DOI 10.1002/prca.201600017 *Proteomics Clin. Appl.* 2016, *10*, 614–620

RESEARCH ARTICLE

Effect of simulated air dive and decompression sickness on the plasma proteome of rats

Jacky Lautridou[1], Vianney Pichereau[2], Sébastien Artigaud[2], Peter Buzzacott[1], Qiong Wang[1], Benoit Bernay[3], Sabrina Driad[1], Aleksandra Mazur[1], Kate Lambrechts[1], Michael Théron[1] and François Guerrero[1]

[1] Laboratoire ORPHY EA 4324, Université de Bretagne Occidentale, Brest, France
[2] LEMAR UMR 6539 CNRS/UBO/IRD/Ifremer, Université de Bretagne Occidentale, Brest, France
[3] Proteogen SFR ICORE 4206, Université de Caen, Caen, France

Received: January 20, 2016
Revised: March 1, 2016
Accepted: April 6, 2016

Purpose: Decompression sickness (DCS) is a poorly understood systemic disease caused by inadequate desaturation following a reduction in ambient pressure. Although recent studies highlight the importance of circulating factors, the available data are still puzzling. In this study, we aimed to identify proteins and biological pathways involved in the development of DCS in rats.

Experimental design: Eighteen male Sprague-Dawley rats were subjected to a same simulated air dive to 1000 kPa absolute pressure and divided into two groups: no DCS or DCS. A third control group remained at atmospheric pressure. Venous blood was collected after hyperbaric exposure and the plasma proteomes from four individuals per group were analyzed by using a two-dimensional electrophoresis-based proteomic strategy.

Results: Quantitative analysis identified nine protein spots with abundances significantly changed (false discovery rate < 0.1) between the tested conditions. Three protein spots, identified as Apolipoprotein A1, Serine Protease Inhibitor A3K (Serpin A3K), and Alpha-1-antiproteinase, appeared increased in DCS animals but displayed only weak changes. By contrast, one protein spot identified as Transthyretin (TTR) dramatically decreased (i.e. quite disappeared) in animals displaying DCS symptoms. Before diving, TTR level was not different in DCS than nondiving group.

Conclusion: These results may lead to the use of TTR as an early biomarker of DCS.

Keywords:
Decompression sickness / Diving / Rat / Transthyretin

1 Introduction

Decompression sickness (DCS) is the most serious danger for Self-Contained Underwater Breathing Apparatus (SCUBA) divers but it may also occur among caisson workers, hyperbaric chamber nurses, tunnellers, high altitude pilots, astronauts performing extravehicular activity, or individuals escaping entrapment from a disabled submarine. Among recreational diving cohorts the estimated incidence of DCS averages 1.55 per 1000 dives [1]. Spinal cord DCS, which is the more severe form of DCS, represents 40–45% of total DCS cases and manifests in a broad array of symptoms that can result in severe morbidity, life-long disabilities, and even death [2]. Among these most serious cases, 20–30% of divers will suffer definitive sequelae. Although the appearance of DCS is linked to the presence of intravascular bubbles, even high amounts of circulating bubbles do not necessarily lead to DCS [3], indicating that the presence of bubbles is not sufficient to evoke DCS, and that other mechanisms, which still are unclear, are involved. Previous studies showed that an increase of oxidative stress, which correlates with the severity of decompression [4], is clearly involved in the dive-induced impairment of vessel function [5, 6], although its implication in the development of DCS is not yet established. An activation of the coagulation cascade has also been shown [4, 7] as well as a release of circulating microparticles [8, 9]. The immune system is also affected by scuba diving even in the absence of symptoms of DCS, as shown by the activation of neutrophils, monocytes, and macrophages while lymphocyte

Correspondence: Jacky Lautridou, Université de Bretagne Occidentale, 6 Avenue Le Gorgeu, 29200 Brest, France
E-mail: Jacky.lautridou@gmail.com

Abbreviations: **BICAM**, ammonium bicarbonate; **DCS**, decompression sickness; **FDR**, false discovery rate; **SCUBA**, self-contained underwater breathing apparatus; **TTR**, Transthyretin

Clinical Relevance

DCS is a poorly understood systemic pathology, with a wide variety of symptoms. Numerous studies have shown that a lot of physiological mechanisms are involved, such as platelet aggregation, endothelial dysfunction, or immune system activation. The study of protein expression could show a link between those mechanisms, allowing a better understanding of this pathology. Furthermore, this work may bring out biomarkers linked to the appearance of DCS and lead to better patient care. Results from this study are therefore relevant both to fundamental and applied research.

activity is suppressed [10–12]. However, even if these factors seem to be linked with DCS, none of these appear determinant in the onset of this pathology.

