Hematological variables in recreational breath-hold divers: a longitudinal study.

Running title: chronic apnea training & hematology

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BACKGROUND:

The influence of regular breath-hold training on hematological variables is not fully understood. We monitored hematological variables in breath-hold divers' (BHDs) and active controls over a year expecting both breath-hold training and seasonal effects.

METHODS:

In 11 recreational BHDs (36 ± 9 yrs, 177 ± 8 cm, 72 ± 9 kg) and 12 active controls (22 ± 2 yrs, 174 ± 8 cm, 70 ± 13 kg) monthly blood samples were analyzed with the hematological module of WADA's athlete biological passport. Hemoglobin mass and plasma volume were measured indirectly by the CO-rebreathing method for the last eight months of the study. Breath-hold training sessions were recorded online. Days without breath-hold training, or the number of hours prior to blood sampling when training was realized within the last 24 h, were recorded.

RESULTS:

Hematology did not differ significantly between BHDs and controls over the study time ($P > 0.05$). However, hematological values varied significantly over time for both groups suggesting seasonal effects. Blood sampling 19h or more after a breath hold training did not indicate any acute effects of breath holding training.

CONCLUSIONS:

In comparison with a physically active lifestyle, regular breath-hold training does not induce significant variations over one year for the hematological module of the ABP.

Key words: hematology, chronic apnea training, anti-doping

Introduction

Breath-hold diving has emerged as an organized sport with different disciplines, the main ones being dynamic, depth, and static. Breath holding performances are defined by horizontal or vertical under water distance covered, for dynamic and depth respectively, and apnea time for static (1). The common denominator is apnea tolerance. To improve their performance breath-hold divers (BHD) seek to control their urge to breathe, to delay their breakpoint. This is achieved by regular apneas, which increase the tolerance to hypercapnia (2, 3) and hypoxemia (4). Apart from leading to hypercapnic hypoxia, breath-holding induces the classic diving response with peripheral vasoconstriction and bradycardia. Even though hypercapnia solicits a stronger urge to breathe, it is the progressive hypoxemia which is the main performance limiting factor since it will eventually lead to loss of consciousness. Arterial blood samples obtained at surfacing after a dive to 42 m in six BHDs showed oxygen tensions as low as 31.6 ± 17.0 mmHg (5).

BHDs are therefore regularly exposed to episodes of hypoxemia. The accompanying diving reflex includes contractions of the spleen expulsing ~ 100 ml of concentrated red cells into the circulation (6). This leads to transient increases of blood hemoglobin concentration ([Hb]) and hematocrit (HCT), lasting between 2 and 10 minutes (7-9). While these acute effects are fairly well described there are few longitudinal studies describing any chronic effects of repeated exposure to hypoxemia during breath-holds in BHDs. During dynamic diving (with locomotion) the hypoxemia was sufficient to stimulate erythropoietin (EPO) release by the kidneys, with blood EPO increasing by 60% up to at least 180 min post apnea (10). Two weeks of breath-hold training in non-BHD increased reticulocyte percentage (Ret%) by 15%, but did not change [Hb] (11). Similarly, six weeks of dynamic breath-hold training resulted in a 28% increase in Ret%, which returned to baseline values one week after the training, while [Hb] remained unaltered (12). Whether the repeated stimulation of erythropoiesis in those studies was accompanied by an increase in total hemoglobin mass (Hbmass) is unknown since Hbmass nor plasma volume (PV) were quantified. With cross-sectional research, Prommer et al. (8) found that trained BHDs do not have greater Hbmass compared to controls. By contrast, Zelenkova (13) found that 7 elite BHDs had a somewhat higher Hbmass compared to controls, but lower Hbmass compared to endurance athletes.

The long-term effects of regular breath-holding on erythropoiesis thus appear to be limited, but remain to be better described (3). The Athlete Biological Passport (ABP) was developed for longitudinal monitoring of individual blood parameters in athletes for anti-doping

purposes (14, 15). Using the ABP we monitored a cohort of BHDs' hematological variations over a year and compared the results to those of an active control group. We tested the hypothesis that regular breath-hold training leads to variations in hematological variables, and more so in BHDs than in controls. We also repeatedly measured total hemoglobin mass (Hbmass) and plasma volume (PV). We hypothesized that breath-hold divers would have higher Hbmass and PV and more pronounced seasonal variation compared to controls.

