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Targeted and untargeted metabolomics applied to occupational exposure to hyperbaric atmosphere

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ABSTRACT

Occupational exposure to hyperbaric atmosphere occurs in workers who carry out their activity in environments where breathing air pressure is at least 10% higher than pressure at sea level, and operations can be divided in Dry or Wet activities. The increased air pressure implies the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), consumption of antioxidants and reduction of antioxidant enzyme activity, causing lipid peroxidation, DNA and RNA damage. The present study was aimed to establish the relation between hyperbaric exposure and metabolic changes due to ROS unbalance, by means of the determination of urinary biomarkers of oxidatively generated damage to DNA and RNA during a controlled diving session. The investigated biomarkers were 8-oxo-7,8-dihydroguanine (8-oxoGua), 8-oxo-7,8-dihydroguanosine (8-oxoGuo), and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo).

The experimental session involved six experienced divers subjected to 3 atmospheres absolute for 30 minutes in two different experiments, in both dry and wet conditions. Urine samples were collected at t = 0 (before exposure) and 30 (end of exposure),90, 240, 480 and 720 minutes. The concentration of 8-oxoGua, 8-oxoGuo, and 8-oxodGuo was determined by isotopic dilution high performance liquid chromatography (HPLC-MS/MS). In all subjects there is an increase of the urinary excretion of 8oxo-Guo and 8oxo-dGuo, in both conditions, after 1.5 - 4 hours from the start of the experiment, and that the values tend to return to the baseline after 12 hours. Besides that, also the nucleic magnetic resonance (NMR)-based untargeted metabolomics was employed for the same objective on the same samples, confirming a different metabolic response in the subjects exposed to dry or wet conditions. In particular, the observed hypoxanthine urinary level increases during the underwater hyperbaric exposure, in agreement with the trend observed for 8-oxoGuo and 8-oxodGuo levels. Present results confirmed the relationship between exposure and oxidative stress and depicted a clear temporal trend of the investigated biomarkers. Due to the possible negative consequences of oxidative stress on workers, present research shows a new line in term of risk prevention.

1. Introduction

Occupational exposure to hyperbaric atmosphere occurs in a variety of workers who carry out their activity in environments where breathing air pressure is at least 10% higher than pressure at sea level (1 atm, normobaric atmosphere). Work operations involving hyperbaric atmospheres can be Dry hyperbaric activities (caisson, tunnel boring, hyperbaric chambers personnel) or Wet activities (oil rig, research and archeology, search and rescue, military), that is the large majority. Typically, the hyperbaric activity requires to pass from a normobaric condition to a hyperbaric one and back again at the end of work shift. In the breathing air mixture the inert nitrogen is about 79%. When external pressure rises (first phase, compression), nitrogen dissolves in body tissues and in the second phase, decompression, reverses this

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tendency when external pressure returns to normal. While the first transition is supposedly troubleless, the latter may induce bodily disorders called decompression sickness (DCS). Many factors modulate probability and severity of the DCS occurrence: mainly pressure and duration of the exposure, but also temperature, physical activity and body composition (US Navy, 2018).

In the compression phase, rising of external pressure determines an increase of breathed oxygen partial pressure and the resulting oxygen toxicity limits the deep and duration of exposure (NOAA Diving Manual, 2017). Differently from nitrogen, oxygen has a metabolic role in cellular respiration that produces a decrease in the partial pressure between inspired and expired oxygen partial pressure. The interplay between these two breathed gases is named “Oxygen window” (OW). The OW is the difference in air pressure from the inspired and the expired air that let the dissolved nitrogen return as a gas in the expired air. This phenomenon becomes greater in the decompression phase because of the difference between internal and external nitrogen partial pressure (Kot et al. 2015). The change in partial pressure of these gases in body tissues gives way to a number of biochemical reactions that are related to these pressure changes, as the increase in reactive oxygen species (ROS) production (Gröger et al. 2009). The increased air pressure implies the formation of ROS and reactive nitrogen species (RNS), consumption of antioxidants and reduction of antioxidant enzyme activity, causing lipid peroxidation, DNA and RNA damage (Gröger et al. 2009). It is well accepted that reactive oxygen species (ROS) mediate O_2 toxicity. ROS are generated as natural by-products of metabolism and they include superoxide (O_2 •), hydrogen peroxide (H_2O_2), hypochlorous acid (HClO), and hydroxyl (HO•). ROS are increased in many organs by hyperoxia. Scavenging antioxidants combat the overproduction of these reactive species. These findings were confirmed in Zwart et al. (2012) as they measured an increase in poly (ADP-ribose) (PAR), which is formed in response to DNA damage, in a saturation[1] diving study.

