



## Article

# Heart Rate Variability at Rest Predicts Heart Response to Simulated Diving

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**Simple Summary:** The diving reflex is a complex response of the cardiovascular system that allows mammals, including humans, to survive immersion in water, as well as hypoxia caused by respiratory arrest. The response is adaptive, preferentially protecting brain tissue from the effects of apnea-induced hypoxia. In everyday life, there are situations in which there is a temporary apnea with simultaneous cooling of the face. This can trigger a hemodynamic response with an increase in blood pressure and a slow heart rate as a result of the diving reflex mechanism. Diving response is mediated by the autonomic nervous system with simultaneous extensive stimulation of the sympathetic and parasympathetic systems, which can evoke life-threatening arrhythmias. A characteristic feature of the cardiac response to diving is the uncertainty in predicting an individual's outcome. The current research examined the poorly understood regulatory oscillations of the heart rhythm and their influence on the course of the cardiac response to diving. The results of the research indicate that the cardiac response to diving is strictly dependent on the autonomic regulation of the heart rhythm under resting conditions. The present work provides a foundation for further research to preventative measures that could cause unfavorable course of cardiodepressive responses.



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**Abstract:** A characteristic feature of the cardiac response to diving is the uncertainty in predicting individual course. The aim of the study was to determine whether resting regulatory heart rate determinants assessed before diving may be predictors of cardiac response in a simulated diving test. The research was conducted with 65 healthy volunteers (37 women and 28 men) with an average age of 21.13 years (20–27 years) and a BMI of 21.49 kg/m<sup>2</sup> (16.60–28.98). The simulated diving test consisted of stopping breathing after maximum inhaling and voluntarily immersing the face in water (8–10 °C) for as long as possible. The measurements included heart rate variability (HRV) analysis before diving and determination of the course of the cardiac response to diving—minimum and maximum heart rate (HR). The results indicate that minimum HR during diving (MIN\_div) is dependent on the short-term HRV measures, which proves the strong influence of the parasympathetic system on the MIN\_div. The lack of dependence of MIN\_div on short-term HRV in women may be associated with differences in neurogenic HR regulation in women and men. In conclusion, cardiac response to simulated diving is strictly dependent on the autonomic regulation of the heart rhythm under resting conditions. The course of the cardiac response to diving and its relationship with resting HRV appears to be gender dependent.

**Keywords:** diving; simulated diving test; heart rate; heart rate variability; healthy individuals

## 1. Introduction

Diving is a popular activity that is undertaken for recreational and sport purposes. Freediving is a form of underwater diving that relies on breath-holding until resurfacing

rather than using a breathing apparatus, such as scuba gear. Breath-hold diving triggers a complex reflex response known as the diving response, the aim of which is to increase the possibility of human survival in the aquatic environment [1]. During diving, an organism has to cope with a drop in the partial pressure of oxygen ( $PO_2$ ) in arterial blood. The risk of insufficient oxygen supply is the appearance of disturbances in cell functioning and even cell death. The retrograde changes caused by oxygen deficiency are associated with the reduction or complete inhibition in oxidative phosphorylation. Cells characterized by a living metabolism, including nerve cells and cardiomyocytes, are exposed the most to hypoxia [2]. During diving, autonomous mechanisms are triggered. The diving response is redistribution of blood with oxygen for vital organs and is therefore referred to as an oxygen-conserving mechanism [3].