Proteomics offers a modern tool to explore protein responses to changes occurring during the dive, especially in terms of posttranslational regulations or modifications. In general, plasma proteins are synthesized in the liver, but they also come from other various tissues and cells. Consequently, plasma protein levels may reflect body responses to a specific stress such as diving. Proteomic analyses have been used in numerous studies and on various themes. Choi et al. [13] used these techniques to characterize up- and downregulations of plasma protein levels upon H1N1-infected patients in order to find early biomarkers of H1N1 influenza viral infection. Trifonova et al. [14] also worked on blood proteome to investigate the effects of long-term confinement in an isolation chamber. Julian et al. [15] used proteomics to show that an exposure to hypoxia enhances enzymatic antioxidant systems in individuals who develop Acute Mountain Sickness. Eftedal et al. [10] used a transcriptomic approach to show that scuba diving significantly affects the blood transcriptome, and by doing so could affect the blood proteome. In this context, plasma can be used as it interacts with the whole organism. Furthermore, a sample of blood can be easily collected and does not require any surgical procedure, which is of interest for a potential future prevention strategy.

The aim of this study was to quantify differences in the proteome of both asymptomatic rats and rats with DCS both before and after a simulated dive to identify specific protein responses to diving stress in order to find early specific biomarkers of DCS, and possibly predict individual DCS susceptibility. This is the first proteomic study in plasma proteins of rats with DCS.

2 Methods

2.1 Animal model

Animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and with the approval of the Université de Bretagne Occidentale Ethics Committee for Animal Experimentation.

Eighteen male Sprague-Dawley rats were obtained from Janvier SAS (France). Animals were housed in the university vivarium for at least 7 days after arrival, two per cage in an environmentally controlled room (temperature 21 ± 1°C, 12–12 h light-dark cycle). They were fed standard rat chow and water.

2.2 Chemicals

Thiourea (VWR Chemicals, ref. M226), Urea (GE Healthcare/Plus One, ref. 17-1319-01), CHAPS (ARESCO, ref. M127), DTT (GE Healthcare/Plus One, ref. 17-1318-02), Bromophenol Blue (Sigma, ref. B0126), Bradford (Bio-Rad ref. 500-0006), Acrylamide (Applichem, ref. A3626), SDS (Merck Millipore, ref. 8.22050), APS (Interchim, ref. UP306098), TEMED (Sigma, ref. T9281), Agarose (Interchim, ref. 31772L), IodoAcetate (GE Healthcare/Amersham, ref. RPN6302), EDTA (Amresco, ref. 0245), Glycerol (VWR Chemicals, ref. 24387-292), Coomassie Blue (GE Healthcare/Plus One, ref. PhastGel Blue R-350/17-0518-01), Methanol (VWR, ref. 20847. 368), Acetic Acid (Sigma, refA6283), and $CuS0_4$ (Acros Organic, ref. 1977110000).

2.3 Simulated dive protocol

Because both age and weight are known to influence the probability of DCS [16, 17], animals used in these experiments were all the same age (11-wks-old) and of similar weights (410 ± 30 g) at the day of the experiment. Each rat was compressed in a 130-liter steel hyperbaric chamber, always in the morning at 7 a.m. to avoid interference with biological rhythm. Animals were randomly assigned to diving or control group. To study the effect of diving and DCS, rats were compressed with air at a rate of 100 kPa/min up to 1000 kPa absolute pressure (equivalent to a depth of 90 meters of sea water) and remained at maximum pressure for 45 min. Thereafter, decompression was performed at a rate of 100 kPa/min before pausing for three decompression stops: 5 min at 200 kPa, 5 min at 160 kPa, and 10 min at 130 kPa. Total hyperbaric exposure duration was 83 min. Following decompression the rats were observed for 1 h for four standard signs of DCS in the rat, specifically respiratory distress, difficulty walking,

paralysis, convulsions, or death [4, 18]. To use the same diving protocol enabled us to compare animals that differed by the occurrence or not of DCS only since they were exposed to the same diving parameters (oxygen partial pressure, total pressure, dive duration). A control group of rats ($n = 6$) was similarly confined but not exposed to elevated ambient pressure, and observed for 1 h before physiological investigation.

2.4 Sampling

Blood samples were collected from each rat before and after diving. One milliliter of blood (i.e. <5% of the animal's total blood volume) was collected by retroorbital sinus on anesthetized animals (Ketamine 80 mg/kg, Xylazine 15 mg/kg intraperitoneally) 14 days before diving in order to ensure full recovery of the hematological status of rats at the day of the hyperbaric exposure [19]. After the simulated dive blood samples were collected immediately after symptoms of DCS or at the end of the observation period by intracardiac puncture in animals anesthetized with intraperitoneal administration of Ketamine/Xylazine (80/15 mg/kg). All samples were centrifuged (5000 × *g*, 10 min, 4°C) and supernatant was collected and stored at −80°C.

