

A comparative evaluation of two decompression procedures for technical diving using inflammatory responses: compartmental versus ratio deco

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Abstract

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Introduction: The aim of this study was to compare two decompression procedures commonly adopted by technical divers: the ZH-L16 algorithm modified by 30/85 gradient factors (compartmental decompression model, CDM) versus the 'ratio decompression strategy' (RDS). The comparison was based on an analysis of changes in diver circulating inflammatory profiles caused by decompression from a single dive.

Methods: Fifty-one technical divers performed a single trimix dive to 50 metres' sea water (msw) for 25 minutes followed by enriched air (EAN50) and oxygen decompression. Twenty-three divers decompressed according to a CDM schedule and 28 divers decompressed according to a RDS schedule. Peripheral blood for detection of inflammatory markers was collected before and 90 min after diving. Venous gas emboli were measured 30 min after diving using 2D echocardiography. Matched groups of 23 recreational divers (dive to 30 msw; 25 min) and 25 swimmers were also enrolled as control groups to assess the effects of decompression from a standard air dive or of exercise alone on the inflammatory profile.

Results: Echocardiography at the single 30 min observation post dive showed no significant differences between the two decompression procedures. Divers adopting the RDS showed a worsening of post-dive inflammatory profile compared to the CDM group, with significant increases in circulating chemokines CCL2 ($P = 0.001$) and CCL5 ($P = 0.006$) levels. There was no increase in chemokines following the CDM decompression. The air scuba group also showed a statistically significant increase in CCL2 ($P < 0.001$) and CCL5 ($P = 0.003$) levels post dive. No cases of decompression sickness occurred.

Conclusion: The ratio deco strategy did not confer any benefit in terms of bubbles but showed the disadvantage of increased decompression-associated secretion of inflammatory chemokines involved in the development of vascular damage.

Key words

Scuba diving; Decompression tables; Inflammation; Chemokines; Bubbles; Echocardiography

Introduction

Decompression sickness (DCS) after scuba diving is probably more common than previously thought.¹ DCS is associated with different pathophysiological conditions. The first is an increase in intravascular inert gas bubbles directly related to the degree of inert gas supersaturation of tissues. These bubbles in turn activate inflammatory responses. Intravascular inert gas bubbles have been linked to the elevation of circulating microparticles (MPs) observed both in humans and in experimental animal models of diving and associated with inflammation and neutrophil activation.² MPs have a physiological role in inflammation.³ Elevated circulating MPs in divers have been clearly linked to neutrophil and endothelial activation, triggering a response cascade able to increase circulating inflammatory molecules.^{4,5} Several studies have recently focused on the effects of decompression on the vascular endothelium, even in divers without DCS.⁶ Altered endothelial function may exert a negative effect on the maintenance of vascular homeostasis after diving. A post-dive decrease of endothelial function has been demonstrated following a single air dive that produced few

post-dive bubbles and no clinical symptoms of DCS.⁷ The alterations of endothelium include an increased expression of endothelial adhesion molecules.² These responses were recorded soon after diving and constitute early physiological responses to decompression.⁸ Moreover, these studies demonstrated that endothelial physiology is modified even after safe dives. These modifications in vascular physiology may be useful, early, sensitive biomarkers able to monitor the adverse effects of decompression linked to inflammation and endothelial activation.

For more than a century, compartmental decompression models (CDM) have been proposed to describe mathematically tissue desaturation mechanisms and thereby limit DCS. These models have been statistically evaluated by DCS cases, and over time have gradually included bubble formation biophysics.^{9,10} Technical divers perform deep mixed-gas 'square' dives, with a relatively long duration at the target depth and very long decompressions, which are often outside the validation of the algorithms used by these divers.¹¹ For these reasons, an increasing number of technical divers use decompression schedules generated without using dive tables, decompression software or a dive

computer in the hopes of producing safer decompression. The basis for calculating these decompression schedule using a 'ratio decompression strategy' (RDS) are relatively simple and generally driven by anecdote. Commonly adopted compartmental decompression algorithms express exponential profiles favouring gradually longer decompression stops approaching the surface. The RDS expresses a 'S'-shaped ascent curve, extending the duration of decompression stops at which the switch to the first oxygen-rich 'deco' gas takes place. This S-shaped ascent curve would also take advantage of a greater number of deep stops aimed to better control microbubble formation.¹² There is widespread belief that bubble algorithms and the RDS, which redistribute decompression in favour of deeper decompression stops, are more efficient than compartmental shallow-stop algorithms. This is despite recent hyperbaric chamber studies not supporting this view.^{13,14}

With regard to the pathophysiological approach to decompression, what we know currently is not enough to predict which decompression procedures are better than others in terms of DCS prevention. At present, the only way to compare different decompression strategies is to test them in underwater practice, but this means monitoring a huge number of dives, which is expensive and difficult to achieve in a reasonable amount of time, especially for technical dives.

This study is based on the assumption that inflammation and modification of vascular physiology, monitored by post-dive circulating inflammatory molecules, can produce biomarkers able to evaluate the quality of decompression, even in the absence of DCS events. We studied two decompression procedures commonly adopted by technical divers, comparing their post-dive inflammatory profiles elicited by the same dive. We focused on the circulating pro-inflammatory cytokines and chemokines involved in endothelial activation. Thus, we propose an innovative approach to compare decompression procedures in underwater practice.

Methods

STUDY POPULATION

The research was conducted on 74 healthy volunteer divers and 25 healthy volunteer swimmers. All subjects provided written informed consent, and the study was conducted in conformity with the principles of the Declaration of Helsinki. The local ethics committee approved the study protocol (Ethics Committee of the Azienda Ospedaliera-Universitaria Pisana; approval number 2805).

Subjects were selected after exclusion of disease and the use of anti-inflammatory drugs (steroidal or non-steroidal) within seven days before diving. Body mass index (BMI) >30 kg·m⁻² was also considered an exclusion criterion. Divers were divided into three groups

based on their dive and decompression procedures: 23 recreational divers (Rec) were 40.0 ± 8.1 (mean ± SD) years old and had a BMI of 24.7 ± 4.2 kg·m⁻²;

23 technical divers adopting a compartmental decompression model (Tech CDM; ZH-L16 algorithm modified with 30/85 gradient factors) had a mean age of 40.5 ± 6.7 years and mean BMI 25.3 ± 2.7 kg·m⁻²;

28 technical divers adopting the ratio deco decompression strategy (Tech RDS) had a mean age of 41.0 ± 4.7 years and mean BMI 22.8 ± 2.3 kg·m⁻².

A group of 25 swimmers (mean age 41.1 ± 9.1 years and mean BMI 24.9 ± 3.4 kg·m⁻²) was enrolled as a control group to assess the effects of exercise alone on the inflammatory profile. Swimmers performed moderate surface exercise (slow breaststroke-style swimming), comparable to that performed by the divers, for a similar duration of 60 min.

DIVES AND DECOMPRESSION PROFILES

All dives were performed with open-circuit scuba equipment and with dry suits to avoid the effects of cold on circulatory and vascular physiology. Bottom temperatures ranged from 17–19°C while surface temperature ranged from 22–26°C. Technical dives (Tech CDM and Tech RDS) were based on the presence of trimix bottom gas (18% oxygen, 45% helium, 37% nitrogen) and two stage bottles, enriched air nitrox 50 (EAN50) and 100% oxygen, with the switch gases fixed at 21 metres' sea water (msw) and 6 msw respectively (switch PO₂ = 1.6 bar). All the dives were performed in the vicinity of the Giannutri Island Marine National Park, Tuscany, Italy. The technical dives were carried out on the ferry wreck, *Nasim II*, at 50 msw for 25 min. A descent shot line and a path line along the wreck were placed up to the point where the ascent began in order to keep the amount of swimming during the dive similar for each diver. Ascent was performed in open water towards the coast. The two technical diving decompression schedules were selected by the Tech divers as typical for the dive; all divers followed closely the prescribed schedules, as verified by analyzing their diving computer records.

The recreational decompression dive was a 30 msw air dive within the no-stop limits prescribed by the US Navy Air Decompression Table. The maximum depth was reached after 2 min, and divers remained at 30 msw up to 25 min of dive time. The ascent was at 10 m·min⁻¹ up to a safety stop at 3 msw for 3 min and then they surfaced at an ascent rate of 3 m·min⁻¹.

The CDM was generated by Deco Planner software based on the Bühlmann algorithm (ZH-L16 algorithm modified with gradient factors 30/85), one of the most commonly used by technical divers (Table 1). This profile (Figure 1) calculates the decompression timing according to the exponential kinetics of the inert gases in tissues. The software considers the behavior of tissues and assigns gradually longer decompression stops as divers near the surface. The ascent

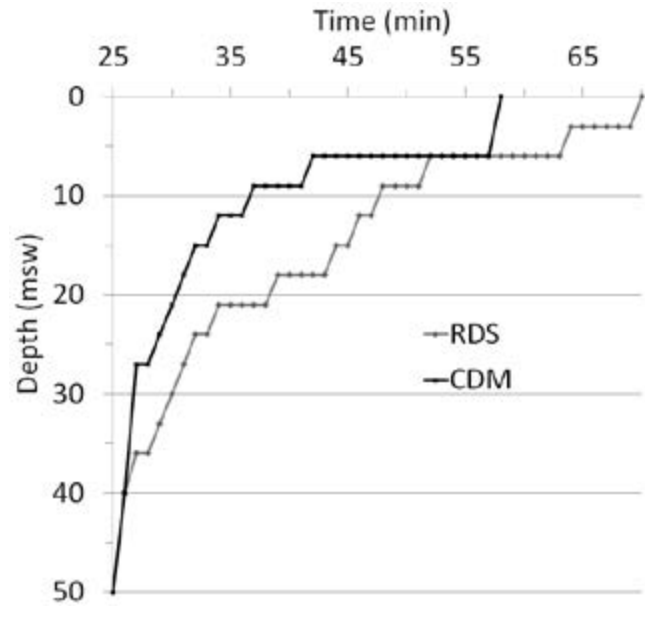
Table 1

Two different decompression schedules – ratio decompression strategy (RDS) and compartmental decompression (CDM) – for a technical dive with a total descent and bottom time of 25 min; depth at which the diver is located (msw) against run time (min)

Run time (min)	RDS (msw)	CDM (msw)
25	50	50
26	40	40
27–28	36	27
29	33	24
30	30	21
31	27	18
32–33	24	15
34–36	21	12
37–38	21	9
39–41	18	9
42–43	18	6
44–45	15	6
46–47	12	6
48–51	9	6
52–57	6	6
58	6	0
59–63	6	
64–69	3	
70	0	

Figure 1

Two technical diving decompression profiles – ratio decompression strategy (RDS, light grey) and compartmental decompression (CDM, dark grey); the descent and bottom-time profile (0–25 min, not shown) was identical for both RDS and CDM dives



to the first decompression stop (27 msw) was 10 m·min⁻¹ and 3 m·min⁻¹ thereafter.

The RDS adopts a coded concept of set points that fixes a ratio of bottom time and decompression time for various depths to calculate the decompression times (40 msw ratio 1:1; 60 msw ratio 1:2; 75 msw ratio 1:3). Additional rules are used to interpolate between set point depths. The total decompression time obtained from the RDS is distributed among decompression stops according to a set of rules (Table 1). The total decompression time for RDS divers was longer than that generated by the CDM profile, but what changed most was the shape of the ascent profile (Figure 1), with lengthening of the time at the gas switch. The decompression develops in several steps with a first deep stop at 75% of the maximum depth (1 min) and a second deep stop at 50% of maximum depth (1 min). These two stops at a conservative depth during the ascent phase are proposed to help to control the critical volume of inert gas which is correlated to the radius of the microbubbles. The ascent to the first decompression stop (37 msw) was 10 m·min⁻¹ and thereafter 3 m·min⁻¹ up to the gas switch and subsequent stops.

BLOOD SAMPLE COLLECTION AND DETECTION OF CYTOKINES

For the diver groups, 5 ml of venous blood was collected from the antecubital fossa of the left arm into a Vacutainer® (BD Science) containing ethylene diamine tetraacetic acid (EDTA). In swimmers, 300 µl of blood was collected

by digital puncture and transferred to Eppendorf tubes containing EDTA. Blood was collected 60 min before and 90 min after diving or swimming. Blood samples were kept at 4°C for 24 h, then centrifuged at 1000 g for 15 min. Plasma was collected and stored at -80°C until analysis. Cytokines present in plasma were quantified in triplicate (plasma dilution 1:4) by using a customized detection panel (BioRad, USA): interleukin 6 (IL-6), interleukin 8 (IL-8); C-X-C motif chemokine 10 (CXCL10), C-C Motif Chemokine Ligand 2 (CCL2), Macrophage Inflammatory Protein-1 beta (MIP-1β) and C-C Motif Chemokine Ligand 5 (CCL5). The assays were performed in 96-well filter plates by multiplexed Luminox®-based immunoassay as previously described,¹⁵ following the manufacturer’s instructions, at the Proteomic Unit CRR, University of Bologna.

Samples were analysed as a single batch, after performing validation and calibration of the instrument (Bioplex Validation & Bioplex Calibration Kits, Biorad, USA). Microsphere magnetic beads coated with monoclonal antibodies against the different target analytes were added to the wells. After 30 min incubation, the wells were washed and biotinylated secondary antibodies were added. After further incubation for 30 min, beads were washed and then incubated for 10 min with streptavidin-PE conjugated to the fluorescent protein, phycoerythrin (streptavidin/phycoerythrin). After washing, the beads (a minimum of 100 per analyte) were analyzed in the BioPlex 200 instrument (BioRad, USA). Sample concentrations were estimated from the standard curve using a fifth-order polynomial equation and expressed as pg·ml⁻¹ after adjusting for the dilution factor (Bio-Plex Manager software 5.0). Samples

below the detection limit of the assay were recorded as zero, while samples above the upper limit of quantification of the standard curves were assigned the highest value of the curve. The intra-assay coefficients of variability (CV) averaged 12%.

BUBBLE ANALYSIS AND GRADING

After surfacing, divers returned to the diving centre by fast boat (20 min trip, seated) then rested seated for 10 min. Finally, they lay supine for 2D echocardiography performed 30 min after surfacing. A 30-sec clip of each of the following echocardiographic views was acquired: apical four-chamber (to evaluate right ventricle and right atrium), heart base short-axis (to evaluate right atrium, right ventricular outflow tract and main pulmonary artery), inferior vena cava and right atrium subcostal scan. A visual search for circulating bubbles was made offline on recorded loops. The use of a single evaluation of circulating bubbles is sub-optimal for proper assessment, but we had to limit ultrasonic evaluations due to protocol constraints.

Echocardiography evaluation was at the time (30 min post dive) that previous reports indicate as the time of peak venous gas emboli (VGE),¹⁶ and each ultrasonic evaluation was protracted for 90 sec (30 sec for each of the three analyzed views) to reduce the likelihood of underestimating bubble grades owing to spontaneous variability of the number of VGE. Bubbles were graded as the maximum in any view by an operator unaware of the decompression procedures followed by the diver, according to the Eftedal-Brubakk grading.¹⁷ Bubble grades were divided into high (grades 3–5) and low bubble grade groups (grades 0–2).

URINE COLLECTION AND ANALYSES

Urine specific gravity has been used to assess hydration status in sportsmen.¹⁸ Urine samples (15 ml) were collected in polypropylene bottles from all divers 60 min before and 90 min after the dive. Combur-Test® strips (Roche, Germany) were immediately used for the detection of leukocytes, proteins, glucose and blood. Analyses were repeated at least twice for each sample. No diver showed values outside the normal range. Urine specific gravity was evaluated in triplicate by using a refractometer (Atago, Japan).

Oxidative damage was analyzed by measuring 8-hydroxy-2-deoxy guanosine (8-OH-dG) and creatinine in urine, which has been used to evaluate the effect of exposure to systemic reacting oxygen insults.¹⁹ Urinary 8-OH-dG (Abcam Inc., USA) was measured in triplicate using a commercially available ELISA kit following the manufacturer's instructions. 8-OH-dG, a frequently used biomarker of oxidative DNA damage, is removed from DNA by the base excision repair pathway, and subsequently transported into saliva, urine and plasma. Creatinine was determined by means of a modified Jaffe reaction (alkaline

picrate method) using the Wako Creatinine-Test (Wako Pure Chemical Ind, Ltd. Japan).

STATISTICAL ANALYSIS

Continuous variables are expressed as mean \pm SD of at least three independent determinations. Normality of distribution was verified with the D'Agostino-Pearson and Shapiro-Wilk tests and the homogeneity of variances (homoscedasticity) with the F-test. Statistical differences between groups were determined by Student's *t*-test. GraphPad Prism 6 (GraphPad Software Inc., San Diego, CA, USA) was used for all analyses. Categorical variables are expressed in total counts and percentage of counts, and were compared using χ^2 tests. Differences were considered significant at $P < 0.05$.

Results

BUBBLE ANALYSIS

Echocardiographic bubble analysis made at one time point (30 min) post dive showed no significant differences between the two groups of technical divers (Figure 2), although high bubble grades (grades 3–4) were more frequent in the RDS group (2/23 in Tech CDM divers vs. 4/28 in Tech RDS divers). There were no statistical differences in bubble grading between the two decompression procedures, either comparing low with high grade frequencies or grade zero against all other grades.

PRO-INFLAMMATORY MARKERS

The 60 min of moderate exercise did not modify the inflammatory profile of swimmers (Figure 3A), whereas the Rec diver group showed a significant increase in circulating CCL2 (1.4 fold; $P < 0.001$) and CCL5 (1.2 fold, $P = 0.003$) after diving; IL-6, IL-8, CXCL10 and MIP-1 β were unaffected (Figure 3B). A similar increase in CCL2

Figure 2

Bubble grades 30 min after surfacing using two different decompression schedules – ratio decompression strategy (RDS) and compartmental decompression model (CDM) for a 50 msw, 25 min bottom time technical dive; no grade 5 bubbling was detected

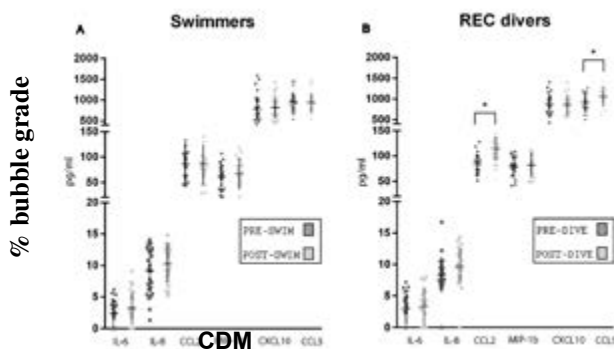
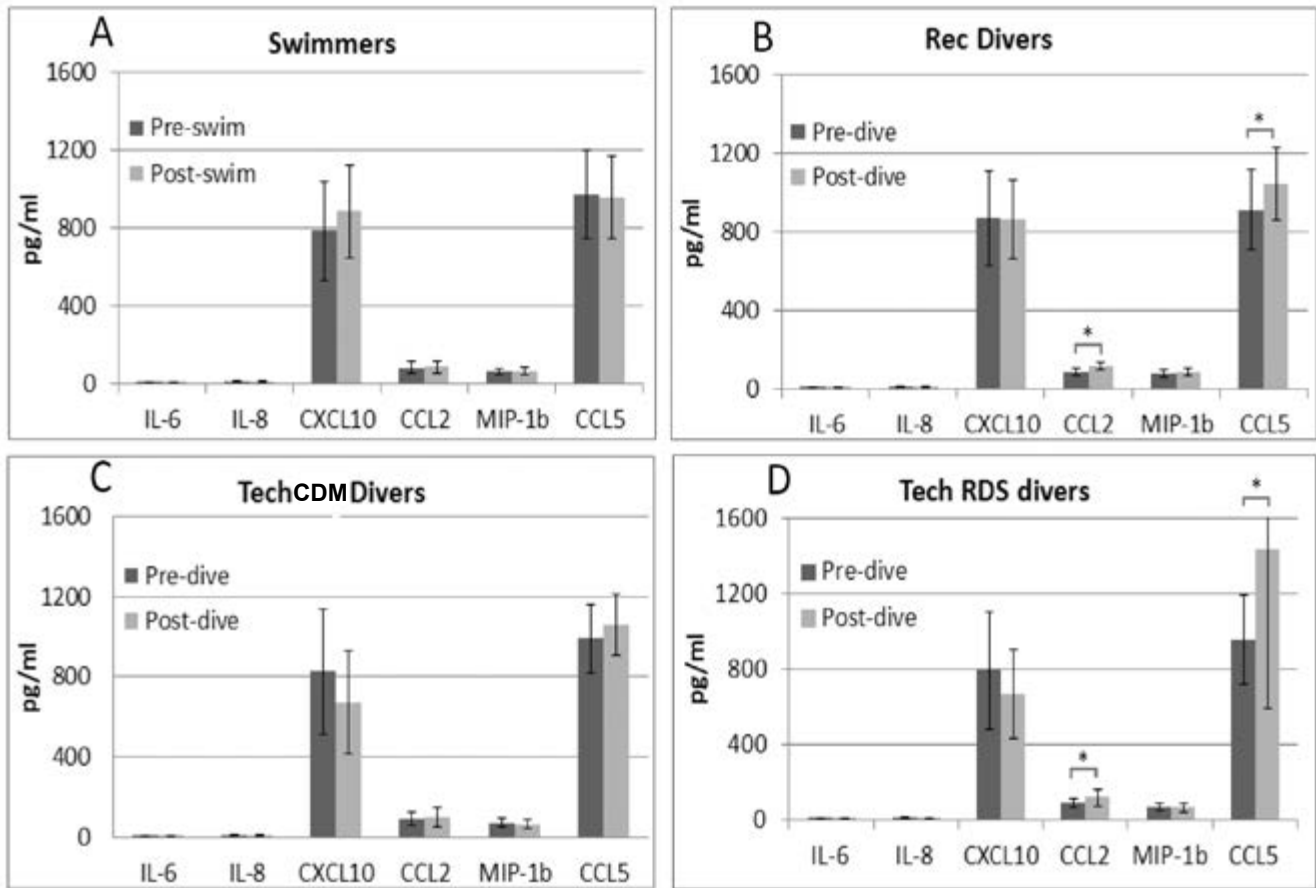


Figure 3

Circulating cytokines and chemokines detected in swimmers before and 90 min after surface swimming, and in three groups of divers (mean +/- SD shown) before and 90 min after surfacing from their different dives: the concentrations of interleukin 6 (IL-6); interleukin 8 (IL-8); C-X-C motif chemokine 10 (CXCL10); C-C motif chemokine ligand 2 (CCL2), macrophage inflammatory protein-1 beta (MIP-1β) and C-C motif chemokine ligand 5 (CCL5) were simultaneously measured in the plasma of swimmers and divers by multiplexed Luminex®-based immunoassay; * indicates statistically significant differences (see text for details)