The diving response is a complex reflex initiated by apnea and cooling of the facial skin, especially the vestibule of the nose and the forehead. The response is triggered by stimulation of arterial chemoreceptors and trigeminal nerve endings located in the skin [4]. This triggers the arterial chemoreceptor reflex and the synergistic trigeminocardiac reflex [5]. The body's reaction to diving is mediated by the autonomic nervous system (ANS) with simultaneous stimulation of the sympathetic and parasympathetic systems. The stimulation of the intracardiac parasympathetic nerves slows down the heart rate (HR) and lengthens the conduction time of the depolarization wave from the atria to the ventricles. At the same time, the intracardiac sympathetic innervation is activated, which has the opposite, positive chronotropic effect [6]. However, there is prevalence of the vagus nerve on heart rhythm, which can be associated with bradycardia with HR near 20–30 beats per minute [7]. On the other hand, sympathetic stimulation is responsible for constriction of peripheral blood vessels and the associated increase in total vascular resistance, which leads to an increase in blood pressure with simultaneous redirection of blood to the body's core. Oxygen supplies are directed to the hypoxia-sensitive brain and heart at the expense of ischemia and hypoxia of the abdominal organs and large oxygen-consuming skeletal muscles. Experienced divers, e.g., pearl divers, mainly Japanese women called AMA divers, can spend up to five minutes underwater thanks to the diving response [8].

Under laboratory conditions, the diving response may be tested by a simulated diving test. It consists of a maximum inhale and submerging the face in the water for the time specified by the research protocol. A characteristic feature of the cardiac response to diving is HR reduction shortly after submerging. However, an increase in HR shortly before the test can be observed, which is a symptom of emotional agitation associated with intracardiac sympathetic activity [9,10].

HRV analysis was used as a tool for assessing the influence of the autonomic nervous system on the heart rhythm, and thus determining the role of neurogenic regulation on the cardiac response to diving. HRV is an established and non-invasive research method that describes the effects of the autonomic nervous system on HR. Thus, it is a tool for determining the profile of neurogenic, extrinsic heart regulation and its influence on HR while diving. Resting heart rhythm is influenced by the sympathetic and parasympathetic activity of the ANS, which determines the short- and long-term variability of sinus rhythm. Moreover, total HRV is the result of other endogenous biological oscillators that include respiratory movements, fluctuations in blood pressure, and hormonal influences [11,12].

The parameters of total HRV reflect the complexity of the regulatory mechanisms of the cardiovascular system. It is widely believed that a reduction in HRV is associated with a reduction in the complexity of control mechanisms, which leads to “stiffening” of the HR, associated with greater regularity of heart rhythm and reduced responsiveness to emerging disturbances in homeostasis [13,14].

Disease processes, aging, lifestyle, the influence of the external environment or neuropsychological conditions can lead to disturbances in the autonomic regulation of heart rhythm and thus affect the variability of HR [13]. There are many reports in the literature of a close relationship between decreased HRV parameters and cardiovascular diseases. HRV reduction is associated with an increased risk of death in post-myocardial infarction pa-

tients. In addition, there is a relationship between HRV parameters and classic risk factors for coronary artery disease, such as arterial hypertension and atherosclerosis [15–17].

A highly individualized and unpredictable course of the reflex response prompted the authors of the study to explore the relationships between the course of the cardiac response to diving with parameters of the HRV at rest in the time and frequency domains [4,18]. Both at rest and during the simulated diving test, HR is under the neurogenic control of the ANS. It can be assumed that the indicators of the autonomic regulation of heart rhythm—HRV indices may correlate with the cardiac response during diving [4]. The primary objective of this study was to assess the maximum and minimum HR during the simulated diving test and study the relationship between them and the HRV indexes calculated from the resting electrocardiogram (ECG) recording. The maximum HR during diving corresponds to the anticipatory HR acceleration associated with sympathetic innervation of the heart. Minimum HR during diving is the equivalent of the parasympathetic influences on the heart that are responsible for the cardiodepressive response of the simulated diving test. It was therefore assumed that the maximum and minimum HR during diving could be used as an indicator of the functional state of the autonomic nervous system during the test [19].

We hypothesized an especially strong relationship between short-term HRV and minimum HR during the diving test, and therefore, resting short-term HRV indices could serve as a predictor of cardiodepressive response during diving. Additionally, we assumed that the relationship of HRV indices with cardiac response to diving is gender-specific (different in males and females).

Finding an association between the HR during the simulated diving test and HRV determinants at rest may be useful in the employment of HRV in predicting cardiac response to diving.

