Does recreational scuba diving have clinically significant effect on routine haematological parameters?

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Abstract

Introduction: Scuba diving represents a combination of exercise and changes in environmental conditions. This study aimed to evaluate changes in haematological parameters after recreational scuba diving in order to identify clinically significant changes.

Materials and methods: The study included males, 17 recreational divers, median age (range) 41 (30-52) years. Blood samples were taken before diving, immediately after diving to 30 meters for 30 minutes, 3 hours and 6 hours after diving. Complete blood counts were analyzed on the Cell Dyn Ruby haematology analyzer. Statistical significance between successive measurements was tested using Friedman test. The difference between the two measurements was judged against desirable bias (DSB) derived from biological variation and calculated reference change values (RCV). The difference higher than RCV was considered clinically significant.

Results: A statistically significant increase and difference judging against DSB was observed: for neutrophils immediately, 3 and 6 hours after diving (18%, 34% and 36%, respectively), for white blood cells (WBCs) 3 and 6 hours after diving (20% and 25%, respectively), for lymphocytes (20%) and monocytes (23%) 6 hours after diving. A statistically significant decrease and difference judging against DSB was found: immediately after diving for monocytes (- 15%), 3 and 6 hours after diving for red blood cells (RBCs) (- 2.6% and -2.9%, respectively), haemoglobin (- 2.1% and - 2.8%, respectively) and haematocrit (- 2.4% and - 3.2%, respectively). A clinically significant change was not found for any of the test parameters when compared to RCV.

Conclusions: Observed statistically significant changes after recreational scuba diving; WBCs, neutrophils, lymphocytes, monocytes increase and RBCs, haemoglobin, haematocrit decrease, probably will not affect clinical decision.

Key words: diving; preanalytical phase; blood cell count; evaluation

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Introduction

With safety advancement through the development of diving equipment and procedures, scuba diving becomes a global sport activity. Scuba diving is characterized by changes in environmental conditions that include hyperbaric exposure, breathing compressed air or other gas mixtures at the elevated pressure, effect of immersion and exposure to cold temperature. In addition, diving implies a demanding physical activity due to the diving equipment weight and increased resistance to movement and represents psychological stress since the human ability to remain under water is physiologically limited (1,2).

Considering millions of people involved in recreational or sport diving (3), the knowledge of expected changes in laboratory tests after diving may contribute to clinical decision making in urgent conditions. It has been estimated that 60% of medical decisions depend on laboratory test results (4) which often include a complete blood

count or haematological parameters in blood. Biological variables such as physical activity, psychological stress and environmental conditions present in diving cause a specific physiological response that could significantly affect haematological test results (5-7).

In previous studies on professional divers, the changes in haematological parameters were observed immediately and 3 hours after surfacing (8-11). However, the effect of recreational scuba diving on the haematological parameters has not yet been investigated. We hypothesized that a single recreational scuba dive to 30 meters for 30 minutes could influence haematological parameters. The aim of this study was to evaluate changes of haematological parameters in recreational scuba divers immediately, 3 and 6 hours after diving in relation to the values obtained before diving in order to provide information on the changes that could influence interpretation of results.

Materials and methods

Subjects

The study was conducted in the Department of Laboratory Diagnostics, Dubrovnik General Hospital, Dubrovnik, Croatia. The study group consisted of 17 male recreational divers, median age (range) 41 (30–52) years, with the diving experience between 5 and 20 years and the number of dives per year less than 20. None of the subjects was a professional athlete and practiced scuba diving during the winter period. Their height, weight and body mass index (BMI), expressed as median and interquartile range (IQR), were 1.80 (1.80–1.85) m, 90 (85–92) kg and 27.5 (24.9–28.4) kgm^{-2}, respectively. The day before the study performed, participants were subjected to medical examination by a hyperbaric medicine specialist that included medical history taking, blood pressure measurement, anthropometric measurements and laboratory tests. All subjects did not have symptoms of any acute or chronic disease and did not take any medications or alcohol 48 hours before and during the study. The subjects were refrained from any form of diving 7 days before the study and any form of exercise 48 hours before the study. Before giving their written consent to participate, the subjects were informed on the purpose and demands of the study and were instructed to take low-fat meals at the same time of day during the study. The study was designed in accordance with the Declaration of Helsinki and was approved by the Institutional Ethical Committee.