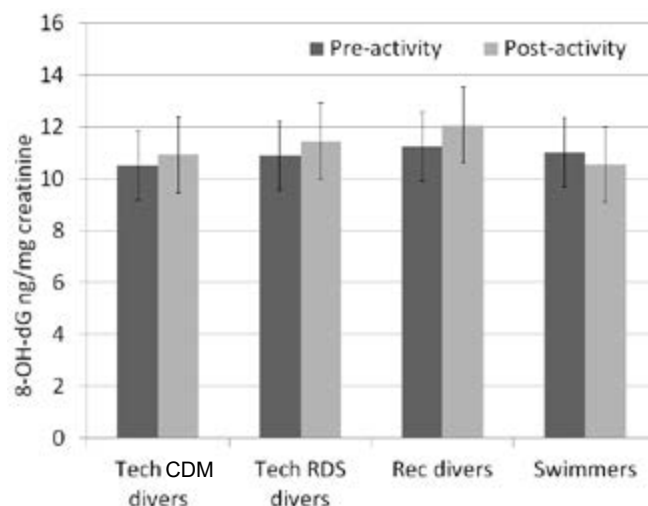
(1.4 fold, $P = 0.001$) and CCL5 (1.5 fold, $P = 0.006$) was observed in Tech RDS divers (Figure 3C). By contrast, Tech CM divers showed only a slight, non-significant decrease in the mean value of CXCL10 (from 827 to 674 $\text{pg}\cdot\text{ml}^{-1}$) and MIP1-β (from 73 to 65 $\text{pg}\cdot\text{ml}^{-1}$) (Figure 3D). Comparing the pro-inflammatory markers in all three groups of divers, it was evident that only Rec and Tech RDS divers showed a worsening of their inflammatory profile, particularly in circulating CCL2 and CCL5 levels, while inflammation was unchanged after diving in Tech CM divers. There was no correlation between bubble grades and circulating CCL2 or CCL5 levels after diving.

URINE ANALYSIS

Most of the divers had an urinary specific gravity above 1.020 before diving (average 1.022) but there were no differences in urinary specific gravity observed pre or post dive among the three diver groups. Increased oxygen exposure during the dives did not modify urinary 8-OH-dG levels in any of the three dive groups (Figure 4).

Figure 4

Urinary 8-hydroxy-2'-deoxyguanosine (8-OH-dG) taken 90 min after surface swimming and in three groups of divers before and 90 min after surfacing from their different dives (mean +/- SD)



Discussion

The RDS is widely used by technical divers for their decompression procedures. Nevertheless, decompression protocols with experimental deep stops added, when tested in simulated dives in hyperbaric chambers, have never shown any real advantages over more traditional compartmental models.^{13,14} However, the conditions under which these laboratory studies were conducted differ from conditions in typical technical diving. Field studies can allow decompression procedures to be evaluated under typical conditions encountered by technical divers.

Studies comparing different decompression models in terms of decompression effectiveness require a vast number of analyzed dives since they are based on statistical analyses of DCS cases and statistical analyses of Doppler and echocardiographic bubble counts. On the other hand, we do not know enough about the pathophysiology of DCS to predict the goodness of fit of decompression models. An in-depth analysis of real decompression accidents clearly shows that the majority of DCS cases reported by DAN occur after dives conducted following appropriately prescribed decompression.²⁰ This suggests that as yet unknown pathophysiological factors are involved in the onset of DCS.

It is known that physical activity may alter circulating IL-6, IL-8, MIP-1 β and other pro-inflammatory molecules.²¹ The modification of the inflammatory profile after scuba diving, but not after the comparable swimming exercise in our study, suggests that it is decompression that causes an increase in some circulating chemokines, namely CCL2 and CCL5, and not with the physical exercise performed during the dive. While CCL2 and CCL5 increased after diving in both the Rec group and in the RDS group, they remained unaffected 90 min after diving in the CDM dive. This suggests that the recreational air dive to 30 msw was more proinflammatory than the CDM dives to 50 msw. This apparent paradox may be explained partially by the documented protective effects of helium on the endothelium.²² Given the increased levels of these two pro-inflammatory chemokines after RDS-controlled dives, we conclude that the RDS ascent profile caused a worsening in diver inflammation compared with the CDM ascent profile. This fits with the chamber evidence of no advantage to deeper stops.^{13,14}

The chemokine CCL5 or RANTES (regulated on activation, normal T cell expressed and secreted) is a member of the CC chemokine family stored in and released from platelets and activated T lymphocytes. Circulating chemokine CCL5 is known to contribute to endothelial activation and the interaction between endothelial cells and monocytes.²³ It was reported recently that CCL5 secretion facilitates endothelial progenitor cell recruitment and increases nitric oxide production in endothelial cells.²⁴ Thus, CCL5 may be considered a good circulating marker of vascular damage.