## 2. Materials and Methods

The conducted research was of a cognitive nature and did not pose a threat to the health of the subjects. The study was carried out with healthy volunteers, recruited among students of the Medical University of Gdańsk. After completing the health questionnaire and medical history, the subjects were instructed about the details of the study. Bodyweight, height, and blood pressure were measured. The exclusion criteria were based on the data from the questionnaire and medical history. Diseases of the respiratory and cardiovascular systems (e.g., cardiac arrhythmia, resting tachycardia, syncope, and hypertension) were exclusion criteria. Disclosure of abnormalities in the ECG during the initial resting recording resulted in exclusion from participation in the study.

The study was conducted in accordance with the Declaration of Helsinki. The protocol was approved by the Independent Bioethics Commission for Research at the Medical University of Gdansk (NKBBN/471/2013). All participants were informed about the procedures, risks, and expected outcomes before starting the experimental procedure and gave their written informed consent for participation.

This study was conducted with 65 healthy volunteers, including 37 women and 28 men. The participants' mean age was 21 years, and ranged from 20 to 27 years. Body mass index (BMI) was 21.49 kg/m<sup>2</sup> (16.60–28.98).

The main element of the experimental procedure was immersion of the face in low-temperature water, combined with a voluntary, longest apnea-simulated diving test. Before starting the test, the subject remained seated for 10 min with elbows and forearms resting on the table. At a given sign, the subject performed a maximal breath and then submerged his/her face in the water (8–10 °C), trying to stay submerged for as long as possible. Before and during the simulated diving test, each participant's ECG was recorded at a sampling rate of 4 kHz using an integrated data acquisition system (ADInstruments Research System, ADInstruments, New Zealand). The examinations were carried out in the presence of an experienced specialist in internal medicine (THW), who continuously monitored the regularity of the heart rate, immediately stopping the test in the event of

cardiac arrhythmias during the prolonged diving. All subjects completed the simulated diving test.

### 2.1. Heart Rate Analysis

The analysis of the cardiac response to diving consisted in determining of the time series of the identified R waves. On this basis, the durations of the RR intervals (RRi) were determined. Then the longest and shortest RRi were used to calculate the minimum (MIN\_div) and maximum (MAX\_div) HR during diving.

### 2.2. HRV Analysis

The heart rate variability analysis was calculated from a 512 RRi segment of the resting ECG using statistical methods and the fast Fourier transform (FFT) method using Kubios HRV Pro software (Kuopio, Finland). Assessment of total heart rate variability was based on the standard deviation of NN (normal to normal) intervals (SDNN) and on the total spectrum power (TP ms<sup>2</sup>) of the HRV frequency analysis. Short-term HRV indices, such as root mean square of successive differences (rMSSD) and pNN50% (the proportion of NN50 divided by total number of NNs), were used to reflect parasympathetic cardiac activity [12]. The power of the spectrum in the high frequency range (HF ms<sup>2</sup>) corresponds to changes in HR with a frequency of 9–24 times per minute (0.15–0.4 Hz). HF (ms<sup>2</sup>) was used as an indicator of the activity of the parasympathetic intracardiac nerves in the HRV frequency analysis. The power of the spectrum in the low frequency range (LF ms<sup>2</sup>) reflects changes in HR with a frequency of 2.4–9 times per minute (0.04–0.15 Hz). It was used to assess the long-term heart rate variability.

### 2.3. Statistical Analysis

The nature of the data distribution was assessed with the Shapiro–Wilk test. Depending on the distribution of the analysed data, parametric or non-parametric tests were used, and the results were presented as mean ± SD. The mean values of the independent samples (man/woman) were compared using the Student's *t*-test or U Mann–Whitney test. The relationships between pairs of independent variables were analysed using the Pearson and Spearman linear regression method. A significance level of  $p < 0.05$  was considered significant. The significance level of statistical analysis was calculated for two-tailed. The HRV analysis data were divided into two subgroups: less and equal ( $\leq_{MED}$ ) and greater ( $>_{MED}$ ) than the median. The median was used to obtain numerically similar subgroups of data, while the division into subgroups was used for a more detailed analysis of the relationship between the minimum and maximum HR during diving and the selected parameters of the HRV analysis in the time and frequency domain. All statistical calculations were performed using statistical package Statistica 10 (StatSoft Inc., Tulsa, OK, USA).