The present study was aimed to establish the relation between hyperbaric exposure and metabolic changes due to ROS overproduction, by means of the determination of urinary biomarkers of oxidatively generated damage to DNA and RNA during a controlled bounce[1] dive session. Since nucleic acids are the main cellular target for several of the deleterious effects in biology including mutagenesis and ultimately carcinogenesis, the three biomarkers studied are 8-oxo-7,8-dihydroguanine (8-oxoGua), 8-oxo-7,8-dihydroguanosine (8-oxoGuo), and 8-oxo-7,8-dihydro-2’-deoxyguanosine (8-oxodGuo) considering the terminology is in accordance with Cadet et al. (2012). Moreover, these metabolites have been also revealed useful oxidative stress biomarkers in other occupational categories demonstrating to provide information for the protection of workers' health even in conditions of compliance with health and safety standards, highlighting reversible effects of chronic exposure at very low doses (Tranfo et al. 2019): therefore we propose their use also for the hyperbaric health risk assessment. On this respect, data reported by Andreoli et al. (2011) showed a high intra- and inter-individual variability for all these biomarkers, particularly for 8-oxoGua, but showing no significant level variations in samples collected at different times during the same day so excluding a production related to circadian rhythm. For this reason a control group was considered not advisable.

Besides that, also the NMR-based untargeted metabolomics was employed for the same objective on the same samples. This represents a snapshot of human health status, identifying metabolic biomarkers of disease useful for diagnosis, prognosis and therapy and establishing the basis for the precision medicine (Wishart, 2016). Recently, the metabolomics is also becoming a useful tool in the occupational exposure research to identify predictive health biomarkers in workers (Vermeulen, 2017).

Finally, present research could lead to the identification of new and individual biomarkers in the prevention of negative health effects in workers occupationally exposed to hyperbaric atmospheres.

2. Material and Methods

As noted above, some hyperbaric exposures can be in Dry environments or Wet ones. From now, the experimental exposure in chamber will identified as Dry and the underwater one as Wet.

2.1. Study population

The group studied included 6 healthy volunteers (5 males and 1 female), experienced divers (instructor level) and aged 43-63 years. All the studied subjects were no smokers, they were exposed to 3 atmospheres absolute for 30 minutes in two different experiments, in both Dry and Wet conditions, as a part of a larger study (INAIL BRIC 2016, ID 25). To characterize the subject an ad hoc online questionnaire was realized in order to know his physical activity level and the diet habits. In particular, standard scores as the International Physical Activity Questionnaire (IPAQ, 2020) for physical activity and Cardioprotective Mediterranean Diet Index (Martínez-González et al. 2004), Predimed score (Martinez-Gonzalez et al., 2012) e MedDiet score (Panagiotakos et al. 2006) for diet habits were measured. Moreover, subject’s body density was estimated via in air plethysmographic measurement (BODPOD, COSMED spa, Rome, Italy).

The day before each experimental session the subjects were requested to consume a light dinner without drinking alcohol. The following morning, 3 hours before the experiment, they had a light and similar breakfast. All subject provided a urine sample before and after diving, at different time points.

The study was a non interventional /observational study on the basis of the definitions of the European Directive 2001/20/EC for which the approval of an ethics committee was not requested (The European Parliament and the Council, 2001); it was conducted according to the declaration of Helsinky and followed the International Code of Ethics for Occupational Health Professionals (International Committee of Occupational Health, 2014) also by INAIL. Collected information were used on aggregate health data of the group of workers with no possibility of individual identification and all subjects signed an informed consent together with the questionnaire.

In Table 1 are reported the main subjects’ characteristics.

2.2. Exposure in Hyperbaric chamber (Dry)

Hyperbaric chamber exposure had a depth of 3 Atmospheres absolute and had a duration of 30 minutes, with 3 minutes of decompression safety stop. Two exposures were done with 3 voluntaries each. Temperature was constant at 25 °C. The structure that hosts the chamber gave ample room for the samples collection. Dive profile was displayed by the chamber instrumentation. During the session the subjects were sitting on a bench.

2.3. Underwater exposure (Wet)

Underwater exposure was organized, for safety reason, in Bracciano Lake, in the town of Trevignano. A camp on the beach was built for samples collection and an underwater buoy system was realized to assure a safe and easy recoverability of scuba divers. The exposure was in two groups of three subjects. A Dive ambulance was floating on the underwater buoy system. An identical Wetsuit was provided to scuba divers to equalize the temperature exposure. Dive time/depth profile was recorded by an individual dive computer that recorded even air exhaustion, besides temperature and cardiac frequency. The dive

[1] Saturation dive is a compression that lasts as long a multi-shift work activity and is opposed to bounce dive in which the compression lasts for the single shift.

Table 1
Characteristics of subjects.

Subject n.	Age (years)	Height (m)	Body Mass (kg)	Body Density (kg/m^3)
1	58	1.65	85.0	0.994
2	63	1.80	97.0	0.978
3	60	1.76	113.0	0.992
4	50	1.80	75.0	0.957
5	53	1.76	84.0	0.964
6	42	1.60	67.0	0.984
Mean (St Dev)	54.33 (7.66)	1.73 (0.08)	86.83 (16.33)	0.975 (0.015)

parameters, depth and duration, were the same of the chamber ones. The water temperature at the bottom of dive was 12 °C while at the surface it was 25°. During the bottom time divers maintained the depth level with the aid of a buoyancy compensator jacket.

2.4. Urine sample collection

Urine samples were collected by the volunteers before exposure (time 0) and afterwards, at different time points: immediately after the end of the exposure (30 minutes), 90 minutes, 240, 480 and 720 minutes (4, 8 and 12 hours) after the beginning of the exposure. Urine samples were collected in sterile plastic containers, aliquoted into three 15 ml screw-cap polypropylene tubes and then transferred refrigerated to the laboratory where they were stored at −20 °C until analysis.

2.5. Targeted metabolite analysis

2.5.1. Chemicals and Supplies

The analytical reference standards of 8-oxoGua. 8-oxodGuo and 8-oxoGuo were purchased by Spectra 2000s.r.l (Rome, Italy). The isotope labelled internal standards ($^{13}C^{15}N_2$) 8-oxodGuo and ($^{13}C^{15}N_2$) 8-oxoGuo were obtained from CDN Isotopes Inc. (Pointe-Claire, QC, Canada). ($^{13}C^{15}N_2$) 8-oxoGua (98%) was obtained from Cambridge Isotope Laboratories Inc. (Tewksbury, MA, USA). Glacial acetic acid 30% NH_3, dimethyl sulfoxide, sodium hydroxide solution (50%–52% in water) and CHROMASOLV® gradient grade 99.9% methanol and acetonitrile for HPLC/MS 99.9% carbon disulfide low benzene content were obtained from Sigma Aldrich (Saint Louis, MO, USA). Purified water was obtained from a Milli-Q Plus system (Millipore Milford, MA, USA). Anotop 10LC syringe filter device (0.2 m pore size, 10 mm diameter) were purchased from Whatman Inc. (Maidstone, UK). A Kinetex Polar C18 column 100 A (150 x 4.6 mm, 2.6 m) supplied by Phenomenex (Torrance, CA, USA) were used throughout the study.

2.5.2. Analysis of urine samples HPLC with tandem mass spectrometry

The urine samples were analyzed on a Series 200 LC quaternary pump (PerkinElmer, Norwalk, CT, USA) coupled with an AB/Sciex API 4000 triple-quadrupole mass spectrometry detector equipped with a Turbo Ion Spray (TIS) probe. The concentration of 8-oxoGua, 8-oxoGuo, and 8-oxodGuo was determined by isotopic dilution HPLC-MS/MS following the method described by Andreoli et al. (2010), with some modifications such as modulation of thawing of the sample, dilution solvents, chromatographic column and mobile phases. Before the analysis, samples were thawed in lukewarm water, at about 37 °C, vortexed, centrifuged at 10,000 ×g for 5 min; the urine supernatant were added of internal standard and were injected into the HPLC-MS/MS system. The precursor/product ionic transitions monitored (positive ion mode) were 168.0 → 140.0 and 171.0→143.0 for 8-oxoGua and its internal standard (($^{13}C^{15}N_2$) 8-oxoGua) 284.3→168.0 and 287.13→171.1 for 8-oxodGuo and its internal standard (($^{13}C^{15}N_2$) 8-oxodGuo), 300.24→168.2 for 8-oxoGuo and 303.24→171.0 for the internal standard (($^{13}C^{15}N_2$) 8-oxoGuo), respectively. The 1.5 version of Analyst® software (AB Sciex, Framingham, MA, USA) was employed for instrument control. The final concentration of the analytes was expressed in μg/g of creatinine to normalize values with respect to urine dilution variability. Urinary creatinine was determined by the method of Jaffè using alkaline picrate test with UV/Vis detection at 490 nm (Kroll et al. 1986).

2.5.3. Data analysis and statistics

Data are presented as geometric mean (GM) ± geometric standard deviation (GSD). Due to the small sample size, we analyzed data with the no parametric Mann-Whitney U test to determine differences between time series. Statistical analysis was performed using IBM SPSS, release 25 (IBM, Armonk, New York, USA), with the significance level set at $p \leq 0.05$.

2.6. Untargeted metabolite analysis

2.6.1. Sample collection

The metabolomic study has been applied to one of the aliquots of the same samples collected for the targeted one. In order to minimize the inter-individual metabolic variability due to gender, only the five male volunteers were included.

