorylation in the respiratory chain)
- The formation of the radicals depends not only to the higher oxygen uptake through physical activity but also on the higher pO2 [1-4].
- the DHBs value, are comparable to the earlier data published by Gronow et al. (2005), and Kähler et al. (2013) [5-6].
- the levels of the oxidative makers after 6h resting compared to the levels prior to diving (t<sub>0</sub>)
- This trend suggests that 6h resting time provides a complete regeneration of the divers

Conclusion:

In this study,
- the feasibility for the parallel detection of different oxidative stress markers was evaluated
- the identification was possible
- also the quantification of each marker compound. Moreover:
  - a trend could be observed between the sample before and after the dive
  - as well as the samples after the dive and after 6 hours resting
  - after the dive, the values were highest
- before and after 6 h, the values were approximately similar.

References: