ORIGINAL ARTICLE

Erythrocyte, haemoglobin and haematocrit do not correlate with apnoea duration among sedentary male

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ABSTRACT

In freediving, divers rely solely on a single breath. The duration of apnoea depends on the oxygen reserves, mostly derived from the air trapped in the lungs and airways. It is necessary to investigate whether erythrocyte and haemoglobin levels correlate with the achievable apnoea duration, considering their roles as oxygen binders and carriers in the blood. This study examines the correlation between erythrocyte and haemoglobin levels and apnoea duration in 12 sedentary males. Erythrocyte and haemoglobin levels were assessed through blood sample examination in a clinical laboratory by professional personnel, while apnoea duration was measured by remaining motionless in a swimming pool at a depth of 0.5 meters, with three measurements taken. Normality test results indicated that all data were normally distributed ($p > .05$). Pearson correlation test results revealed no correlation between apnoea duration and erythrocyte levels, haemoglobin, or haematocrit ($p > .05$). From the research findings, it can be assumed that these three blood biochemical variables cannot be categorized as oxygen reserves and only function as oxygen transportation media.

Keywords: Sport medicine, Blood, Breath-hold, Diving, Sport performance.

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INTRODUCTION

Diving can be categorized into two distinct types: freediving, which does not involve the use of underwater breathing apparatus, and SCUBA diving, which relies on the utilization of a Self-Contained Underwater Breathing Apparatus (SCUBA). These two divergent types of diving are characterized by difference of techniques and procedures. In the context of SCUBA diving, divers are able to maintain continuous respiration throughout the entire dive. It is important for divers to refrain from breath-holding during the dive session in order to mitigate physiological problem. Conversely, in freediving practices, where divers initiate the dive only by relying to a single breath taken at the water's surface, followed by breath-holding throughout the dive duration. Both types of diving encounter the same environmental conditions, physical consequences at depth and causing physiological stress (Eichhorn & Leyk, 2015), where divers are confronted with significant changes of water pressure and the potential disturbances or injuries to the tympanic membrane in ear, commonly referred to as barotrauma (Jones & Wyatt, 2019; Rozycki et al., 2018).

In freediving, the diver holds their breath (apnoea) during the duration of the dive, without any exchange of ventilation between the body and the environment. The only breath-related activity that might occur is exhalation, placing the diver's body at risk of experiencing oxygen deficiency (hypoxia) leading to blackout (Lindholm & Lundgren, 2009; Pearn et al., 2015). Throughout the dive, there is no supply of oxygen from respiration, yet the body continues to consume oxygen and produce carbon dioxide incessantly. The diver relies solely on the air taken in through a single deep breath while still at the water's surface. Consequently, physiological factors within the diver's body become the primary determinants of how the body manages oxygen, directly influencing apnoea duration and, naturally, the duration of the dive.

Several studies have been conducted regarding physiological factors correlated with apnoea capability. Lung capacity has been found to be associated with an individual's ability to hold their breath. A study involving 30 male subjects indicated that There is a strong positive correlation between lung vital capacity and apnoea duration (Putra, Pratama, et al., 2020) A strong positive correlation indicates the significant role of lung vital capacity as the main provider of oxygen reserves during freediving. The body's oxygen utilization capacity (VO_{2Max}) is also recognized to be linked to breath-holding ability. A study involving 36 male subjects revealed that There is a negative correlation between VO_{2max} and apnoea duration. A higher VO_{2Max} corresponds to greater oxygen consumption by the body per minute, rendering the body more extravagant in oxygen utilization (Putra, Karwur, et al., 2020).

Oxygen is utilized to fulfil the energy metabolism requirements within cells. Oxygen is transported through blood vessels by haemoglobin of red blood cells (Guyton & Hall, 2016; Sherwood, 2015). Because of its role in oxygen binding and distribution, the levels of red blood cells and haemoglobin in the blood are also presumed to correlate with the apnoea capabilities of divers. Researchers hypothesize that higher concentrations of haemoglobin and red blood cells in the blood may signify a larger reserve capacity of oxygen flowing within the bloodstream, ready to be utilized by the tissues it passes through. The aim of this study is to explore the correlation between red blood cells and haemoglobin levels and breath-holding abilities. The findings of this research are expected to serve as a reference for freedivers in considering their red blood cell and haemoglobin levels in their training programs and dive preparations.

METHODS

Study population

This study recruited 14 male subjects using purposive sampling method. The subjects in this study were students from the physical education program selected based on inclusion criteria. The inclusion criteria applied were being male, having chest circumference between 75-85 cm, body fat percentage not exceeding 13%, no history of heart or respiratory diseases, not being in an ill or medicated condition, having no fear of swimming pool depths, and expressing willingness to participate as subjects, demonstrated by signing an informed consent form. This research was approved by the Ethics Commission of Satya Wacana Christian University with ethical clearance number 094/KOMISIETIK/EC/9/2022.

Protocol

The researchers recruited subjects who met the inclusion criteria within the Salatiga City area. Upon obtaining consent, to ascertain subject suitability, measurements of chest circumference and body fat percentage were conducted, along with interviews and medical history assessments to identify any disease history and psychological issues related to swimming pool depths.