Materials and methods

Participants

Fifteen BHDs (11 men and 4 women) were invited to participate. Four were excluded: two due to medical reasons and two because not reaching the inclusion criterion of an average minimum of one breath-hold training per week. A final sample of 11 healthy non-smoking BHDs (8 men and 3 women; 36 ± 9 yrs, 177 ± 8 cm, 72 ± 9 kg) was studied. They had at least four years of breath-hold training experience and engaged in an average of 1.3 ± 0.5 breath-hold training sessions per week. All were Swiss apnea club members, regularly participated in national and international competitions, and planned to compete in the Swiss championships in May 2019. Eight finally participated in the championships in the various disciplines: static, dynamic with fins and dynamic with no fins. Static time was 273 ± 47 s (258 ± 55 s for the women (n=2) and 278 ± 50 s for the men (n=6)), dynamic distance with fins was 112 ± 30 m (137 ± 53 m for the women (n=2) and 103 ± 19 m for the men (n=6)) and without fins 89 ± 26 m (125 m for the woman (n=1) and 82 ± 22 m for the men (n=6)). Twelve sports science students at the University of Lausanne were invited to participate as a control group (7 men and 5 women; 22 ± 2 yrs, 174 ± 8 cm, 70 ± 13 kg). They participated in-a maximum of 8 h physical training per week. Five were running on a regular basis, four were playing football, two were playing volleyball in local club and one was doing triathlon. They had no prior experience in breath-hold training. The students were somewhat younger than the BHDs ($P < 0.001$), while height ($P = 0.55$) and body mass ($P = 0.82$) were similar. All participants were healthy, medication free and lived < 800 m. They were informed about the procedure and risks of the study and provided fully informed written consent to participate. The study protocol was approved by the regional research ethics committee (CER-VD, Lausanne, Switzerland, #2018-01019) and conducted in respect of the Declaration of Helsinki.

Blood analyses

Between September 2018 and November 2019, the participants came monthly to the laboratory to provide a blood sample. World Anti-Doping Agency (WADA) blood collection guidelines were strictly followed (16, 17) using the same protocol as described elsewhere (18). Briefly, blood samples were collected at least 120 min after any structured physical activity. Blood was collected from an antecubital vein with a 21G short manifold butterfly needle (Sarstedt Safety-Multifly®, Sarstedt AG, Nuembrecht, Germany) in EDTA-coated tubes (Sarstedt S-Monovette 2.7 ml, Sarstedt AG, Nuembrecht, Germany), the equivalent of the BD Vacutainer®: K2-EDTA (K2) CE cat. no. 368856, as recommended in anti-doping guidelines. Blood samples were stored at 4° C and analyzed, after 15 min of homogenization on a roller system, with a fully automated flow cytometer (Sysmex XN1000, Sysmex Europe GmbH, Norderstedt, Germany) between 30 min – 12 h after blood collection. Internal quality controls were run in duplicate before each batch of samples (Sysmex XN-Checks, levels 1, 2, and 3). Test results were validated after two successive measures within a very narrow range for [Hb] (i.e. $\pm$ 0.1 $g \cdot dl^{-1}$) and for Ret% (i.e. $\pm$ 0.15) (15). The following blood variables were obtained: hematocrit (HCT), hemoglobin concentration [Hb], red blood cell count (RCB), reticulocyte percentage (Ret%), reticulocyte count (Ret#), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) (19), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (standard deviation) (RDW-SD), and immature reticulocyte fraction (IRF). In addition, Hbmass and volumes (i.e. plasma volume (PV), red blood cell volume (RBCV) and total blood volume (TBV)) were determined with a fully automated blood volume analyzer (OpCo: Detalo Instruments, Birkerod, Denmark) based on the carbon monoxide (CO) rebreathing technique, as described elsewhere (18, 20). Hbmass and blood volumes measurements could only be performed over the eight last visits to the laboratory due to technical reasons.

ABP Profiles

An individual longitudinal ABP profile for each participant was created as described elsewhere (18). Briefly, blood variables were entered into the ADAMS-training module in order to generate individual ABP profiles (21). The OFF-score (i.e. [Hb] x 10) - 60 x $\sqrt{Ret\%}$) and the Abnormal Blood Profile Score (ABPS) (i.e. a combination of Ret%, [Hb], HCT,

RBC, MCV, MCH and MCHC) were obtained via the ADAMS-training algorithm (21). After applying population-based upper and lower limits for the first sample, for the successive samples the reference ranges were adapted to the athlete's own values by use of a Bayesian statistical approach. An Atypical Passport Finding (ATPF) was generated when a) a [Hb] and/or OFF-score value of the last entered sample fell outside the lower and upper intra-individual limits or b) when the last 2 to 5 [Hb] and/or OFF-score values deviated from the expected range (a so-called "sequence ATPF"). For the first case, the applied specificity was 99% (i.e. 1:100 chance or less that the deviation was due to normal physiological variation). For the latter, the applied specificity was 99.9% (i.e. 1:1000 chance or less that the sequence deviation was due to normal physiological variation). In addition, for each participant we calculated the shortest distances to lower and upper personal limits, calculated by the Bayesian model, for each ABP biomarker of each sample (18).

Breath-hold training quantification

BHDs recorded their breath-old training via a training monitoring interface (Training Peaks™ (TP), PeaksWare, Lafayette, Colorado, USA). Breath-hold training type, i.e. static/dynamic, with/without water immersion was recorded. Breath-hold training sessions were categorized into six categories: 1) dynamic breath-holds (i.e. with or without fins in a swimming pool); 2) static apnea training with face immersion; 3) mix of dynamic breath-hold training and static with face immersion in the same session; 4) deep apnea training in open water; 5) dry dynamic breath-hold training (i.e. on an ergometer, while running or cycling); and 6) dry static breath-holds. Breath-hold training was entered after the first visit so that training activities prior to the first blood sample could not be considered. The number of days/hours without apnea training before a blood sample was quantified.

Statistical analyses

Descriptive values are given as means and standard deviations. Anthropometric data (i.e. age, height, body mass) and the shortest distances to the individual upper and lower limits for each ABP biomarker (i.e. [Hb], Ret%, OFF-score and ABPS) for both groups were compared with the independent sample t-test, the Welsh test or the Mann Whitney non-parametric test, according to variance homogeneity and deviation from normality. Boxplots representing median, first and third quartiles with whiskers indicating variability outside the

first and third quartiles are presented for the ABP biomarkers, Hbmass and PV for each group.

Hematological variables (i.e. HCT, [Hb], RCB#, Ret%, Ret#, MCV, MCH, MCHC, RDW-SD, IRF%), the OFF-score, and the ABPS of the 12 monthly blood samples were analyzed with a mixed model (with participant as random factor) to test the effect of group and time (fixed factors). Visual inspection of residual plots allowed excluding any obvious deviations from homoscedasticity or normality. Breath-hold training influence was investigated in two different ways: 1) using as covariate the number of days without breath-hold training before blood sample; and 2) using as factor a dichotomous variable on the average time between the training and the blood test for the subgroup that had one in the last 24 hours. We performed two series of mixed models (subject as random factor) to test the effect of time and a variable of breath-hold training (as covariate or fixed factors).

Hbmass and PV, monitored for the eight last months of the study, were analyzed with a mixed model (participant as random factor) to test the effect of group and time (fixed factors). Polynomial contrasts were used for time in mixed models, employing the Bonferroni method. The level of significance was set at $P < 0.05$. All statistical analyses were conducted with open source statistical software (Jamovi version 1.0.5.0, Jamovi Project Software, retrieved from https://www.jamovi.org.

Results

BHDs' training

On average 67.6 ($\pm$ 28.6) breath-hold training sessions were realized over the study period by each BHD. They consisted of: 1) 52% dynamic breath-holds (i.e. with or without fins in a swimming pool); 2) 9% static apnea trainings with face immersion; 3) 8% mix of dynamic breath-hold training and static with face immersion in the same session; 4) 13% of deep apnea training in open water; 5) 9% dry dynamic breath-hold training (i.e. on an ergometer, while running or cycling); and 6) 9% dry static breath-holds.

Hematological values

All participants had 12 blood samples collected over the study with the exception of one diver who missed one (total of 275 blood samples). Time elapsed between two blood samples was 30.5 $\pm$ 3 days for the divers and 31.3 $\pm$ 6 days for the control group ($P = 0.77$).

Distribution of the values for each ABP biomarkers (i.e [Hb], OFF-score, Ret% and ABPS) and Hbmass and PV for each group are presented in Figure 1. Individual mean and standard deviation of hematological values for each participant are presented in Table 1.