2.6.2. Sample preparation

1200 μL of urine were centrifuged at 3500 g for 15 min at 4 °C to remove the cellular debris. 100 μL of a 3-trimethylsilyl-propionic-2,2,3,3-d_4 acid (TSP) in phosphate-buffered saline-D_2O solution (2 mM final concentration) as internal standard were added to 1 mL of centrifuged samples and the pH was measured and adjusted at pH 7 by adding NaOH or HCl. 600 μL of sample were submitted to the NMR analysis.

2.6.3. 1H NMR spectroscopy

^{1}H-NMR spectra were acquired at 298 K using a Bruker AVANCE 400 spectrometer (Bruker BioSpin GmbH, Karlsruhe, Germany) equipped with a magnet operating at 9.4 Tesla and at 400.13 MHz for ^{1}H frequency.

The identification step was achieved by two-dimensional experiments: ^{1}H-^{1}H Homonuclear Total Correlation Spectroscopy (TOCSY), ^{1}H-^{13}C Heteronuclear Single Quantum Correlation (HSQC) and Heteronuclear Multiple Bond Correlation (HMBC) on selected samples and confirmed by literature comparison.

One-dimensional NMR spectra were processed and quantified by using the ACD Lab 1D-NMR Manager ver. 12.0 software (Advanced Chemistry Development, Inc., Toronto, ON, Canada); 2D-NMR spectra were processed by using Bruker Top Spin ver. 3.1 (Bruker BioSpin GmbH) and MestreC ver.4.7.0.0 (Mestrelab Research SL, Santiago de Compostela, Spain). The NMR spectra were manually phased, baseline corrected and referenced to the chemical shift of the TSP methyl resonance at $\delta = 0.00$.

The quantification of metabolites was obtained by comparing the integrals of their diagnostic resonances with the internal standard TSP integral and normalized for their number of protons. Metabolite levels were expressed as μmol mmol^{-1} of creatinine, referred at its $\delta = 4.05$ resonance.

2.6.4. Data analysis and statistics

To evaluate differences in results between Dry and Wet conditions, a Partial Least Square-Discriminant Analysis (PLS-DA) was applied to the entire dataset, who was previously autoscaled before further data processing. The multivariate analysis was carried out by using Unscrambler 10.5 software (CAMO, Oslo, Norway).

Table 2
Concentrations of the oxidation biomarkers in Dry conditions (μg/g creatinine).

Dry experiment						
Time (minutes)	**0**	**30**	**90**	**240**	**480**	**720**
8-oxoGua *(μg/gcreatinine) n = 6*						
GM	42.73	38.8	40.08	-	-	-
GSD	2.43	2.00	2.29	-	-	-
8-oxoGua *(μg/gcreatinine) n = 4*						
GM	40.11	42.15	56.73	22.31	29.36	25.03
GSD	1.97	2.17	2.23	1.57	1.45	1.88
8-oxodGuo *(μg/gcreatinine) n = 6*						
GM	3.91	4.52	5.02	-	-	-
GSD	1.90	2.02	1.89	-	-	-
8-oxodGuo *(μg/gcreatinine) n = 4*						
GM	4.00	5.51	6.40	4.69	3.63	3.80
GSD	1.79	2.09	1.58	1.22	1.83	1.90
8-oxoGuo *(μg/gcreatinine) n = 6*						
GM	16.27	16.37	24.38	-	-	-
GSD	1.83	1.87	1.99	-	-	-
8-oxoGuo *(μg/gcreatinine) n = 4*						
GM	14.68	17.71	27.43	15.37	14.15	15.11
GSD	1.41	2.17	1.96	1.47	1.88	1.83

Table 3
Concentrations of the oxidation biomarkers in Wet condition (μg/g creatinine).

Wet experiment						
Time (minutes)	**0**	**30**	**90**	**240**	**480**	**720**
8-oxoGua *(μg/gcreatinine) n = 6*						
GM	22.60	22.41	22.01	26.86	22.30	25.92
GSD	1.74	1.20	1.42	1.18	1.31	2.14
8-oxodGuo *(μg/gcreatinine) n = 6*						
GM	2.92	2.93	3.76	5.87	4.90	4.20
GSD	1.33	1.13	1.35	1.22	1.48	1.70
8-oxoGuo *(μg/gcreatinine) n = 6*						
GM	20.54	20.27	22.67	35.39	30.76	11.35
GSD	1.39	1.32	1.40	1.25	1.38	1.63

3. Results and discussion

3.1. Physical activity and Diet Habits

Data from the standard IPAQ, reported a level of weekly physical activity of 1689 ± 947 MET/week. This value compared with the World Health Organization (WHO) recommendations classified our subjects in a moderate level. All subjects, matched by sex and age, ranged from moderate to high level of physical activity exerted except one who resulted at low level. Regarding diet habits, food intake was recorded in three different questionnaires to assess, in particular, the adherence with Mediterranean diet. Data acquired did not show differences respect to general Italian population regarding the diet habits. All subjects presented a good match to the Mediterranean diet as described in Panagiotakos et al., 2006.