## 3. Results

### 3.1. Anthropometric Data

Table 1 presents anthropometric data, including age, body mass, height, body mass index (BMI), and body water and body fat percentages.

**Table 1.** Anthropometric data.

	All	Women	Men	P [w vs. m]
Age	21.13 ± 1.34	21.03 ± 0.91	21.30 ± 1.89	0.4700 NS
Body mass (kg)	65.12 ± 11.25	59.25 ± 7.18	74.73 ± 10.08	0.0000 ***
Height (m)	1.74 ± 0.09	1.69 ± 0.05	1.82 ± 0.08	0.0000 ***
BMI—body mass index (kg/m <sup>2</sup> )	21.49 ± 2.33	20.81 ± 2.03	22.62 ± 2.39	0.0033 ***
%Fat (%)	22.05 ± 7.80	23.70 ± 5.38	18.76 ± 10.64	0.0316 *
%H <sub>2</sub> O (%)	54.28 ± 3.93	52.67 ± 3.36	57.70 ± 2.68	0.0000 ***

\*  $p < 0.05$ ; \*\*\*  $p < 0.001$ ; and NS—not significant.

### 3.2. HR Response to Simulated Diving

Immersion in water resulted in HR acceleration followed by a cardiodepressive response (Table 2). The maximum HR during diving was  $112.98 \pm 16.23 \text{ min}^{-1}$  and was higher in women ( $117.44 \pm 16.38 \text{ min}^{-1}$  vs.  $107.09 \pm 14.25 \text{ min}^{-1}$ ). In all subjects, we noticed a decrease in HR during the simulated diving test. The minimum HR during diving (MIN\_div) was  $52.66 \pm 8.16 \text{ min}^{-1}$  and was significantly lower in men compared to women ( $49.35 \pm 7.73 \text{ min}^{-1}$  vs.  $55.17 \pm 7.64 \text{ min}^{-1}$ ). In the current study, arrhythmias occurred rarely and concerned only a few cases (five times). It concerned single ventricular extrasystoles (three times) and partial lengthening of atrioventricular conduction (two times). In each case of arrhythmia, the simulated diving test was stopped immediately and the heart rhythm returned to regular. Since arrhythmias appeared at the end of the diving, there were no reasons to exclude these trials from the study.

**Table 2.** The HR response to simulated diving in healthy men and women.

	All	Women	Men	P [w vs. m]
MIN_div ( $\text{min}^{-1}$ )	$52.66 \pm 8.16$	$55.17 \pm 7.64$	$49.35 \pm 7.73$	0.0036 **
MAX_div ( $\text{min}^{-1}$ )	$112.98 \pm 16.23$	$117.44 \pm 16.38$	$107.09 \pm 14.25$	0.0098 **

\*\*  $p < 0.01$ .

### 3.3. HRV Analysis

HRV measures in the time domain in the resting state are presented in Tables 3 and 4. Total heart rate variability, as measured by SDNN, was  $60.51 \pm 22.37 \text{ ms}$ . There was a significant difference in the subgroup of values higher than the median -SDNN<sub>>MED</sub>. In the group of men, it was  $87.28 \pm 21.09 \text{ ms}$ , and in the women, it was  $69.54 \pm 13.48 \text{ ms}$ .

**Table 3.** Analysis of heart rate variability—HRV in the time domain.

	All	Women	Men	P [w vs. m]
SD_NN (ms)	$60.51 \pm 22.37$	$56.48 \pm 17.07$	$65.83 \pm 27.31$	0.0953 NS
rMSSD (ms)	$44.78 \pm 24.02$	$41.33 \pm 18.63$	$49.34 \pm 29.44$	0.5762 NS
pNN50% (%)	$19.92 \pm 16.51$	$19.38 \pm 15.23$	$20.63 \pm 18.33$	0.9735 NS

NS—not significant.