The researchers then scheduled appointments with subjects who met the inclusion criteria to carry out apnoea duration measurements in the swimming pool and haematological examinations at the Prodia Clinical Laboratory in Salatiga City. All subjects were instructed not to consume caffeine and alcohol, avoid staying up late starting 24 hours, avoid exercise or any moderate physical activity 12 hours before the apnoea duration measurement in the swimming pool.

Body temperature, blood pressure, and capillary oxygen saturation (SpO2) examinations were conducted on the same day prior to the apnoea duration measurements and haematological examinations. The body temperature, blood pressure, and SpO2 assessments were carried out to ensure that all subjects were not in an ill condition during the apnoea duration measurements and haematological examinations. The examination of body fat levels was conducted using non-invasive Bio-Impedance Analysis method employing Omron HBF-375. Capillary oxygen saturation was measured utilizing a pulse oximeter applied to the subject's index finger, while body temperature was measured using a non-contact infrared thermometer. Blood pressure was measured using a digital sphygmomanometer. If the body temperature exceeds 37.5 degrees Celsius, systolic blood pressure is below 90 mmHg and above 125 mmHg, or the diastolic blood pressure is below 70 mmHg and above 95 mmHg, or capillary oxygen saturation below 94%, the subject is not allowed to proceed with the apnoea test and haematological examination.

The apnoea duration test in the swimming pool were conducted by the researchers. The duration of apnoea was measured by calculating the longest time a subject could hold their breath underwater while sitting still in a pool with a depth of 0.5 meters from the water surface. The apnoea duration measurement was performed through 3 trial attempts, and the longest time was recorded. Timing commenced as the head entered the water and concluded upon the head's emergence from the water. A digital stopwatch was used for timing purposes.

Haemoglobin (Hb) measurements were conducted on subjects before assessing apnoea duration. The Hb measurement was performed using the Cyanmethemoglobin method by trained professionals at the Prodia Clinical Laboratory. Blood collection was conducted once per subject, using 20 µL (microliters) of blood. Following blood collection, the blood was stored in a container placed in a cooling box along with other blood samples before being tested using the Cyanmethemoglobin method.

The Cyanmethemoglobin method involves diluting the blood with a Drabkin solution, inducing erythrocyte haemolysis, which subsequently converts haemoglobin into cyanide haemoglobin. The resulting solution is then examined using a spectrophotometer (colorimeter), where the absorbance value correlates with the haemoglobin concentration in the blood. If the obtained haemoglobin concentration result meets the criteria, the subject's ability to hold their breath (apnoea) will be assessed. Results of the haematological examinations were generated in duplicate. The original result sheets were given to the subjects, while the duplicate sheets were retained by the researchers as research data.

Statistical analysis

Data obtained were then tested for normality and correlation using SPSS version 25. To determine the normal distribution of the data, the Shapiro-Wilk test was employed since the number of subjects in this study is less than 50 individuals. The criterion used to assess normality is that if the *p*-value is greater than .05, the data can be considered normally distributed.

For correlation analysis between Hb levels and apnoea duration, the Pearson correlation coefficient was used. The criterion for determining the correlation between Hb levels and apnoea duration is that if the *p*-value is less than .05, it can be concluded that there is a correlation between haemoglobin levels and breath-holding duration (apnoea).

RESULT & DISCUSSION

From 14 subjects, it was found that one subject experienced anaemia and one subject had erythrocyte concentration above normal, consequently leading to the exclusion of two subjects from the analysis. Thus, the total number of subjects meeting the inclusion criteria amounted to 12 Subjects. Table 1 indicates that the subjects in this study are similar in age, weight, body fat percentage, and chest circumference.

Table 1. Descriptive statistic.

Variable	**Mean ± SD**	**Min**	**Max**
Age (yr.)	23.58 ± 1.3	21	25
Body Height (cm)	166.67 ± 5.17	157	178
Body Weight (kg)	53.18 ± 3.87	45.7	59.1
Chest Circumference (cm)	79.0 ± 2.37	76	82
Body Fat (%)	9.94 ± 1.79	7.7	12.4
SpO2 (%)	97.0 ± 1.34	95	99
Haematocrit (%)	45.03 ± 1.95	41	47
Erythrocyte (10^6/uL)	5.11 ± 0.33	4.53	5.61
Haemoglobin (g/dL)	15.35 ± 0.86	14.2	16.9
Apnoea duration (sec)	18.07 ± 6.93	9.66	31.23

Table 2. The result of normality test.

	Shapiro-Wilk		
	Statistic	df	Sig.
Apnoea duration	.917	12	.264
Erythrocyte	.933	12	.412
Haemoglobin	.956	12	.726
Haematocrit	.944	12	.548

Table 3. Result of Pearson correlation test.

		Erythrocyte	**Haemoglobin**	**Haematocrit**
Apnoea duration	Pearson Correlation	.121	.201	-.185
	Sig. (1-tailed)	.354	.266	.283
	N	12	12	12

The result of the normality test indicates that the data from all variables are normally distributed ($p > .05$). The result of the Pearson correlation test (1-tailed) indicates that there is no significant correlation ($p > .05$) between the apnoea duration and the levels of erythrocytes, haemoglobin, and haematocrit.