3.2. Targeted metabolite analysis

The GM of the concentrations, with their relative GSD, of the three urinary biomarkers measured for each subject, at each time point, are reported in Table 2 for the Dry experiment separately for all the subjects (n = 6) that gave the urine samples until the 90 minutes time point and for the 4 subjects (out of the 6) who gave the urine samples until the 720 minutes time point. The zero time is set at the beginning of the dive. The timing of the sampling is not exactly the same among subjects, due to the individual timing for removing the wetsuit and to reach the toilet, but this variability has been considered negligible with reference to the time intervals between the sampling points. Related to targeted analysis, present results at time 0 are within the range of data found in a general population group tested by Tranfo et al. (2017).

In the Dry experiment 8-oxoGuo and 8-oxodGuo at 90 min showed significant different values respect to time 0 ($p < 0.05$); after the 90 minutes time point two subjects did not provide the urine samples, and therefore values measured after 240, 480 and 720 min are lower than those measured after 90 minutes but the statistical test is not applicable. Anyway, this can be interpreted as a raising peak of the values of 8-oxoGuo and 8-oxodGuo after 90 minutes from the exposure, that seem to decrease very slowly after 240 minutes (4 hours).

In Table 3 the GM of the concentrations, with their relative GSD, of the three urinary biomarkers measured for each subject, at each time point, are reported for the wet experiment.

In Wet condition, the values measured at 0, 30 and 90 minutes are lower than those found at 240 and 480 minutes (4 and 8 hours) for 8-oxoGuo and 8-oxodGuo ($p < 0.05$). The values measured at 480 are significantly different and higher from those at 720 min only for 8-oxoGuo ($p < 0.01$). This can be interpreted as a raising peak of the values of 8-oxoGuo and 8-oxodGuo at 240 minutes (4 hours), whose concentrations lower to the baseline after 12 hours only for 8-oxoGuo; for the other biomarkers there is a decreasing trend but it is not statistically significant.

In order to better visualize the biomarkers concentrations temporal trend, the mean values have been plotted vs time for the two experiments in Fig. 1 (Dry) and 2 (Wet). Due to the variability among subjects, the biomarkers are depicted scaled by value at 0 min and data reported as percentages. In the Dry experiment, the maximum was reached at 90 min whilst in the Wet one peaks were recognised later, at 240 min (Fig. 2).

From the above figures it can be seen that, even where differences are not statistically significant, there is an increase of the urinary excretion of 8-oxoGuo and 8-oxodGuo, in both experiments, after 90-240 minutes and that the values tend to return to the baseline after 12 hours. This seems to confirm the hypothesis of Haldane (Boycott et al. 1908) that after 12 hours from the dive the excess of nitrogen would be completely eliminated. The increase and the following decrease are to be ascribed to the hyperbaric exposure, as a circadian rhythm for the excretion of the considered biomarkers must be excluded (Andreoli et al. 2011) and both the experimental conditions (Dry and Wet) show similar trends even with slight differences. Likely, these differences can be ascribed to the temperature and to the low physical activity exerted during the dive in wet conditions. Moreover, wetting the face is known to trigger vagal response and physiological adjustment (NOAA, 2017).

These results are in agreement with what reported in Tranfo et al. (2019), were 8-oxoGuo is highlighted as the more suitable among the nucleic acids oxidation biomarkers to study the effect of a working shift, as it seems to be the most sensitive to short-term exposure variations; the excretion of 8-oxoGua is very variable and more complex to understand, and a convincing interpretation of results is still difficult.

Results are also comparable with another controlled experiment in underwater divers (Bosco et al. 2018) where six subjects performed two immersions at 6 hours distance, and a statistically significant increase was found only for urinary 8-oxodGuo values measured before the beginning of the experiment and after the second immersion. In the light of present results, those variations could be interpreted as the consequence of the first dive.

3.3. Untargeted metabolite analysis

NMR profiling of urine of subjects exposed to hyperbaric atmospheres has individuate some metabolites with statistically significant differences between Dry and Wet sessions. In the Table S1 the NMR signal assignment was reported. The concentrations of the metabolites were expressed as μmol/mmol of creatinine.

A PLS-DA analysis showed a good separation between urine samples collected in Dry and Wet conditions, with $R^2 = 0.85$ and $Q^2 = 0.67$ (Fig. 3).