**Table 4.** Analysis of heart rate variability—HRV in the time domain for groups divided by the median.

		All	Women	Men	P [w vs. m]
SD_NN (ms)	≤MED.	$43.80 \pm 8.88$	$44.11 \pm 8.90$	$44.38 \pm 10.64$	0.9854 NS
	>MED.	$77.74 \pm 18.65$	$69.54 \pm 13.48$	$87.28 \pm 21.09$	0.0115 *
rMSSD (ms)	≤MED.	$27.87 \pm 7.65$	$27.90 \pm 7.79$	$27.93 \pm 7.92$	0.9274 NS
	>MED.	$62.22 \pm 22.63$	$55.51 \pm 16.01$	$70.74 \pm 27.41$	0.1744 NS
pNN50% (%)	≤MED.	$7.90 \pm 5.51$	$8.07 \pm 5.80$	$6.46 \pm 4.42$	0.6488 NS
	>MED.	$33.94 \pm 13.72$	$31.32 \pm 12.72$	$34.80 \pm 15.68$	0.8345 NS

\*  $p < 0.05$ ; and NS—not significant.

The short-term variability indexes were  $44.78 \pm 24.02$  and  $19.92 \pm 16.51$  for rMSSD and pNN50%, respectively. There were no significant differences between women and men independent of the division into subgroups.

Tables 5 and 6 represent HRV measures in the frequency domain in the resting state. Measures of total heart rate variability in the frequency domain analysis are the total range of power spectral density, total power (TP). TP ( $\text{ms}^2$ ) was  $4016.13 \pm 3099.80 \text{ ms}^2$  and was higher in the men subgroup of values higher than the median. TP<sub>>MED</sub> was  $7744.70 \pm 3683.16 \text{ ms}^2$  in men and  $4905.17 \pm 2027.73 \text{ ms}^2$  in women.



**Table 5.** Analysis of heart rate variability—HRV in the frequency domain.

	All	Women	Men	P [w vs. m]
Total Power (ms <sup>2</sup> )	4016.13 ± 3109.80	3368.07 ± 2112.47	4872.51 ± 3954.41	0.3236 NS
LF (ms <sup>2</sup> )	2269.90 ± 1857.76	1867.00 ± 1436.92	2802.30 ± 2216.16	0.0645 NS
HF (ms <sup>2</sup> )	1177.15 ± 1288.87	1000.80 ± 886.21	1410.18 ± 1670.79	0.8998 NS
SD <sub>1</sub>	31.79 ± 17.02	29.34 ± 13.20	35.03 ± 20.86	0.5644 NS
SD <sub>2</sub>	99.60 ± 34.39	92.89 ± 26.65	108.47 ± 41.39	0.0700 NS

NS—not significant.

**Table 6.** Analysis of heart rate variability—HRV in the frequency domain for groups divided by the median.

		All	Women	Men	P [w vs. m]
Total Power (ms <sup>2</sup> )	≤MED.	1867.30 ± 667.92	1911.87 ± 663.92	2000.31 ± 1069.45	0.6228 NS
	>MED.	6232.12 ± 3085.56	4905.17 ± 2027.73	7744.70 ± 3683.16	0.0175 *
LF (ms <sup>2</sup> )	≤MED.	1029.15 ± 401.93	953.99 ± 368.44	1214.71 ± 570.03	0.2992 NS
	>MED.	3549.42 ± 1905.17	2830.73 ± 1521.06	4389.88 ± 2108.79	0.0008 ***
HF (ms <sup>2</sup> )	≤MED.	368.42 ± 175.78	415.03 ± 209.75	324.45 ± 148.86	0.2223 NS
	>MED.	2011.14 ± 1408.35	1619.11 ± 910.08	2495.91 ± 1799.09	0.3327 NS
SD <sub>1</sub>	≤MED.	19.81 ± 5.44	19.83 ± 5.54	19.87 ± 5.65	0.9274 NS
	>MED.	44.15 ± 16.03	39.39 ± 11.34	50.19 ± 19.41	0.1774 NS
SD <sub>2</sub>	≤MED.	73.55 ± 16.97	72.19 ± 16.46	76.05 ± 18.99	0.5973 NS
	>MED.	126.46 ± 26.03	114.73 ± 15.26	140.90 ± 30.55	0.0194 *