This study's findings surprisingly indicate the absence of correlation between apnoea duration and erythrocytes, haemoglobin, and haematocrit, thereby refuting the researcher's hypothesis. There is a possibility that erythrocytes and haemoglobin solely serve as oxygen transport media, whereas the bound oxygen within them in the blood vessels cannot be considered as oxygen reserves. This might be due to the easy binding and release of oxygen from haemoglobin anywhere in the body depending on the partial pressure of gas at that location, or the rapid distribution of oxygen from the lungs to the cells, making the oxygen in transit within the blood vessels not significant as a reserve (E. Barrett et al., 2012; Wagner, 2023).

The presence of carbon monoxide (CO) gas also needs to be considered as an interfering factor because once inhaled, it will strongly bind with haem in haemoproteins such as haemoglobin (Hb) and myoglobin (Mb), which are crucial for oxygen binding (Mao et al., 2021). Furthermore, a study (Maehira et al., 2022) suggests that haemoglobin bound to carbon monoxide (HbCO) exhibits greater physicochemical stability in aqueous conditions compared to when it is bound to oxygen (HbO_2). As a result, if CO gas is present in the blood, it can easily displace the O2 bound to haemoglobin and occupy its position in the bond with iron (Fe). Another issue is that the human body also produces CO (Nakahira & Choi, 2015). Approximately 10 mL of this gas is naturally generated throughout the human body each day during the metabolism of haem, an iron-containing molecule, by the enzyme haem oxygenase (Katsnelson, 2019).

In the context of actual sea diving, in addition to the accumulation of CO produced within the body, CO gas can also originate from surface air contaminated by the exhaust emissions of ship engines used by divers. In SCUBA diving, CO gas can come from the compressor engine used to fill the tanks; the exhaust fumes from the compressor engine are often inadvertently drawn into the tanks during filling. It is not uncommon for divers to detect the smell of diesel combustion in the air tanks they use.

As the duration of apnoea progresses, it does not allow the exchange of oxygen in the lungs with the environment outside the body, there is no new oxygen intake and there is no way for carbon dioxide and carbon monoxide to leave the body (Cheng, 2015; Wagner & Shah, 2020). As time goes on, the amount of dissolved carbon dioxide will increase, it can replace the position of oxygen in binding with haemoglobin and fill the blood vessels with carbon dioxide. If that happens, the amount of oxygen distributed by the blood will decrease because the blood is dominated by carbon dioxide. Because the exchange of oxygen and carbon dioxide in the alveoli continues to occur, over time carbon dioxide will also dominate in the lungs and airways.

The exact mechanism of the process of carbon dioxide dominance in the blood and lungs is not yet known. Does carbon dioxide dominate the lungs first and then after the partial pressure of carbon dioxide in the lungs increases the blood is also dominated, or does carbon dioxide dominate the blood first and then because of the increase in the partial pressure of carbon dioxide in the blood the lungs are also dominated? Further studies are needed to answer the exact mechanism of this process.

If apnoea activity cannot expect haemoglobin and erythrocytes to act as oxygen reserve media but only as oxygen distributors, then this means that apnoea performance is almost completely dependent on the availability of oxygen in the lung space and airways. Taking into account the results of previous studies (Putra, Pratama, et al., 2020) that lung capacity is positively correlated with the duration of apnoea, it is possible that lung capacity is the most dominant factor that determines the length of apnoea duration that can be performed. Apart from that, previous studies also found that body fat levels were negatively correlated with the duration of apnoea (Putra et al., 2022). The mechanism underlying this is not yet known for certain. Is it the presence of visceral fat that physically squeezes the lungs and occupies space that should be occupied by the lungs or is there a certain biochemical mechanism in the blood that involves blood fat, affecting erythrocytes and haemoglobin and thus affecting the distribution of oxygen in the blood.

It is also not yet known whether this only applies to sedentary individuals and whether there are differences in individuals who have routinely undergone certain types of exercise such as aerobic, anaerobic and specific exercise for apnoea. Further studies are needed studying this in trained subjects.

CONCLUSION

Haemoglobin, erythrocyte and haematocrit levels do not correlate with the duration of apnoea implying that these three blood biochemical variables cannot be expected to act as an oxygen reserve medium but actually carry out their main function as an oxygen distributor.

AUTHOR CONTRIBUTIONS

Kukuh Pambuka Putra (study design, data collection, statistical analysis, manuscript preparation). Bayu Anugroho (study design, data collection, statistical analysis). Ferry Fredy Karwur (study design, statistical analysis, manuscript preparation)

SUPPORTING AGENCIES

In this study, there is collaboration between the researchers and Prodia Clinical Laboratory, Salatiga. The researchers obtained a discount for the examination of all blood samples.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

INFORMED CONSENT STATEMENT

The researchers declare that have obtained consent from all subjects involved in this study.

DATA AVAILABILITY STATEMENT

All data supporting the findings of this study, available within the Supplementary Information.

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