2.5 IEF and SDS-PAGE analysis

For proteomic analysis, approx. 10 μL of plasma (500 μg of proteins) from four animals in each group (control, No DCS, DCS) were added to 250 μL of Destreak rehydration solution (GE Healthcare) with 1% of IPG buffer pH 4–7 (GE Healthcare). IPG strips (pH 4–7, 13 cm; GE Healthcare) were passively rehydrated with the protein solution in wells for 14 h. Isoelectric focusing was conducted using an Ettan IPGphor3 IEF (GE Healthcare) with the following protocol: 250 V for 15 min, 500 V for 2 h, gradient voltage increased to 1000 V for 1 h, gradient voltage increased to 8000 V for 2.5 h, 8000 V for 3 h, and finally reduced to 500 V. IPG strips were then placed in an equilibration solution (SDS 2%, urea 6 M, EDTA 0.5 M, bromophenol blue 20 mg, Tris–HCL 1.5 M, Glycerol 30%) with DTT (10 mg/mL) for 15 min, and in the same equilibration solution with IAA (48 mg/mL) for the next 15 min in order to trigger the carbamidomethylation of cysteine residues. IPG strips were placed on top of 12% polyacrylamide gels in TGS 1X (Tris 250 mM, Glycine 1920 mM, SDS 1%), which were run in thermoregulated electrophorese unit at 10°C (SE 600; Hoefer Inc.) at 10 mA per gel for 1 h and then 30 mA per gel until complete migration. Gels were subsequently stained with Coomassie Blue (PhastGel R350, GE Healthcare) and unspecific coloration was destained with an aqueous solution containing 30% methanol and 7% acetic acid. The resulting gels were scanned with a transparency scanner (G:BoxChemi XL 1.4; SynGene) in gray scale with 16-bit depth and a resolution of 100 dpi.

2.6 Gel image analysis and statistical analysis of protein's abundance

Images were aligned and spots were detected and quantified using the ProgenesisSameSpots software (version 3.3, Nonlinear Dynamics) with manual alignment completed by automated algorithm. All detected spots were manually checked and artifact spots were removed. Data were exported as volume raw values and statistical analyses were conducted in R [20] using the package prot2D [21] from the Bioconductor suite [22]. Data were normalized (quantile normalization) and the samples from animals presenting a symptom of DCS after diving ($n = 4$) were compared to those from asymptomatic ones ($n = 4$). For comparisons, we used a moderated *t*-test, which is a modified *t*-test, for which the standard errors were moderated across spots, increasing the reliability of the test [21, 23]. Once the values of moderated *t*-test were calculated, *p*-values were corrected using a false discovery rate (fdr), in order to take into account multiple comparison issues. Spots with an fdr threshold <0.1 were considered as differentially expressed.

2.7 Protein identification by MS

Proteins for which abundance changed between groups were excised from gels and prepared for analysis by MS. Gel pieces were first washed in 50 mM ammonium bicarbonate (BICAM), and then dehydrated in 100% ACN. Gel pieces were vacuum-dried, and rehydrated with BICAM containing 0.5 μg MS-grade porcine trypsin (Pierce Thermo Scientific), and incubated overnight at 37°C. Peptides were extracted from the gels essentially as described in Artigaud et al. [24], by alternative washing with 50 mM BICAM and ACN, and with 5% formic acid and ACN. Between each step, the supernatants were pooled, and finally concentrated by evaporation using a centrifugal evaporator (Concentrator 5301, Eppendorf).

MS experiments were carried out on an AB Sciex 5800 proteomics analyzer equipped with TOF/TOF ion optics and an OptiBeamTM on-axis laser irradiation with 1000 Hz repetition rate. The system was calibrated immediately before analysis with a mixture of Angiotensin I, Angiotensin II, Neurotensin, ACTH clip (1–17), and ACTH clip (18–39) and mass precision was better than 50 ppm. After tryptic digestion, the dry sample was resuspended in 10 μL of 0.1% TFA. A 1 μL volume of this peptide solution was mixed with 10 μL volumes of solutions of 5 mg/mL α-cyano-4-hydroxycinnamic acid matrix prepared in a diluant solution of 50% ACN with 0.1% TFA. The mixture was spotted on a stainless steel Opti-TOF 384 target; the droplet was allowed to evaporate before introducing the target into the mass spectrometer. All acquisitions were taken in automatic mode. A laser intensity of 3400 was typically employed for ionizing. MS spectra were acquired in the positive reflector mode by summarizing 1000 single spectra (5 × 200) in the mass range from 700 to 4000 Da. MS/MS spectra were acquired in the positive MS/MS

Table 1. Mean weight by DCS outcome

Group	Control	DCS	Asymptomatic
Weight (g)	468 ± 10	470 ± 26	454 ± 25

reflector mode by summarizing a maximum of 2500 single spectra (10 × 250) with a laser intensity of 4200. For the MS/MS experiments, the acceleration voltage applied was 1 kV and air was used as the collision gas. Gas pressure medium was selected as settings. The fragmentation pattern was used to determine the sequence of the peptide.