INSERT FIGURE 1

INSERT TABLE 1

Over the 12 assessments, the average values for the hematological variables between groups are presented in Table 2. There was no significant influence of group for any hematological variable ($P > 0.05$). There were significant effects of time for all hematological variables ($P < 0.05$) with the exception of the ABPS ($P = 0.99$). Polynomial contrasts for time were mostly quadratic (Table 2). Interaction group x time was significant for Ret% ($P < 0.001$), Ret# ($P = 0.004$) and IRF% ($P = 0.013$). For those variables, a linear decrease with time appeared for controls, and stability for the BHDs.

INSERT TABLE 2

ABP profiles

A typical BHD ABP profile is presented in Figure 2. The hematological profiles of all BHD and control group participants are available as Supplementary Figures I to X for the divers and XI to XXII for the control group. In the full data set, covering a year, only one point ([Hb]) fell outside of the individual ABP limits thus generating an ATPF in the ADAMS-training hematological module. The acute variation in [Hb] for this subject occurred concomitantly with a change in PV between the fourth and the fifth measurement (see supplementary Figure 10 for details).

INSERT FIGURE 2

The average shortest distances to the individual limits of the ABPS variables were not significantly different between groups ([Hb]: 1.2 ± 0.3 vs. 1.2 ± 0.4 g·dl^{-1} ($P = 0.87$), OFF-score: 18.5 ± 4.3 vs. 18.8 ± 5.8 ($P = 0.86$), Ret%: 0.48 ± 0.16 vs. 0.49 ± 0.16 ($P = 0.89$) and ABPS: 0.94 ± 0.21 vs. 0.93 ± 0.31 ($P = 0.93$) for BHD and controls respectively).

Influence of breath-hold training

The time elapsed between a breath-hold training session and blood sampling averaged 3.5 ± 2 days (range: 0-6 days). This elapsed period of time was not related to variations of the hematological variables (i.e. [Hb], HCT, Ret%, OFF-score and ABPS) ($P > 0.05$) nor to Hbmass or PV ($P > 0.05$). There was a relationship between an increased number of days without apnea training and an increase in the IRF% (F (1,107) = 5.6, $P = 0.02$, b = 0.16). When considering only the training session that was the closest to blood sampling in all BHDs (average time between training and blood sample: 19.4 ± 2.3 h) there was no influence observed of the latter training session on the hematological variables, Hbmass or PV ($P > 0.05$). For further details, all variables and statistics of the relationships with breath-hold training are presented in supplementary file (Supplementary Table 1).

Discussion

This is the first study collecting and analyzing monthly blood samples over one year in recreational BHDs for comparison with samples of an active control group. We tested the hypothesis that breath-hold training induces changes in hematological variables in BHDs over time. The main findings of this study were that: a) over a one-year period, hematological values varied significantly over time for both BHDs and an active control group; b) the time effects were present for all blood variables, with polynomial contrasts (quadratic effects) on most variables, suggesting seasonal variations; c) Ret% and IRF were stable in BHDs over the study time despite regular apnea training; and d) BHDs do not have different hematological characteristics in comparison to an active control group.

Variations in hematological variables of elite athletes over a competitive season have been reported before, with notable temporary decreases in [Hb], HCT and Ret% after heavy training periods (22). These hematological variations seem more pronounced for more endurance-type sports like cycling, running and swimming as compared to intermittent-type sports such as football and rugby (22), even though Diaz et al., (2011) observed a certain stability in Ret% and [Hb] in elite triathletes followed over four consecutive seasons

In addition to the significant effects of time found in our study, for all hematological variables – with the exception of the ABPS – we found that the polynomial contrasts for time were mostly quadratic. These findings are suggestive of a seasonal effect that was not

different between BHDs and controls. The ABP analytical module thus seems sufficiently robust to not flag such variations as potentially the result of blood doping practices.

There was variability in the distribution of ABP biomarkers values in BHDs, which were also present in the control group (Figure 1). Regarding the primary (i.e. [Hb], OFF-score) and secondary (i.e. Ret% and ABPS) biomarkers of the ABP, the distances between measured variables to the individual limits of the ABP were quite short and did not differ significantly between groups. We observed one ATPF on a primary biomarker of the ABP (i.e. [Hb]) in a BHD (supplementary Figure 12). This participant had been on vacation in a hot country during which he had refrained from any endurance exercise or breath-holding training to then return to cold January weather prior to coming to the laboratory for blood sampling. We tentatively explain the drop in PV for this athlete in January compared to his prior and subsequent values to be the result of a combination of detraining and acute cold exposure (24). Both environmental heat exposure and increased endurance training load are known to increase PV and thereby affect concentration-dependent hematological variables such as [Hb] (18). This underlines the relevance of documenting an athlete's location and training, or better, measuring PV, for a more nuanced appreciation of an athlete's hematological values, something particularly relevant in an anti-doping context.

In the case of BHDs one should also make sure that the acute effects of spleen contraction have subsided, since this is known to cause acute temporary changes in [Hb], HCT and Ret%. A study of Richardson et al., (2005) showed that 3 maximal repeated apneas, without immersion and separated by 2 min of recovery, acutely increased [Hb] in divers, skiers and untrained humans (25). Such effects have been reported when apnea was practiced with (26, 27) and without face immersion (7, 28). The observed increases in HCT and [Hb] are thought to be transient, lasting between 2 and 10 min (25, 29). However, the exact minimum time needed to allow a complete return to baseline levels is not known. Our study was not designed to determine this, but in our BHDs a minimum of 19 h separating the blood sampling from a prior breath-hold training appeared to exclude any significant variation in the monitored hematological variables. This suggests that for anti-doping purposes leaving a pause of at least 19 hours after a breath-hold training would be sufficient to prevent confounding from the acute effects of splenic contraction. We found that IRF% increased as a function of the number of days without breath-hold training, illustrating a tenuous link

between a variation in IRF% and breath-hold training sessions. Others showed that IRF% was unchanged 30 min after a breath-hold training session to then decrease by 20% from baseline value after 4 h (30). Taken together, this may suggest that breath-hold training sessions activate the release of hemoglobin by splenic contraction (thus altering acutely [Hb], HCT and IRF%) (7) instead of generating new precursors of RBC to cope with transient hypoxemia/hypoxia.

Our study design has some limitations. First, we could not record PV or Hbmass during the first four months of the study, due to technical issues. Then, we did not directly monitor iron stores in our participants and therefore cannot exclude that some might have been iron deficient, which could have negatively influenced new RBC production (31). However, the hematological variables allowed us to exclude any clear iron-deficiency anemia. While active blood doping by the participants cannot be excluded, its likelihood would seem extremely low given the limited rational to blood dope in our participants and the results of the ABP monitoring (32). The small number of women in our study and the influence of the menstrual cycle on blood composition (33) do not allow us to draw any conclusions of sex-specific effects. Finally, the non-elite level of our BHDs precludes generalization to the elite.

From a performance and in an anti-doping perspective, the acute effects of breath-holding on [Hb] and Ret% could be used by athletes to 1) enhance their performance as breath-holding leads to a „natural" auto-transfusion, as 50% of the 200-250 mL of packed RBC that are stored in the spleen can be released into the circulation upon breath-holding (27), increasing blood O_2 transport capacity (30); or 2) to use breath-holding right before an anti-doping control to increase their [Hb] and Ret%, so as to mask any actual blood doping at other times of anti-doping controls where the athlete would refrain from breath-holds prior to sampling. Whether the latter approach is feasible and how the resulting variations of the monitored blood parameters by ABP would play out remains to be investigated directly.

Conclusions

In conclusion, we report that regular breath-hold training does not induce significant long term hematological variations in comparison to a being physically active, while seasonal variations occur in blood parameters in both divers and controls. The ABP can be used in

breath hold divers by leaving at least 19 h between the last apnea training and blood sampling.

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Authors' contributions.
Raphael Faiss and Martial Saugy have given substantial contributions to the conception or the design of the manuscript, Tiffany Astolfi and Raphael Faiss to data acquisition. All authors participated in the analysis and interpretation of the data. All authors have participated to drafting the manuscript, Tiffany Astolfi revised it critically. All authors read and approved the final version of the manuscript.

