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FoCus - Food Chain Plus

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

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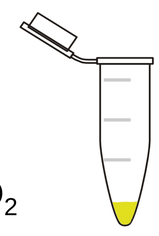
Introduction

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-prostaglandine-$F_{2\alpha}$); 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.

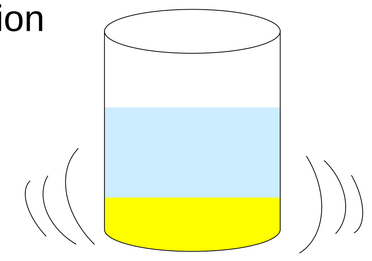
Experimental:

- 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 (pO_2 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

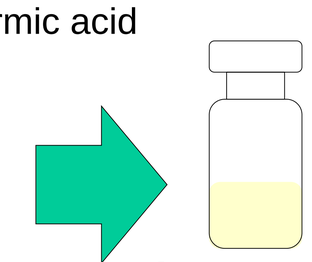


Urine

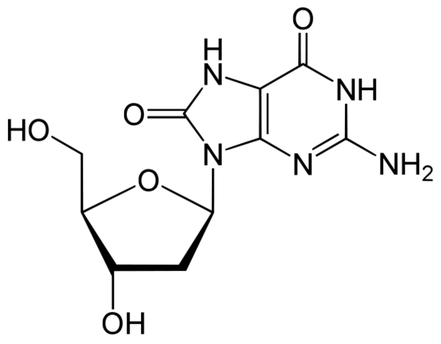
<u>Dilute and shoot preparation</u>:
250 µl urine,
add 750 µl of an aqueous formic acid solution



Shaking



Aliquotation into HPLC vials



8-oxo-2-deoxyguanosine

Sample Analysis:

<u>UHPLC Method:</u>
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

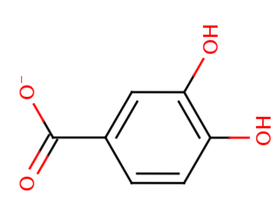
minutes	% B
0	0
0.5	0
10	95
10.5	95
11	0
15	0

<u>QToF-MS method</u>

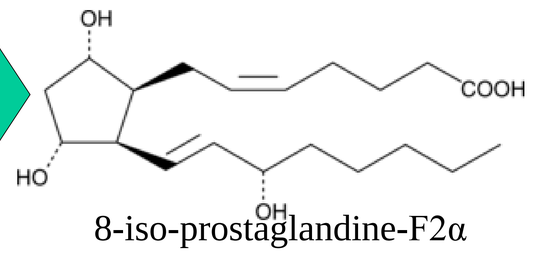
- Electro-spray ionization source
- negative mode
- Optimize for small molecules (90-300 m/z)

Transfer Area			
Funnel 1 RF	180.0 V_{pp}	Funnel 2 RF	180.00 V_{pp}
isCID Energy	0.0 eV	Hexapole RF	90.0 V_{pp}
Quadrupole			
Ion Energy	10.0 eV	Low Mass	50.00 m/z
Collision Cell			
Collision Energy	10.0 eV	Collision RF	150.0 V_{pp}
Transfer Time	60.0 µs	Pre pulse Storage	5.0 µs

isCID:In-source-collision-induced-dissociation
eV: Electron voltages
m/z Mass to charge-ratio
V_{pp} : Voltage peak-to-peak
RF: Radio frequency