Database searching was performed using the MASCOT 2.4.0 program (Matrix Science). Two databases were used: a homemade list of well-known contaminants (keratins and trypsin; 21 entries) and an updated compilation of the Swiss-Prot database with *Rattus norvegicus* as the selected species.

The variable modifications allowed were as follows: carbamidomethylation of cystein, K-acetylation, methionine oxidation, and dioxidation. "Trypsin" was selected as enzyme, and three miscleavages were also allowed. Mass accuracy was set to 300 ppm and 0.6 Da for MS and MS/MS mode, respectively.

3 Results

3.1 DCS outcome

The hyperbaric protocol induced 33.3% of DCS (DCS: $n = 6$; No DCS: $n = 12$). Mean weight by DCS outcome is shown in Table 1. No weight difference was observed between groups. None of the control rats ($n = 6$) showed clinical signs of DCS during the observation period.

3.2 2DE proteomic analysis of plasma proteins expressed differently in DCS rats

Plasma proteins extracted from DCS and non-DCS rats after diving were separated by 2DE. Electrophoregrams were analysed by using progenesis Same Spot (version 3.3). In all, 226 proteins spots could be aligned on all gels, and their relative intensities (normalized volumes) extracted for statistical analysis by using the recently developed prot2D R package. No differences were found between control group and asymptomatic group (data not shown). Nine protein spots showed an abundance significantly changed (fdr < 0.1) between asymptomatic and DCS rats. Eight of these nine proteins were identified by MS/MS and are listed in Table 2. Among these proteins, the protein spot number 182 dramatically decreased (up to sixfold) in animals displaying DCS symptoms but was not changed by diving itself (Figs. 1 and 2). MS/MS identified the protein 182 as transthyretin (TTR). To further assess whether this protein was already absent before the hyperbaric exposure in these animals or was decreased during DCS, we compared the plasma proteome of these animals before DCS, to that of the control group. No significant differences in the proteome were found between these two groups, indicating that the occurrence of DCS was associated with a postdiving drop in the level of this protein.

Furthermore, spot TTR(m) (cf. Fig. 1) was also identified as TTR by MS. The position of spot TTR(m) compare to spot 182 (similar on the x axis but lower on the y axis) suggests that this is a monomeric form of TTR with similar pI but a lower molecular weight (MW). The results showed no difference of relative intensity of this spot.

4 Discussion

This study identifies differences among the proteome of rats before and after a single exposure to hyperbaric conditions. Plasma proteins were separated by IEF and SDS-PAGE, and identified by MS/MS. We showed that rats with DCS have significant changes in their proteome by comparing them with the asymptomatic ones. Indeed, nine spots representing five proteins were differentially expressed after the simulated dive in the plasma of rats which displayed signs of DCS. A particular protein, TTR, nearly disappeared among rats with DCS. This raised the question of the presence of this protein before diving. The plasma proteome of these rats before diving was not different from the proteome of control rats

Table 2. Proteins identification

Description		MASCOT score	Mass (kDa)	Coverage (%)	#Peptides (specific + duplicates)	Fold (asymptomatic/DCS)
140	Alpha-1-antiproteinase	57,55	46 106	7,40	3 + 0	0, 57
142	Alpha-1-antiproteinase	97,13	46 106	9,31	4 + 2	0, 70
158	Serine protease inhibitor A3K	174,81	46 532	12,77	4 + 2	2, 34
182	TTR OS = *Rattus norvegicus*	826,91	15 709	67,35	8 + 7	6, 18
191	Alpha-1-macroglobulin	580,99	167 019	8,83	10 + 7	1, 93
222	Alpha-1-macroglobulin	369,63	167 019	6,13	8 + 3	0, 7
296	Apolipoprotein A-I	505,28	30 043	40,66	9 + 5	0, 68
301	Apolipoprotein A-I	463,06	30 043	39,93	8 + 5	0, 28

Proteins of interest from rat plasma samples have been identified by MS/MS. Fold column describe relative changes of protein levels of asymptomatic group compared to DCS group.

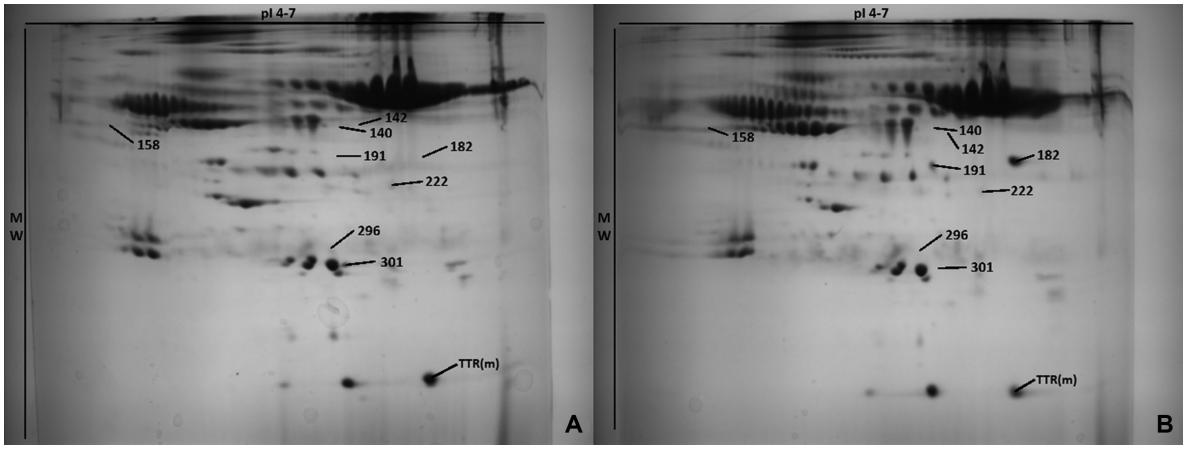

Figure 1. Representative 2DE Gels (pH 4–7, SDS-PAGE 12%) for *Rattus norvegicus* plasma proteins from DCS group (A) and asymptomatic group (B).

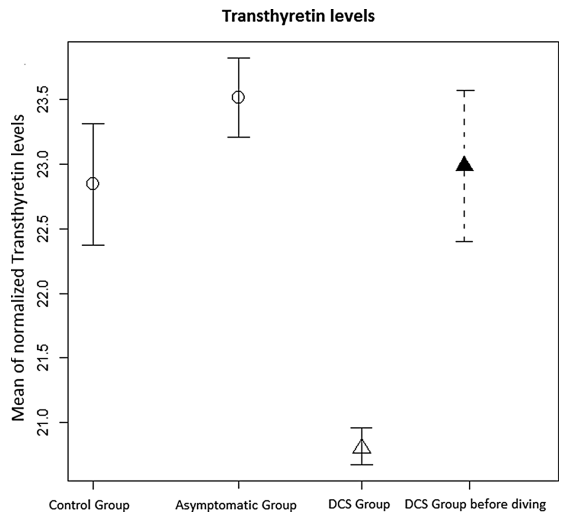

Figure 2. Normalized volumes of protein spot 182 (TTR) among each group. Plasma proteins of rats were separated on bidimensional gel electrophoresis (pH 4–7, 12% acrylamide). Volumes of protein spot 182 (TTR) were measured and normalized for each group (DCS before and after diving, Asymptomatic and Control group). Dotted line with black triangle represents DCS group before diving. Mean differences are significant between DCS group after diving and DCS group before diving ($p < 0.01$), control group ($p < 0.01$) and asymptomatic group ($p < 0.001$). There is no significant difference between the DCS group before diving and the control group or asymptomatic group.

thus showing that TTR disappeared during the development of DCS.

The proteins that were identified in this study are rather abundant in the plasma [25]. It is important to keep in mind that bidimensional electrophoresis is technically biased toward most abundant proteins. However, the protocol used in this study has allowed the separation of 226 protein spots that have not been cloaked by any major protein. Furthermore, the use of kits to precipitate these abundant proteins led to strongly biased results and therefore was not kept in the final protocol. Taking into account these considerations, the proteomic protocol described in this study is a simple and efficient bidimensional electrophoresis protocol to screen a wide variety of plasma proteins.

Apolipoprotein A1 was present in two spots, both increased in animals which suffered DCS as compared to asymptomatic rats. Apolipoproteins are blood proteins linked to lipoproteins, ensuring their solubilization. Variations in ApoA1 affect cholesterol metabolism, which in turn alters the risk of heart disease. Apo1/ApoB ratio is used as a marker of cardiovascular risk, Apo1 being constitutive of High Density Lipoprotein (HDL) and considered as an anti-inflammatory and antioxidant protein. However, HDL protective activity may be altered during acute inflammation, converted into a dysfunctional and proinflammatory particle which fails to prevent Low Density Lipoprotein (LDL) oxidation [26]. Variations in ApoA1 abundance are also associated with amyloid pathologies [27]. In the present study, ApoA1 was increased in the plasma obtained from animals suffering DCS, suggesting that HDL levels might be linked with the occurrence of DCS. The variations of Apolipoproteins observed in this study may be a consequence of changes in lipid metabolism, which would be consistent with the hypothesis that lowering cholesterol levels might influence DCS occurrence on both physiological and biophysical aspects by an activation of eNOS and a diminution of bubble formation [28]. These changes in lipid metabolism are still hypothesized and must be addressed in future studies.

Alpha-1-antiproteinase, or alpha-1-antitrypsine (A1AT), is a protease inhibitor belonging to the serpin (Serum Protease Inhibitor) family, which protects tissues from inflammatory processes [29]. Its concentration may increase upon acute inflammation. In the present study, the DCS group showed a 1.8- (spot 142) and 1.4- (spot 140) fold increase of A1AT, which may either suggest an involvement of this protein in the mechanisms of DCS, or may show inflammatory processes during diving. However, our study also shows a decrease of SerpinA3K (alpha-1-antichymotrypsin). This protein also

belongs to the serpin family and its level would rise upon acute inflammatory processes [30]. This protein protects the lower respiratory tract from neutrophils and mast cells by inhibition of Cathepsin G and Chymase activity. This function of SerpinA3K may have protected asymptomatic animals from DCS (2.3-fold protein level among asymptomatic group compared to DCS group). Furthermore, Chymase and Cathepsin G can convert Angiotensin 1 into Angiotensin 2. It has been shown that Angiotensin 2 induces liberation of procoagulant microparticles, and thus may lead to thrombosis events [31]. Therefore, downregulation of Serpina3K among DCS rats may affect both the renin–angiotensin system and DCS outcome. These findings are consistent with current hypotheses about DCS mechanisms. Additional studies are needed to further confirm or not the link between these protein and DCS.

TTR or prealbumin is a homotetrameric protein with a mass of 55 kDa, with four identical monomers of 127 amino acids [32]. This protein functions as a transport protein for two thyroid hormones, thyroxine (T4) and triiodothyronine (T3), and for retinol. In our study, the amount of tetrameric TTR strongly decreased in animals experiencing DCS (73-fold). The decrease of tetrameric TTR was previously described in several pathologies including amyloid diseases such as familial polyneuropathy, senile systemic amyloidosis, and amyloidotic cardiomyopathy [33]. This protein is also used as a negative acute-phase marker; TTR concentrations markedly decrease in case of acute inflammation. Some studies described dissociation of the tetrameric TTR into its monomeric form when exposed to low pH [34], conversely in the case of amyloidotic pathology the TTR is aggregated, resulting in a nonfunctional "amyloidogenic state" [35–37]. Interestingly, in our study we identified the monomeric TTR in our gels (spot TTR(m) Fig. 1) but no significant difference in quantity was observed between DCS and asymptomatic animals (data not shown). Furthermore, TTR-linked diseases involved a variation in TTR gene sequences and the existence of different TTR isoforms [38, 39]. Further studies will focus on the search of mutation in TTR gene that could influence the stability of tetramers, and thus could be related to DCS. These studies will also point out the presence or not of TTR aggregates in the plasma of DCS rats.

The exact mechanism linking TTR and DCS are still to be determined. However, considered as a negative acute-phase marker, TTR decrease midst rats in DCS observed in this present study is consistent with the current hypotheses about proinflammatory events during diving, showing that hyperbaric conditions could trigger an acute inflammation, which would lead to DCS.

5 Conclusions

In conclusion, this study found that a variation of the plasma proteome of rats is associated with DCS. This variation seems to highlight proinflammatory processes occurring among animals in DCS. This work identified a set of plasma proteins whose level was modified during DCS. In particular, we evidenced a dramatic drop in TTR concentration in the plasma of rats that suffered DCS. This protein could be used as an early biomarker of DCS. However, the mechanism linking the decrease in TTR concentration and DCS remain to be determined. In the future, we will focus on the determination of the TTR genes structures in relation with the TTR multimers stability in the different DCS phenotypes. Future studies will also focus on the specific role of the dive itself, to investigate whether diving modifies the plasma proteome, regardless of DCS occurrence.

This research was part of the PHYPODE project and received funding from the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme FRP/2007-2013/ under REA grant agreement No. 264816 PHYPODE.

The authors have declared no conflict of interest.

6 References

[1] Ranapurwala, S. I., Bird, N., Vaithiyanathan, P., Denoble, P. J., Scuba diving injuries among Divers Alert Network members 2010–2011. *Diving Hyperb. Med.* 2014, *44*, 79–85.

[2] Vann, R. D., Butler, F. K., Mitchell, S. J., Moon, R. E., Decompression illness. *Lancet Lond. Engl.* 2011, *377*, 153–164.

[3] Bakovic, D., Glavas, D., Palada, I., Breskovic, T. et al., High-grade bubbles in left and right heart in an asymptomatic diver at rest after surfacing. *Aviat. Space Environ. Med.* 2008, *79*, 626–628.

[4] Lambrechts, K., Pontier, J.-M., Mazur, A., Theron, M. et al., Mechanism of action of antiplatelet drugs on decompression sickness in rats: a protective effect of anti-GPIIbIIIa therapy. *J. Appl. Physiol. Bethesda Md 1985* 2015, *118*, 1234–1239.

[5] Obad, A., Valic, Z., Palada, I., Brubakk, A.O. et al., Antioxidant pretreatment and reduced arterial endothelial dysfunction after diving. *Aviat. Space Environ. Med.* 2007, *78*, 1114–1120.

[6] Wang, Q., Guerrero, F., Mazur, A., Lambrechts, K. et al., Reactive oxygen species, mitochondria, and endothelial cell death during in vitro simulated dives. *Med. Sci. Sports Exerc.* 2015, *47*, 1362–1371.

[7] Pontier, J.-M., Vallée, N., Ignatescu, M., Bourdon, L., Pharmacological intervention against bubble-induced platelet aggregation in a rat model of decompression sickness. *J. Appl. Physiol. Bethesda Md 1985* 2011, *110*, 724–729.

[8] Yang, M., Kosterin, P., Salzberg, B. M., Milovanova, T. N. et al., Microparticles generated by decompression stress cause central nervous system injury manifested as neurohypophysial terminal action potential broadening. *J. Appl. Physiol. Bethesda Md 1985* 2013, *115*, 1481–1486.

[9] Thom, S. R., Milovanova, T. N., Bogush, M., Bhopale, V. M. et al., Microparticle production, neutrophil activation, and intravascular bubbles following open-water SCUBA diving. *J. Appl. Physiol. Bethesda Md 1985* 2012, *112*, 1268–1278.

[10] Eftedal, I., Ljubkovic, M., Flatberg, A., Jørgensen, A. et al., Acute and potentially persistent effects of scuba diving on

the blood transcriptome of experienced divers. *Physiol. Genomics* 2013, *45*, 965–972.

[11] Madden, D., Thom, S. R., Milovanova, T. N., Yang, M. et al., Exercise before scuba diving ameliorates decompression-induced neutrophil activation. *Med. Sci. Sports Exerc.* 2014, *46*, 1928–1935.

[12] Thom, S. R., Yang, M., Bhopale, V. M., Huang, S., Milovanova, T. N., Microparticles initiate decompression-induced neutrophil activation and subsequent vascular injuries. *J. Appl. Physiol. Bethesda Md 1985* 2011, *110*, 340–351.

[13] Choi, S., Lim, J., Kim, Y., Song, M. J. et al., Plasma proteomic analysis of patients infected with H1N1 influenza virus. *Proteomics* 2014, *14*, 1933–1942.

[14] Trifonova, O. P., Pastushkova, L. K., Samenkova, N. F., Chernobrovkin, A. L. et al., The study of the proteome of healthy human blood plasma under conditions of long-term confinement in an isolation chamber. *Bull. Exp. Biol. Med.* 2013, *155*, 37–39.

[15] Julian, C. G., Subudhi, A. W., Hill, R. C., Wilson, M. J. et al., Exploratory proteomic analysis of hypobaric hypoxia and acute mountain sickness in humans. *J. Appl. Physiol. Bethesda Md 1985* 2014, *116*, 937–944.

[16] Buzzacott, P., Lambrechts, K., Mazur, A., Wang, Q. et al., A ternary model of decompression sickness in rats. *Comput. Biol. Med.* 2014, *55*, 74–78.

[17] Webb, J. T., Pilmanis, A. A., Balldin, U. I., Fischer, J. R., Altitude decompression sickness susceptibility: influence of anthropometric and physiologic variables. *Aviat. Space Environ. Med.* 2005, *76*, 547–551.

[18] Arieli, R., Boaron, E., Abramovich, A., Combined effect of denucleation and denitrogenation on the risk of decompression sickness in rats. *J. Appl. Physiol. Bethesda Md* 1985 2009, *106*, 1453–1458.