TABLES

Table I. *Mean and standard deviation of hematological variables for each divers and participant in the control group.*

	W/M	[Hb] [g/dl]	HCT [%]	RBC [10^6/ l]	Ret [%]	Ret# [10^6/ l]	MCV [fL]	MCH [pg]	MCHC [g/dL]	RDW-SD [fL]	IRF [%]	OFF-score [a.u.]	ABPS [a.u.]	PV* [mL]	Hbmass* [g]	Hbmass* [g.kg^-1]
Divers 1	W	13.2 ± 0.3	38.8 ± 1.2	4.3 ± 0.1	0.96 ± 0.13	0.042 ± 0.005	89.5 ± 1.47	30.5 ± 0.3	34.1 ± 0.5	37.5 ± 0.96	2.5 ± 1.7	73.9 ± 6.1	-1.73 ± 0.14	3259 ± 197	710 ± 46	10.7 ± 0.7
Divers 2	M	15.5 ± 0.5	43.3 ± 1.8	4.9 ± 0.2	1.25 ± 0.31	0.061± 0.017	87.7 ± 0.94	31.7 ± 0.7	36.1 ± 0.9	38.8 ± 2.13	4.5 ± 1.6	88.7 ± 6.3	-0.76 ± 0.61	4154 ± 204	1110 ± 36	11.1 ± 0.3
Divers 3	W	13.0 ± 0.5	37.8 ± 1.5	4.4 ± 0.2	0.88 ± 0.16	0.038 ± 0.066	85.8 ± 0.93	29.5 ± 0.2	34.4 ± 0.4	39.0 ± 0.95	4.6 ± 1.4	74.5 ± 7.6	-1.91 ± 0.12	3256 ± 132	683 ± 44	10.5 ± 0.4
Divers 4	M	14.5 ± 0.6	42.0 ± 2.0	4.9 ± 0.2	0.91 ± 0.18	0.045 ± 0.007	85.3 ± 1.13	29.5 ± 0.22	34.5 ± 0.4	41.5 ± 1.00	6.8 ± 1.9	91.1 ± 7.1	-1.49 ± 0.51	3583 ± 342	880 ± 54	13.3 ± 0.9
Divers 5	M	15.5 ± 0.5	43.5 ± 1.6	5.2 ± 0.2	1.06 ± 0.15	0.054 ± 0.007	84.4 ± 0.80	30.0 ± 0.29	35.6 ± 0.4	36.4 ± 0.61	5.2 ± 2.1	95.4 ± 6.0	-1.12 ± 0.47	3361 ± 177	931 ± 40	14.4 ± 0.7
Divers 6	M	13.5 ± 0.5	39.4 ± 1.6	4.5 ± 0.2	1.07 ± 0.11	0.048 ± 0.006	88.1 ± 1.34	30.3 ± 0.26	34.3 ± 0.4	37.0 ± 0.89	6.9 ± 2.2	74.2 ± 5.7	-1.77 ± 0.14	4796 ± 409	1048 ± 43	14.1 ± 0.3
Divers 7	M	13.2 ± 0.2	39.2 ± 0.9	4.4 ± 0.1	0.84 ± 0.17	0.036 ± 0.007	89.6 ± 1.14	30.2 ± 0.28	33.7 ± 0.5	40.0 ± 1.16	3.0 ± 1.1	76.9 ± 6.5	-1.57 ± 0.19	4401 ± 217	981 ± 35	12.1 ± 0.3
Divers 8	M	14.2 ± 0.4	40.6 ± 1.1	4.6 ± 0.1	1.21 ± 0.16	0.055 ± 0.008	88.4 ± 1.03	31.0 ± 0.25	35.1 ± 0.35	37.8 ± 1.12	7.7 ± 1.4	78.1 ± 4.7	-1.57 ± 0.17	3849 ± 179	918 ± 53	11.3 ± 0.5
Divers 9	M	14.3 ± 0.4	42.1 ± 1.3	4.6 ± 0.1	0.90 ± 0.16	0.042 ± 0.007	91.8 ± 1.16	31.1 ± 0.25	33.9 ± 0.97	42.5 ± 0.72	5.3 ± 1.9	87.7 ± 3.8	-1.31 ± 0.23	3328 ± 282	800 ± 16	11.7 ± 0.2
Divers 10	M	15.0 ± 0.7	42.3 ± 1.9	5.3 ± 0.3	1.52 ± 0.15	0.080 ± 0.008	79.7 ± 0.6	28.3 ± 0.32	35.5 ± 0.42	34.4 ± 0.51	4.4 ± 1.5	78.5 ± 9.9	-2.27 ± 0.31	4234 ± 554	1089 ± 51	13.9 ± 0.6
Divers 11	W	12.8 ± 0.5	37.2 ± 1.6	4.3 ± 0.1	1.50 ± 0.21	0.064 ± 0.051	86.6 ± 1.2	29.8 ± 0.27	34.4 ± 0.49	38.7 ± 1.03	7.0 ± 2.2	56.7 ± 8.6	-1.78 ± 0.16	3081 ± 314	623 ± 28	10.2 ± 0.4
Control 1	W	13.0 ± 0.5	38.2 ± 1.8	4.3 ± 0.2	1.44 ± 0.28	0.062 ± 0.001	88.2 ±1.1	29.9 ± 0.27	33.9 ± 0.46	39.8 ± 0.95	4.0 ± 1.9	58.0 ± 9.6	-1.88 ± 0.46	3355 ± 212	719 ± 26	9.3 ± 0.3
Control 2	M	15.0 ± 0.5	44.7 ± 1.6	5.4 ± 0.2	1.30 ± 0.10	0.070 ± 0.006	82.3 ± 0.9	27.7 ± 0.35	33.6 ± 0.50	34.8 ± 0.77	7.1 ± 1.8	81.9 ± 5.9	-1.11 ± 0.67	3469 ± 308	948 ± 45	11.1 ± 0.5
Control 3	W	13.1 ± 0.6	38.6 ± 2.0	4.6 ± 1.9	1.16 ± 0.12	0.052 ± 0.005	84.5 ± 1.4	28.6 ± 0.20	33.8 ± 0.53	37.5 ± 1.44	3.9 ± 1.5	66.0 ± 7.7	-1.11 ± 0.78	2826 ± 188	603 ± 15	9.6 ± 0.3
Control 4	M	15.8 ± 0.3	45.5 ± 1.3	5.4 ± 0.1	1.08 ± 0.19	0.057 ± 0.001	84.9 ± 1.1	29.6 ± 0.2	34.8 ± 0.42	35.8 ± 0.88	3.9 ± 1.7	96.2 ± 7.6	-1.39 ± 0.62	4254 ± 569	1197 ± 51	11.1 ± 0.6
Control 5	W	13.1 ± 0.3	38.4 ± 0.8	4.3 ± 0.1	1.35 ± 0.13	0.057 ± 0.006	90.3 ± 0.7	30.7 ± 0.3	34.0 ± 0.29	41.8 ± 1.12	5.9 ± 2.1	60.9 ± 2.9	-1.31 ± 0.62	2792 ± 104	591 ± 22	9.8 ± 0.3