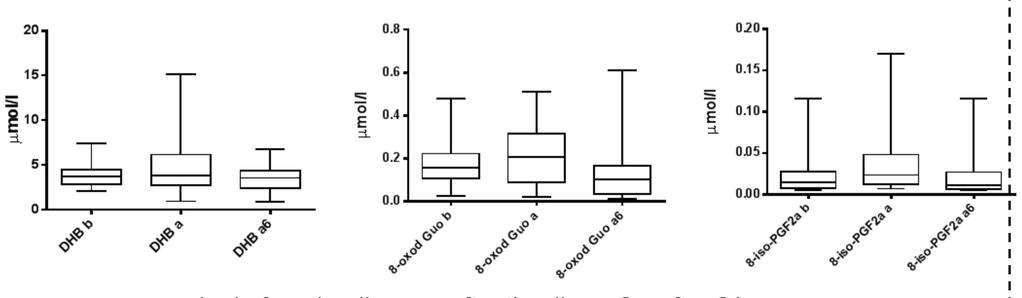


DHBs



8-iso-prostaglandine-F2α

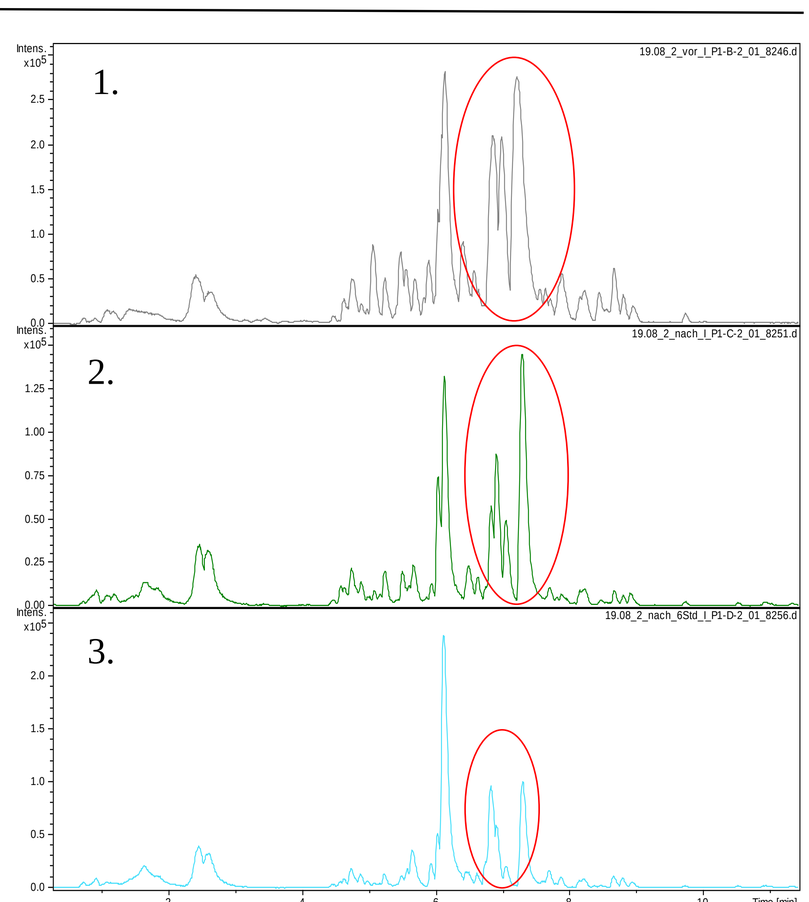
Results:



b =before the dive; a = after the dive; a6 = after 6 hours
8-iso PGF2a = 8-iso Prostaglandin F2a; 8-oxodGuo = 8-oxo-2-deoxy Guanosine;
DHB = Sum of the dihydroxylated benzoated

Median for the samples with n=63, shown in µmol/L / mg creatinine

Base peak chromatograms (BPC) of an example sample
1. = before the dive,
2. =after the dive and
3. = 6 h after the dive;
the red cycle shows a region of the chromatogram, in which the detected masses were different between before, after to after 6 h.



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

	8-Iso-prostaglandin F2α	8-oxo-2-deoxy-guanosine	Dihydroxylated Benzoates
LOD	0.013 µmol/L	0.016 µmol/L	0.387 µmol/L
LOA	0.025 µmol/L	0.032 µmol/L	0.774 µmol/L
LLOQ	0.042 µmol/L	0.055 µmol/L	1.310 µmol/L

LOD: Limit of detection; LOA: Limit of acquisition; LLOQ Lower limit of quantifications

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 pO_2 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 pO_2 [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 6 h resting compared to the levels prior to diving (t_0)
- This trend suggests that 6 h resting time provides a complete regeneration of the divers

Conclusion:

In this study,

- the feasibility for the parallel detection of different oxidative stress makers 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:

[1] Boveris A, Chance B. The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. *Biochem J* 134(3), 707–716 (1973).
[2] Turrens JF. Mitochondrial formation of reactive oxygen species. *The Journal of Physiology* 552(2), 335–344 (2003).
[3] Carpagnano GE, Kharitonov SA, Foschino-Barbaro MP, Resta O, Gramiccioni E, Barnes PJ. Supplementary oxygen in healthy subjects and those with COPD increases oxidative stress and airway inflammation. *Thorax* 59(12), 1016–1019 (2004). 10.1136/thx.2003.020768.
[4] Ferrer MD, Sureda A, Batle JM, Tauler P, Tur JA, Pons A. Scuba diving enhances endogenous antioxidant defenses in lymphocytes and neutrophils. *Free Radic. Res.* 41(3), 274–281 (2007).
[5] Kahler W, Koch I, Wohlrab C, Kowalski J, Witte J, Koch A. Influence of hyperoxia and physical Exercise on *OH-radical stress in humans as measured by dihydroxylated benzoates (DHB) in urine. *Undersea Hyperb Med* 40(3), 231–238 (2013).
[6] Gronow G, Kähler W, Koch A, Klause N. Benzoate Hydroxylation. In: *Oxygen Transport to Tissue VI* (566). Okunieff P, Williams J, Chen Y (Eds.), Springer US, 223-229 (2005).