[19] Descat, F., Hématologie du rat: hémogramme et myélogramme. *OATAO* 2002.

[20] R Development Core Team, R: A language and environment for statistical computing. Vienna, Austria. GBIF.ORG n.d.

[21] Artigaud, S., Gauthier, O., Pichereau, V., Identifying differentially expressed proteins in two-dimensional electrophoresis experiments: inputs from transcriptomics statistical tools. *Bioinforma. Oxf. Engl.* 2013, *29*, 2729–2734.

[22] Gentleman, R. C., Carey, V. J., Bates, D. M., Bolstad, B. et al., Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol.* 2004, *5*, R80. DOI: 10.1186/gb-2004-5-10-r80.

[23] Smyth, G. K., Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. *Stat. Appl. Genet. Mol. Biol.* 2004, *3*, Article 3.

[24] Artigaud, S., Lavaud, R., Thébault, J., Jean, F. et al., Proteomic-based comparison between populations of the Great Scallop, Pecten maximus. *J. Proteomics* 2014, *105*, 164–173.

[25] Hortin, G. L., Sviridov, D., Anderson, N. L., High-abundance polypeptides of the human plasma proteome comprising the top 4 logs of polypeptide abundance. *Clin. Chem.* 2008, *54*, 1608–1616.

[26] G, H. B., Rao, V. S., Kakkar, V. V., Friend turns foe: transformation of anti-inflammatory HDL to proinflammatory HDL during acute-phase response. *Cholesterol* 2011, 2011, 274629.

[27] Traynor, C. A., Tighe, D., O'Brien, F. J., Leavey, S. F. et al., Clinical and pathologic characteristics of hereditary apolipoprotein A-I amyloidosis in Ireland. *Nephrol. Carlton Vic* 2013, *18*, 549–554.

[28] Duplessis, C. A., Fothergill, D., Investigating the potential of statin medications as a nitric oxide (NO) release agent to decrease decompression sickness: a review article. *Med. Hypotheses* 2008, *70*, 560–566.

[29] Mackiewicz, A., Kushner, I., Baumann, H., *Acute Phase Proteins Molecular Biology, Biochemistry, and Clinical Applications*, CRC Press, 1993.

[30] Kalsheker, N., Morley, S., Morgan, K., Gene regulation of the serine proteinase inhibitors alpha1-antitrypsin and alpha1-antichymotrypsin. *Biochem. Soc. Trans.* 2002, *30*, 93–98.

[31] Cordazzo, C., Neri, T., Petrini, S., Lombardi, S. et al., Angiotensin II induces the generation of procoagulant microparticles by human mononuclear cells via an angiotensin type 2 receptor-mediated pathway. *Thromb. Res.* 2013, *131*, e168–e174.

[32] Lundberg, E., Bäckström, S., Sauer, U. H., Sauer-Eriksson, A. E., The transthyretin-related protein: structural investigation of a novel protein family. *J. Struct. Biol.* 2006, *155*, 445–457.

[33] Sörgjerd, K., Dissertation Thesis, Molecular aspects of transthyretin amyloid disease. Linköping University, Sweden, 2008.

[34] Ferrão-Gonzales, A. D., Souto, S. O., Silva, J. L., Foguel, D., The preaggregated state of an amyloidogenic protein: hydrostatic pressure converts native transthyretin into the amyloidogenic state. *Proc. Natl. Acad. Sci. USA* 2000, *97*, 6445–6450.

[35] Inoue, S., Kuroiwa, M., Saraiva, M. J., Guimarães, A., Kisilevsky, R., Ultrastructure of familial amyloid polyneuropathy amyloid fibrils: examination with high-resolution electron microscopy. *J. Struct. Biol.* 1998, *124*, 1–12.

[36] Kelly, J. W., The alternative conformations of amyloidogenic proteins and their multi-step assembly pathways. *Curr. Opin. Struct. Biol.* 1998, *8*, 101–106.

[37] Lai, Z., Colón, W., Kelly, J. W., The acid-mediated denaturation pathway of transthyretin yields a conformational intermediate that can self-assemble into amyloid. *Biochemistry (Mosc.)* 1996, *35*, 6470–6482.

[38] McCutchen, S. L., Colon, W., Kelly, J. W., Transthyretin mutation Leu-55-Pro significantly alters tetramer stability and increases amyloidogenicity. *Biochemistry (Mosc.)* 1993, *32*, 12119–12127.

[39] McCutchen, S. L., Lai, Z., Miroy, G. J., Kelly, J. W., Colón, W., Comparison of lethal and nonlethal transthyretin variants and their relationship to amyloid disease. *Biochemistry (Mosc.)* 1995, *34*, 13527–13536.