Control 6	M	15.4 ± 0.4	43.8 ± 1.5	5.2 ± 0.2	1.14 ± 0.17	0.059 ± 0.009	84.0 ± 0.86	29.6 ± 0.33	35.2 ± 0.4	37.5 ± 0.9	4.3 ± 1.5	89.9 ± 3.8	-1.34 ± 0.88	2774 ± 173	749 ± 28	12.3 ± 0.5
Control 7	M	13.6 ± 0.4	39.9 ± 1.3	4.5 ± 0.2	1.17 ± 0.18	0.052 ± 0.007	89.3 ± 1.59	30.4 ± 0.31	34.1 ± 0.4	38.3 ± 1.3	7.6 ± 1.6	71.5 ± 6.6	-1.52 ± 0.50	3995 ± 283	903 ± 18	11.5 ± 0.4
Control 8	W	14.2 ± 0.7	41.3 ± 2.2	4.6 ± 0.2	1.42 ± 0.27	0.065 ± 0.013	90.2 ± 1.07	31.1 ± 0.30	34.5 ± 0.5	39.5 ± 0.8	5.2 ± 1.9	71.2 ± 8.5	-1.29 ± 0.86	2810 ± 300	644 ± 32	10.8 ± 0.6
Control 9	W	12.9 ± 0.4	37.7 ± 1.5	4.5 ± 0.2	1.15 ± 0.26	0.051 ± 0.011	84.1 ± 1.11	28.8 ± 0.35	34.2 ± 0.4	35.2 ± 1.2	4.2 ± 2.1	64.7 ± 9.5	-1.31 ± 0.62	3252 ± 222	665 ± 13	9.6 ± 0.3
Control 10	M	15.8 ± 0.8	43.7 ± 2.5	5.1 ± 0.2	1.07 ± 0.16	0.054 ± 0.008	85.1 ± 1.44	30.8 ± 0.28	36.2 ± 0.6	35.2 ± 1.3	5.6 ± 1.6	96.3 ± 9.2	-1.57 ± 0.59	3374 ± 384	881 ± 25	11.6 ± 0.4
Control 11	M	15.6 ± 0.3	44.7 ± 0.9	5.0 ± 0.1	1.05 ± 0.16	0.052 ± 0.008	90.1 ± 1.19	31.4 ± 0.27	34.8 ± 0.6	40.6 ± 1.0	5.6 ± 1.3	94.3 ± 5.1	-1.65 ± 0.60	3543 ± 205	977 ± 42	13.9 ± 0.7
Control 12	M	14.1 ± 0.4	41.0 ± 1.2	4.6 ± 0.1	1.03 ± 0.14	0.047 ± 0.007	89.2 ± 1.0	30.6 ± 0.38	34.3 ± 0.5	41.3 ± 1.0	6.3 ± 1.8	79.9 ± 3.7	-1.67 ± 0.55	3689 ± 282	878 ± 42	11.8 ± 0.6