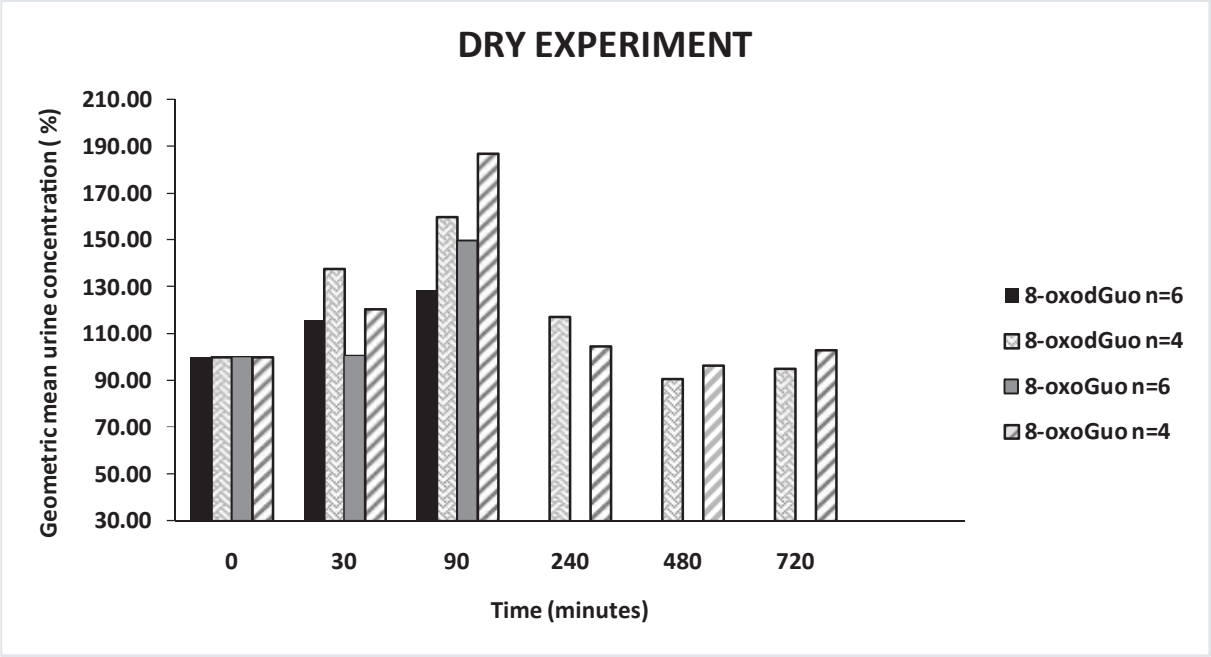

Fig. 1. Concentration of 8-oxoGuo, and 8-oxodGuo, in Dry experiment. Concentration at time 0 min is considered as 100%.

The variables that resulted statistically significant for the discrimination ($p < 0.05$) were: erythro-2,3-dihydroxybutyrate, choline, trans-aconitate and hypoxanthine for Wet condition, and 3-hydroxyisovalerate, acetate, citrate, methyl-guanidine, scillo-inositol, tartarate, tyrosine, formate, trigonelline for Dry one. The metabolite that best discriminate between the two sessions is hypoxanthine. In (Fig. 4) it is reported the boxplot for the urinary levels of hypoxanthine associated to Wet and Dry experiments; the statistical significance for the selected metabolite was determined by Wilcoxon Rank Test and achieved $p < 0.001$.

Moreover, hypoxanthine trend in the Wet experiment, as shown in (Fig. 5), highlights that urinary levels of this metabolite increase during the immersion, reaching a maximum peak around 90 minutes after the diving, and then slowly decreases to the starting levels after 6 – 12 hours. For the Dry experiment a trend cannot be recognized due to the low levels, very close to the baseline.

Differences in hypoxanthine trend between Dry and Wet conditions are likely due to the physical activity workload and to environmental changes during the experiments (Sarver et al. 2017). Indeed, as reported in Sarver et al. (2017) cooling muscles as well as physical exercise exerted could induce higher level of this purine metabolite (Fisher-Wellman and Bloomer, 2009).