As platelet degranulation enhances the release of circulating CCL5, it has been proposed also as a potential index for evaluating decompression stress.²⁵ Our results suggest that CCL5 could be a circulating marker of the endothelial activation involved in decompression stress, linking platelet activation and endothelial dysfunction, two events clearly involved in decompression physiology.²⁶ CCL2, also called monocyte chemoattractant protein 1 (MCP-1), is a pro-inflammatory chemokine involved in tissue inflammation and produced by tissue injury.²⁷ Interestingly, circulating CCL2 and CCL5 concentrations increase in hypertension²⁸ and have been considered as 'early endothelial chemokines' given their role in vascular inflammation.²⁹

It remains possible that the increase in cytokine levels after the RDS dives compared to the CDM group could be attributed to the longer exposure to the environment and high oxygen partial pressures in the breathing gases. Nevertheless, the similar increase in cytokines in the Tech RDS and Rec groups argues against this possibility since Rec divers were exposed to the environment for a shorter period and did not breathe oxygen-enriched decompression gases. As we found no detectable changes in 8-OH-dG levels during these dives, we conclude that the hyperoxia associated with the dive profiles did not give rise to systemic oxidative stress of any importance.

Increased circulating chemokines and higher bubble grades may be two phenomena that are physiologically disconnected. That is, bubble development and the increased inflammation likely induced by vascular modifications might be independent phenomena, both able to enhance divers' susceptibility to develop DCS. However, endothelial physiology, which also depends on individual genetics, is certainly linked to the inflammatory response trigger elicited by circulating bubbles. Further studies will be necessary to correlate circulating chemokines with differences in accepted measures of decompression stress such as the incidence of DCS, VGE evolution or different dive profiles with unequivocal differences in decompression stress.

Urine specific gravity measurements demonstrated that moderate dehydration before diving was common even in highly experienced technical divers. This finding suggests divers are not able to adopt proper hydration strategies in the hours preceding their dives. Dehydration certainly worsens during diving, due to physical exercise, immersion diuresis and loss of water vapour with breathing. The consequent increase in plasma osmolality may concentrate bubbles and also circulating pro-inflammatory molecules. Ninety min after the end of the dive, urine specific gravity tended to decrease in all divers, since they were able to urinate and drink after surfacing from their dives.

We are aware that our study has important limitations, namely the quite low number of divers enrolled, the fact that only a single dive profile – 50 msw, 25 min bottom

time – was tested and the use of a single evaluation of circulating bubbles, which is likely to be sub-optimal. On the other hand, its strength is that it analyzed non-professional divers in the conditions commonly encountered during their recreational dives.

Conclusions

This study does not establish any association between the decompression model chosen and the likelihood of DCS. A single echocardiographic observation of bubble grades is insufficient to draw any useful comparison between CDM and RDS-controlled decompression for this 50 msw dive. However, the RDS has the disadvantage of decompression-associated increased secretion of chemokines involved in the development of vascular damage. This increased secretion of pro-inflammatory chemokines seems related to the decompression system rather than to the longer exposure to high partial pressures of oxygen that RDS divers undergo. Tech RDS and Rec Divers showed very similar inflammatory profiles after the dives. Overall, our findings contradict the idea that adding longer and/or deeper stops is useful to achieve a more effective decompression. A major limitation is that only a single dive profile – 50 msw, 25 min bottom time – was studied and the findings cannot be extrapolated to other dive profiles.

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