Table 2. *Mean and standard deviation of hematological variables for the divers group and control group with various effects (time, group,, time X group).*

	[Hb]	HCT	RBC	Ret	Ret#	MCV	MCH	MCHC	RDW-SD	IRF	OFF-score	ABPS	PV*	Hbmass*	Hbmass*
	[g/dl]	[%]	[10^6/ l]	[%]	[10^6/ l]	[fL]	[pg]	[g/dL]	[fL]	[%]	[a.u].	[a.u|.	[mL]	[g]	[g.kg^{-1}]
Divers group	14.1 ± 1.0	40.5 ± 2.5	4.7 ± 0.4	1.10 ± 0.29	0.052 ± 0.015	87.0 ± 3.2	30.2 ± 0.9	34.7 ± 0.9	38.5 ± 2.4	5.3 ± 2.3	78.2 ± 12.0	- 1.57 ± 0.5	3794 ± 616	902 ± 161	12.20 ± 1.6
Control group	14.3 ± 1.2	41.5 ± 3.2	4.8 ± 0.4	1.20 ± 0.23	0.056 ± 0.010	86.9 ± 3.0	29.9 ± 1.1	34.5 ± 0.8	38.1 ± 2.6	5.3 ± 2.1	77.6 ± 15.1	- 1.43 ± 0.7	3372 ± 543	824 ± 176	11.60 ± 1.5
Group effect	0.699	0.428	0.527	0.285	0.269	0.943	0.533	0.347	0.767	0.861	0.888	0.288	0.078	0.311	0.452
Time effect	<.001 Q	<.001 Q, C	<.001 Q	<.001	0.039	<.001 L, C	0.121	<.001 L, C	0.052	<.001 L, H	<.001 Q, C	0.992	<.001	0.001 Q	0.003
Time x Group	0.540	0.507	0.672	<.001 L	0.004 L	0.280	0.619	0.471	0.062	0.013 L, H	0.008	0.090	0.682	0.303	0.137

Table 2. Average divers group and control group hematological variables over 12 months. Values reported as means ± SD. [Hb]: hemoglobin concentration; HCT%: hematocrit; RBC: red blood cell count; Ret%: reticulocytes; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin content; RDW-SD: red cell distribution width (standard deviation); IRF: immature reticulocytes fraction; OFF-score; ABPS: abnormal blood profile score; PV: plasma volume; Hbmass: hemoglobin mass. a.u.: arbitrary units. *: were monitored during the last eight months of the study. P-value for Group, time and Time X Group effect are presented. Polynomial contrasts for Time: L=linear, Q=quadratic, C=cubic, H=order higher than 3.

TITLES OF FIGURES

Figure 1. Distribution of the values for each ABP biomarkers: **(A)** hemoglobin concentration ([Hb]), **(B)** OFF-score, **(C)** reticulocytes (Ret%) and **(D)** abnormal blood profile score (ABPS) and **(E)** hemoglobin mass (Hbmas) and **(F)** plasma volume (PV) for each group.

Figure 2. Representation of the Athlete Biological Passport (ABP) hematological profile for diver 10, with **(A)** [Hb]: hemoglobin concentration; **(B)** OFF-score, **(C)** Ret%: reticulocytes percentage; **(D)** ABPS: abnormal blood profile score; over the 12 months of the study design. Solid line represents the athlete's values, dotted line represents the upper limits and lower limits calculated by an adaptive Bayesian model (see methods section for details).