\*  $p < 0.05$ ; \*\*\*  $p < 0.001$ ; and NS—not significant.

The low frequency power component, LF (ms<sup>2</sup>), was  $2269.90 \pm 1857.76$  ms<sup>2</sup>. There was also a significantly higher value of the LF measure in the men subgroup of values higher than the median (LF<sub>>MED</sub>). It was  $4389.88 \pm 2108.79$  ms<sup>2</sup> in men and  $2830.73 \pm 1521.06$  ms<sup>2</sup> in women.

The high frequency power component, HF (ms<sup>2</sup>), was  $1177.15 \pm 1288.87$  ms<sup>2</sup>. There were no significant differences between men and women.

The SD<sub>1</sub> and SD<sub>2</sub> measures were  $31.79 \pm 17.02$  and  $99.60 \pm 34.39$ , respectively. SD<sub>2</sub> differed between men and women in the subgroup of values higher than the median. SD<sub>2>MED</sub> was  $140.90 \pm 30.55$  in men and  $114.73 \pm 15.26$  in women.

### 3.4. Dependence of Cardiac Response to Diving on Resting HRV

The correlation coefficients between cardiac response to diving (MIN\_div; MAX\_div) and the selected resting HRV measures in the time-domain analysis are presented in Table 7. The results indicate that MIN\_div is dependent on the short-term HRV measures. This relationship is inversely proportional; an increase in short-term variability is accompanied by a decrease in MIN\_div. The correlations of MIN\_div with rMSSD and pNN50% were highest in the men subgroup whose rMSSD and pNN50% values were higher than the median, rMSSD<sub>>MED</sub>; and pNN50%<sub>>MED</sub>. The correlation coefficients for these pairs were  $-0.7758$  and  $-0.6556$ , respectively. The dependence of MIN\_div on short-term HRV is confirmed by the results of the correlation with HRV measures in the frequency domain analysis and with the use of non-linear methods of assessing heart rate variability (Table 8).

**Table 7.** Correlation coefficients between MIN\_div and the selected parameters of HRV in the time domain.

		All		Women		Men	
		MIN_div	MAX_div	MIN_div	MAX_div	MIN_div	MAX_div
SD_NN	≤MED.	0.1841 NS	−0.0116 NS	0.0228 NS	−0.0894 NS	0.3758 NS	0.0901 NS
	>MED.	−0.5139 **	−0.1293 NS	−0.3519 NS	0.0670 NS	−0.4466 NS	−0.2527 NS
rMSSD	≤MED.	−0.1223 NS	−0.1019 NS	−0.3473 NS	−0.3045 NS	0.0945 NS	0.19656 NS
	>MED.	−0.5676 ***	−0.0901 NS	−0.4468 NS	0.2363 NS	−0.7758 *	−0.0505 NS
pNN50%	≤MED.	−0.1756 NS	−0.0269 NS	0.2501 NS	−0.2150 NS	0.0945 NS	−0.1032 NS
	>MED.	−0.4797 **	−0.0720 NS	−0.0505 NS	0.1558 NS	−0.6556 *	−0.2759 NS

\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; and NS—not significant.

**Table 8.** Correlation coefficients between MIN\_div; MAX\_div and the selected parameters of HRV in the frequency domain.