Study design and blood collection

The subjects were longitudinally evaluated through four blood samples in one day. The experimental dive was conducted at the seaside in April 2016. During the study, the air temperature was between 16 and 20 °C, and the sea temperature was 16 °C at the surface and 14 °C at the bottom (30 meters). The divers were equipped with wetsuits and open-circuit breathing equipment, and air was used as a breathing gas. They were instructed to moderate their loads and remain in the group during the dive.

The first blood sample was collected between 09:15 and 9:45 am within one hour after awaking and after a light meal (t_0). Immediately after blood collection, the divers performed an immersion at a maximum depth of 30 meters for a total time of 30 minutes. Diving profile (Figure 1) was downloaded from dive computers for analysis of the dive depth and duration, and sea temperature. The second blood collection was carried out between 10:45

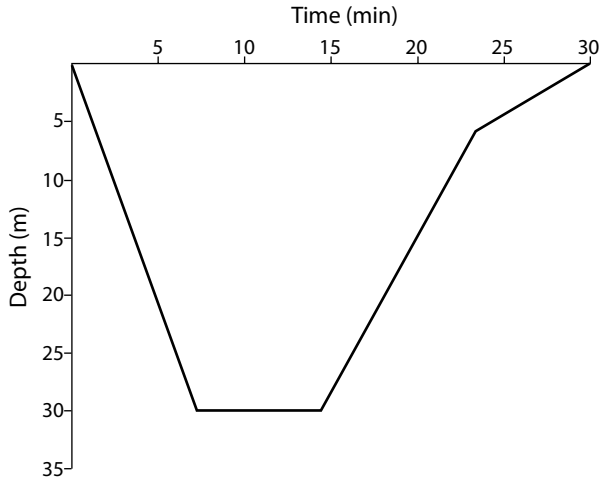

Figure 1. Diving profile: analysis of the dive depth and duration.

and 11:15 am within 30 minutes after surfacing (t_1 - 90 minutes after t_0). The third sample was taken between 01:45 and 02:15 pm (t_2 - 3 hours after t_1) approximately an hour after meal, and the last sample taken between 04:45 and 05:15 pm (t_3 - 6 hours after t_1). All blood samples were drawn from the antecubital vein of subjects, using 20 G straight needle (Vacuette, Greiner Bio-One GmbH, Kremsmünster, Austria), directly into vacuum tubes with K_2EDTA (Vacuette, Greiner Bio-One GmbH, Kremsmünster, Austria). Phlebotomy was performed after at least 15 minutes of resting in a seated position by the expert phlebotomists according to the national recommendations for venous blood sampling by the Croatian Society of Medical Biochemistry and Laboratory Medicine (12).

Methods

All haematological analyses were performed within an hour after blood sampling on the same Cell Dyn Ruby haematology analyzer (Abbott, Illinois, USA). The following haematological parameters were measured: white blood cell (WBC) count, WBC differential blood count including neutrophil, lymphocyte, monocyte, eosinophil and basophil count, red blood cell (RBC) count, haemoglobin, mean corpuscular volume (MCV), RBC distribution width (RDW), platelet (PLT) count and mean platelet volume (MPV), while haematocrit, mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from the measured parameters. The samples were processed the same way as all routine samples. After the analysis, plasma of each blood sample was checked visually and none of the sample had haemolysis, icterus and lipemia. During the study internal quality control was carried out in the morning before sample analysis and in the afternoon after the sample analysis using the manufacturer's control (Cell-Dyn 26 Plus Control, Abbott, Wiesbaden, Germany). Analytical imprecision (CV_A) was calculated from internal quality control results in the period from April 1st to April 30th 2016 and judged against desirable specification for imprecision (DSI) given in the 2014 updated database on biological variation (13).

Statistical analysis

Considering the small number of participants (less than 30) and according to recommendations regarding sample size (14), a statistically significant difference between pre-diving and post-diving measurements was tested using non-parametric Friedman test and the data were presented as median and interquartile range (IQR). The percentage differences between the pre-diving and post-diving measurements were calculated, for each parameter and for each subject, according to the formula: difference (%) = $((t_n - t_0) / t_0) \times 100$, where t_n is value after diving and t_0 is value before diving. The mean difference (%) was calculated as the mean between the subjects for each parameter and judged against desirable bias (DSB) derived from biological variation (13) and calculated reference change values (RCV). RCV was calculated according to the following formula: $RCV = 2^{1/2} \times Z \times (CV_A^2 + CV_I^2)^{1/2}$, where Z is 1.96 for those parameters where rise or fall (change) is equally considered (two-sided) in our study for all parameters. Chosen Z value stands for standard deviation for desired probability of $P < 0.05$. The mean difference (%) higher than RCV was considered clinically significant.

Statistical analyses were performed using MedCalc statistical software version 16.4.3 (MedCalc, Ostend, Belgium). The level of significance was set at $P < 0.05$.

Results

The results of the study are presented in Table 1. A statistically significant increase and difference judging against DSB (13) was observed: for neutrophil count immediately, 3 and 6 hours after diving (18%, 34% and 36%, respectively), for WBC count 3 and 6 hours after diving (20% and 25%, respectively), for lymphocyte count 6 hours after diving (20%) and for monocyte count 6 hours after diving (23%). A statistically significant decrease and difference judging against DSB (13) was found: immediately after diving for monocyte count (- 15%), 3 and 6 hours after diving for RBC count (- 2.6% and – 2.9%, respectively), and haemoglobin (- 2.1% and – 2.8%, respectively) and haematocrit (- 2.4% and – 3.2%, respectively).

Table 1. Influence of recreational scuba diving on haematological parameters

Haematological parameter (unit)	CV_A † (%)	DSI (%)	DSB (%)	RCV (%)	t_0	t_1	Mean difference‡ (%)	t_2	Mean difference‡ (%)	t_3	Mean difference‡ (%)	P§
WBC (x 10^9/L)	1.4	5.7	6.1	31.8	6.88 (5.74–7.80)	6.54 (5.86–8.90)	4.7	7.88 (6.99–9.43)*	**20.4**	8.22 (6.62–10.28)*	**24.5**	< 0.001
NEU (x 10^9/L)	1.7	8.6	9.3	47.6	3.57 (3.30–4.78)	4.39 (3.47–5.19)*	**17.8**	4.73 (3.96–6.20) *	**33.9**	4.89 (3.94–6.37) *	**35.6**	<0.001
LYMP (x 10^9/L)	3.5	5.1	9.2	29.8	2.13 (1.61–2.52)	1,90 (1.56–2.17)	– 7.3	2.24 (2.01–2.59)	9.0	2.55 (2.13–3.03) *	**19.6**	0.002
MONO (x 10^9/L)	6.4	8.9	13.2	52.4	0.45 (0.40–0.63)	0.35 (0.32–0.40)*	**– 15.3**	0.50 (0.37–0.63)	8.4	0.52 (0.47–0.74)*	**22.8**	< 0.001
EOS (x 10^9/L)	10.0	10.5	19.8	64.5	0.15 (0.08–0.21)	0.12 (0.07–0.20)	– 16.7	0.12 (0.08–0.22)	– 10.8	0.16 (0.09–0.21)	0	0.063
BASO (x 10^9/L)	9.1	14.0	15.4	81.6	0.08 (0.06–0.09)	0.09 (0.07–0.12)	2.1	0.09 (0.07–0.09)	5.4	0.08 (0.07–0.09)	6.1	0.215
RBC (x 10^{12}/L)	1.0	1.6	1.7	9.3	5.22 (5.15–5.39)	5.22 (5.11–5.39)	– 0.4	5.08 (4.94–5.28)*	**– 2.6**	5.11 (4.94–5.27)*	**- 2.9**	< 0.001
HGB (g/L)	0.7	1.4	1.8	8.1	155 (151–159)	154 (150–159)	– 0.5	152 (147–155) *	**– 2.1**	152 (145–155) *	**- 2.8**	< 0.001
HCT (L/L)	1.2	1.4	1.7	8.2	0.468 (0.456–0.483)	0.468 (0.449–0.488)	– 0.1	0.460 (0.454–0.467)*	**– 2.4**	0.461 (0.440–0.468)*	**- 3.2**	< 0.001
MCV (fL)	**0.8**	0.7	1.3	4.5	89.8 (87.8–93.0)	90.0 (88.1–93.4) *	0.4	89.9 (88.2–92.0) *	0.2	89.4 (88.2–91.5)	- 0.3	< 0.001
MCH (pg)	**1.1**	0.7	1.4	4.9	29.4 (29.1–30.7)	29.4 (29.2–30.6)	0.1	29.7 (29.0–30.7)	0.5	29.6 (29.1–30.4)	0.3	0.373
MCHC (g/L)	**1.3**	0.5	0.4	4.6	329 (324–335)	327 (325–332)	– 0.3	331 (326–335)	0.3	332 (328–334) *	**0.7**	0.024
RDW (CV%)	1.8	1.8	1.7	10.9	11.9 (11.6–12.7)	12.0 (11.8–12.5)	– 0.2	11.9 (11.6–12.4)	– 1.0	12.0 (11.7–12.6)	- 0.9	0.067
PLT (x 10^9/L)	3.4	4.6	5.9	27.0	213 (192–264)	210 (189–249)	– 2.8	216 (195–258)	– 0.4	224 (193–265)	2.0	0.059
MPV (fL)	**2.6**	2.2	2.3	13.9	7.4 (6.7–8.4)	7.3 (6.6–9.1)	1.6	7.5 (6.6–8.7)	0.3	7.5 (6.7–8.1)	0.3	0.401

Values are presented as median (interquartile range). The difference between measurements was tested using Friedman test.

CV_A – analytical coefficient of variation calculated from internal quality control results in the period from April 1st to April 30th 2016. †Bold values represent values greater than DSI derived from biological variation (13). DSI – desirable imprecision. DSB – desirable bias. RCV – reference change value. t_0 – time point before diving. t_1 – time point after diving. t_2 – 3 hours after diving. t_3 – 6 hours after diving. ‡Bold values for mean difference (%) represent values that are greater than DSB derived from biological variation (13). WBC – white blood cell count. NEU – neutrophil count. LYMP – lymphocyte count. MONO – monocyte count. EOS – eosinophil count. BASO – basophil count. RBC – red blood cell count. HGB – haemoglobin concentration. HCT – haematocrit. MCV – mean corpuscular volume. MCH – mean corpuscular haemoglobin. MCHC – mean corpuscular haemoglobin concentration. RDW – RBC distribution width. PLT – platelet count. MPV – mean platelet volume. *Significant differences in comparison to t_0 value ($P < 0.05$). §$P < 0.05$ was considered statistically significant.

Observed statistically significant increase for MCV immediately and 3 hours after diving and for MCHC 6 hours after diving should be taken with caution, since analytical imprecision for this parameters was less than DSI given in the 2014 updated database on biological variation (13). Furthermore, when the mean difference (%) between the two measurements was judged against DSB derived from biological variation (13), significant difference for MCV and MCHC was not found.

In order to take into account the analytical and biological variation, we calculated the reference change values (RCV). When the mean difference (%) between the two measurements was judged against calculated RCV, a clinically significant difference was not found in any of the tested haematological parameters.

Discussion

Although the clinical significance was not found in our study, our results show interesting changes for the WBC and RBC parameters that accompany the recreational scuba diving. The diving resulted in neutrophil count elevation immediately after surfacing, and the values stayed increased up to 6 hours. The effect on WBC and lymphocytes was delayed; while the WBC increase was observed after 3 hours, the lymphocyte count was found to be increased after 6 hours. Interestingly, monocyte count found to be decreased immediately after surfacing, 6 hours after diving was also increased. The neutrophil increase in the divers is an expected result, since physical exercise causes acute inflammatory response and neutrophil mobilization from marginated pool (7). Besides physical activity, physiological stress in divers, due to the increased release of stress hormones such as catecholamines and cortisol, may contribute to the neutrophil mobilization (15-17). Additionally, cold exposure during diving can induce an immune response and may affect leukocyte mobilization (18). The neutrophil peak (36%) recorded 6 hours after diving in our study indicates a significant immune response that usually accompanies long duration intense exercise (19). However, long duration demanding exercise is followed by lymphocyte decrease in the recovery phase, that was related to the "open window" in the susceptibility to infection (20,21). Hence, important information in our study is that recreational diving to 30 meters for 30 minutes was not followed by lymphocyte count decrease; furthermore, it resulted in lymphocytes increase 6 hours after diving. The reason for lymphocyte increase 6 hours after diving can only be speculated as a consequence of the combination of physical activity, physiological stress, hyperbaria, hyperoxia and exposure to cold. Considering the fact that mild monocytosis often accompanies neutrophil increase in the inflammatory response induced by exercise (22), especially in cold exposure (18), interesting and unexpected result in our study was monocyte decrease immediately after diving. The possible reason for that may lie in transendothelial migration caused by the alterations in vascular/endothelial function observed after one single dive as well as successive dives (23-25).

Although recreational scuba diving differs in many aspects to professional diving (2), some of our results are in agreement to the results obtained in several studies (8-10). Ferrer and Sureda have shown a neutrophil increase 3 hours after diving to 40 meters for 25 minutes and also WBC increase after diving to 50 meters for 35 minutes (9,10). However, immediately after diving, they found no difference for neutrophil count, which was observed in our study (9,10). Although the diving depth in these studies was higher than in our study (30 meters), it is reasonable to assume that discrepancy in speed of the neutrophil mobilization is a result of the difference of the inflammatory and hormonally response between recreational and professional divers. On the other hand, Glavas *et al*. have reported WBC and neutrophil increase with lymphocyte decrease immediately after diving to 54 meters for 100 minutes (8). Bearing in mind that total diving time in study by Glavas *et al.* was far longer than in the above mentioned and our study (approximately 30 minutes), it suggests that the main reason for neutrophil and WBC increase and for lymphocyte decrease could lay in the diving duration.

As far as regards the RBC parameters, we observed statistically significant decrease and difference judging against DSB (13) for RBC count, haemoglobin and haematocrit 3 and 6 hours after diving. The cause of RBC decrease could be explained by fragility of erythrocyte membranes as a consequence of the oxidative damage, which resulted in the haemoglobin and haematocrit decrease. It should be emphasized that scuba diving is characterized by demanding physical activity in hyperoxia resulting from hyperbaric exposure during diving and oxygen availability at high pressure, both of which could induce oxidative stress by increasing free radical production (26). Since the erythrocyte membranes are very vulnerable to peroxidative damage and erythrocytes are unable to repair damaged components as proteins by re-synthesis, they are very sensitive to oxidative stress (27). It is interesting to note that aforementioned studies found no difference for RBC count and haemoglobin in professional divers (8,9,11), while haematocrit decrease was only observed after diving to 40 meters (11). Possible reasons for the discrepancy between our and these results may be a consequence of the increase in antioxidant enzyme activities during consecutive dives in professional divers (9,11).

It should be mentioned that all blood samples in our study were taken after a meal to minimize the impact of the meal or prolonged fasting on haematological parameters (approximately t_0 and t_3 1 hour after meal, t_1 2 hours after meal, t_4 4 hours after meal) (28,29). To ensure objective criteria for clinical significance of the observed changes, we calculated the RCV for each parameter, since RCV takes into account the analytical variation of laboratory analyzers and intra-individual biological variation. When the mean difference (%) between the two measurements was judged against calculated RCV, we found that there was no clinically significant change in any of the tested parameters.

To the best of our knowledge, this is the first investigation that assesses post-diving variations of routine haematological parameters in recreational divers and with the information for clinical relevance of observed changes. Our results in some aspects showed a different effect on WBC and RBC parameters in comparison to previous studies on professional divers. The limitations of this study are small number of participants and unstandardized food intake. Additionally, our study did not provide data for how long observed changes remain present. Although observed changes in our study probably will not influence clinical judgment, the monocyte decrease immediately after diving, the absence of lymphocyte decrease in the recovery phase and decrease for RBC count, haemoglobin and haematocrit deserve attention in further research.

Conclusion

The results of our study has shown that observed changes after recreational scuba diving; WBCs, neutrophils, lymphocytes, monocytes increase and RBCs, haemoglobin, haematocrit decrease, probably will not influence the clinical judgment of the test results of haematological parameters.

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Potential conflict of interest

None declared.

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