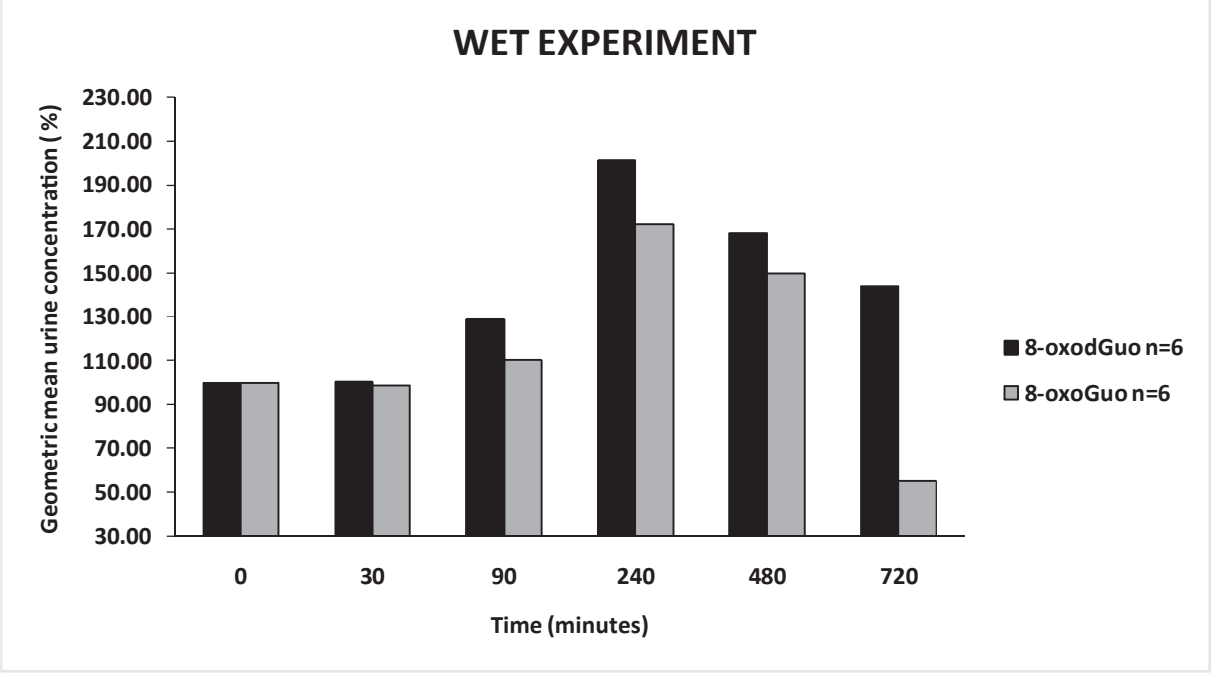

Fig. 2. Concentration of 8-oxoGuo, and 8-oxodGuo, in Wet experiment. Concentration at time 0 min is considered as 100%.

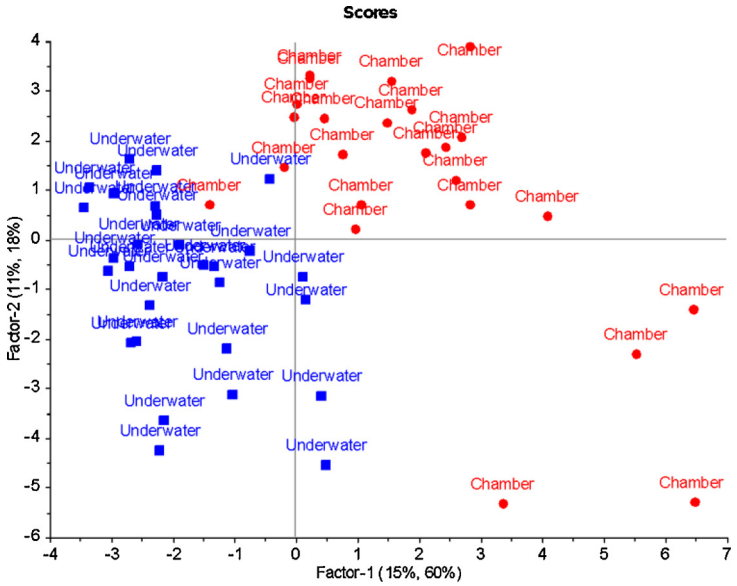

Fig. 3. PLS-DA for the two experimental sessions.

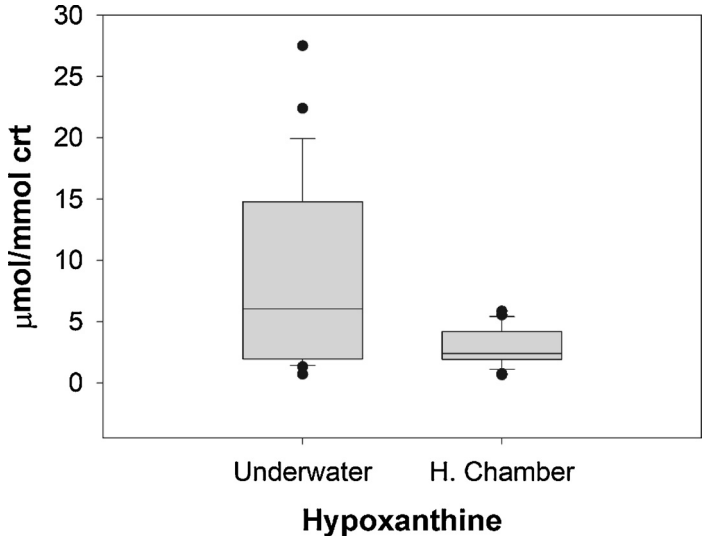

Fig. 4. Boxplot of hypoxanthine urinary levels in Wet and Dry conditions.