		All		Women		Men	
		MIN_div	MAX_div	MIN_div	MAX_div	MIN_div	MAX_div
Total Power (ms <sup>2</sup> )	≤MED.	0.0431 NS	−0.0725 NS	0.0315 NS	−0.0543 NS	0.1736 NS	0.0285 NS
	>MED.	−0.5174 **	−0.1352 NS	−0.2796 NS	0.2260 NS	−0.4224 NS	−0.0945 NS
LF (ms <sup>2</sup> )	≤MED.	0.1560 NS	−0.1443 NS	0.1789 NS	−0.3771 NS	0.1428 NS	0.0065 NS
	>MED.	−0.4160 *	−0.2364 NS	0.3828 NS	−0.3353 NS	−0.1672 NS	−0.0945 NS
HF (ms <sup>2</sup> )	≤MED.	−0.2065 NS	0.2465 NS	−0.2175 NS	0.0728 NS	−0.3010 NS	0.2483 NS
	>MED.	−0.6440 ***	−0.2100 NS	−0.3085 NS	0.1124 NS	−0.6263 *	−0.2043 NS
SD <sub>1</sub>	≤MED.	−0.1226 NS	−0.0986 NS	−0.3491 NS	−0.2957 NS	−0.0945 NS	0.1956 NS
	>MED.	−0.5676 ***	−0.0901 NS	−0.4468 NS	0.2363 NS	−0.7758 **	0.0505 NS
SD <sub>2</sub>	≤MED.	0.0060 NS	−0.1848 NS	−0.1403 NS	−0.2052 NS	0.2131 NS	−0.1428 NS
	>MED.	−0.4853 **	−0.0218 NS	−0.2321 NS	0.1011 NS	−0.3322 NS	0.1252 NS

\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; and NS—not significant.

There were high correlation coefficients between MIN\_div and short-term HRV represented by HF (ms<sup>2</sup>) and SD<sub>1</sub>. In the men subgroup whose HF (ms<sup>2</sup>) and SD<sub>1</sub> values were higher than the median (HF<sub>>MED.</sub>; SD<sub>1>MED.</sub>), the correlation coefficients were −0.6263 and −0.7758, respectively. The relationships of the maximum HR during the dive (MAX\_div) with the measures of total, short- and long-term heart rate variability did not show significant correlation coefficients.

#### 4. Discussion

There are two new findings of this study: (1) the short-term variability of the HR before the test is a predictor of a cardiodepressive reaction during diving and (2) the heart response to simulated diving differs between men and women.

Christoforid et al. assessed the activity of the intracardiac nerves of the ANS in professional divers [20]. Thirteen swimmers with several years of free diving experience (without oxygen apparatus) and thirteen people without swimming experience (control group) participated in the study. The HRV analysis was performed using a 24 h ECG record. In the group of divers, significantly higher values of all HRV indices were obtained. The total variability of the heart rate expressed by SDNN and TP (ms<sup>2</sup>) was higher in the group of divers by 38.1% and 7.2%, respectively. On the other hand, rMSSD and HF (ms<sup>2</sup>), describing short-term heart rate variability, were higher by 61.23% and 74.9%, respectively. The results of the study also show a 23.9% lower minimum heart rate and a 20.6% lower average heart rate at rest in athletes. The slowest HR was recorded in one of the study participants during the night hours, 32 min<sup>−1</sup>. The above-mentioned study may be a premise for the relationship between high activity of intracardiac ANS at rest and better adaptation of the body to diving. Moreover, short-term variability of HR at rest can be an expression of the regulatory potential of parasympathetic heart control.