4. Conclusions

Even if these experiments are very preliminary and need to be confirmed by further investigations,

we think that at this stage results are already meaningful and innovative and suggest some conclusive considerations. The inter-individual variability seems to reproduce the susceptibility to diving illness, that are ascribed, in turn, to constitutional and genetical factors as reported by Levett and Millar (Levett and Millar, 2008).

There is a statistically significant increase of the urinary excretion of two of the considered biomarkers, 8-oxoGuo and 8-oxodGuo, both in Dry and Wet experiments, even if with some differences in the peak timing and among the subjects. The values tend to return to the baseline after 720 minutes (12 hours). Other oxidatively generated damage biomarkers are being studied by the scientific community, not considered in this paper, as oxidative damage to lipids and proteins, that shall be considered in a further study.

The untargeted metabolomics has evidenced the increased excretion of some metabolites after exposure, whose meaning shall be further investigated. Besides, it confirmed a different metabolic response in the subjects exposed to Dry or Wet conditions. In particular, the observed hypoxanthine urinary level increases during the underwater hyperbaric exposure, in agreement with the trend observed for 8-oxoGuo and 8-oxodGuo levels, showing the potential of this approach to identify new, early predictive effect biomarkers.

Effect biomarkers are usually correlated to dose biomarkers of exposure: in the case of exposure to chemical agents for example, their metabolites are measured to assess the individual exposure. In the case of divers, the exposure is determined by the time and depth of the immersion, but dose biomarkers are not available. The determination of the effect biomarkers before and after the immersion help to understand if there is a measurable effect and how long after the immersion it is visible; the extent of this effect, if there are differences among workers and categories of workers and which conditions can modulate this effect, like the temperature or the physical activity, or the fitness of the subjects.

There is no or poor literature on this subject, and this is a small study, so we are not yet able to make recommendations in order to reduce nucleic acid oxidation after diving, but putting the basis to be able to do this is the objective of the study. These is one of the first

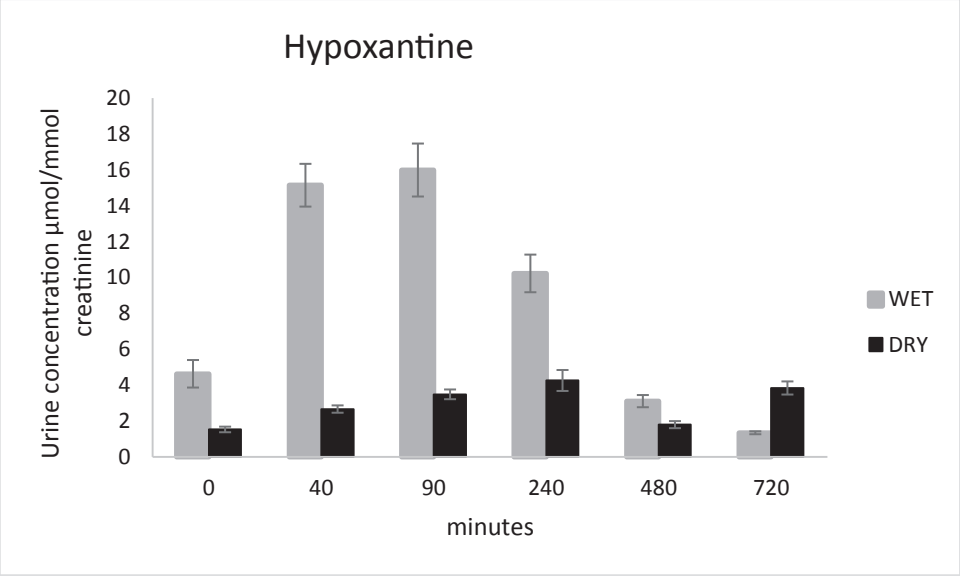

Fig. 5. Hypoxanthine trend in Dry and Wet sessions.

controlled experiment on exposure to hyperbaric atmospheres in volunteers, no-professional divers, in conditions comparable with those of occupational exposures: they will be the basis to study the effect of this risk agent in professional divers, who are regularly trained and could present different anthropometric characteristics.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Declaration of Competing Interest

The authors report no declarations of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.toxlet.2020.03.022.

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