Our study's results indicate the dependence of the slowest heart rate during the dive, MIN\_div, on short-term variability at rest, expressed both by HRV indices in the time and

frequency domains. There was a high, negative correlation coefficient between MIN\_div and the rMSSD, pNN50%, HF, or SD<sub>1</sub> index. This means there is a close relationship between intracardiac activity of the parasympathetic system at rest and the minimum HR during the diving test. It is especially noticeable in subjects with high values (greater than the median) of short-term variability—rMSSD<sub>>MED.</sub>, pNN50%<sub>>MED.</sub>, HF<sub>>MED.</sub>, or SD<sub>1</sub><sub>>MED.</sub>. The highest correlation coefficient was noted in men with high rMSSD values (rMSSD<sub>>MED.</sub>). The correlation coefficient for the relationship of MIN\_div–rMSSD<sub>>MED.</sub> in men was  $-0.7758$ . Lower MIN\_div values were noted in men compared to women ( $49.35 \pm 7.73 \text{ min}^{-1}$  vs.  $55.17 \pm 7.64 \text{ min}^{-1}$ ). Thus, it can be concluded that the greater activity of the intracardiac fibres of the vagus nerve at rest, the greater reduction in HR during the simulated diving test, and the short-term variability of the HR before the test may be predictors of a cardiodepressive reaction while diving.

The lack of dependence of MIN\_div on the determinants of short-term variability of HRV (before the diving test) in women may be associated with differences in neurogenic heart rate regulation in women and men. It should be noted that there was significantly higher minimum and maximum HR during the diving test in women, which can be an expression of lower activity of the parasympathetic system in the cardiodepressive reaction and a higher influence of the sympathetic system on the heart in the anticipatory effect.

The HRV analysis indicated differences in the spectral power density for short- and long-term variability between men and women. Koenig and Thayer conducted a meta-analysis comparing the HRV indices in men and women in 172 studies [21]. The results of the studies indicate a higher mean HR in women along with lower mean values of RR intervals. Women showed a lower total variability of heart rhythm, represented both by index of the time domain, SDNN, as well as the frequency domain, TP (ms<sup>2</sup>). Further differences relate to the power of the spectral density of the low and high oscillations of HRV. Lower LF rates and higher HF were reported in women compared to men. These results indicate a higher activity of intracardiac parasympathetic activity in women, which in turn is in contradiction to the statistically higher HR at rest in this group of respondents. However, the cited meta-analysis included studies that differed in ECG recording time, as well as the position of the examined person and the frequency of breathing. Research procedures and the method of HRV analysis are of great importance for the determined indicators and the interpretation made on its basis [22].

In the present study, higher short-term variability (rMSSD, pNN50%, HF, or SD<sub>1</sub>) and higher total variation (SDNN) in men was observed. However, the lack of statistical significance of differences between women and men does not allow us to attribute higher intracardiac parasympathetic activity in men. The literature does not provide unequivocal answers regarding the differences in HRV indicators in women and men, which indicates the need for further research in this area.

The increase in HR observed before the test is called the anticipatory effect. The increase in HR may persist for a few seconds after the face is immersed in water, and it is associated with the body's response to the stress associated with the performed test [8]. The stimulation of the intracardiac sympathetic nerves accelerated the heart rhythm to  $112.98 \pm 16.23 \text{ min}^{-1}$ . The obtained results indicate a significantly higher anticipatory effect in women ( $117.44 \pm 16.38 \text{ min}^{-1}$  vs.  $107.09 \pm 14.29 \text{ min}^{-1}$ ). There was no direct correlation between parasympathetic activity (expressed by HRV indices of short-term variability) and MAX\_div. This is probably due to the dependence of an anticipatory effect on sympathetic activity. Moreover, no correlation was found between MAX\_div and any HRV indices, which does not exclude the participation of increased sympathetic activity on MAX\_div, but may result from no clear reflection of sympathetic activity by HRV analysis indicators [23].

According to most of the literature data, the standard cardiac response to a simulated diving test is a reduction in HR [24]. Interesting data are presented in the meta-analysis by Schipke et al., where the cardiodepressive reaction in response to diving was analysed. Based on the inclusion and exclusion criteria, eight studies were selected with a total of



182 subjects aged 19–27 who performed a simulated diving test in similar conditions and according to a similar procedure to that performed in this study. The results indicate that the calculated average heart rate reduction during the test was 59 beats per minute (bpm) and ranged from 48 to 67 bpm [25]. There is also a report on the extreme slowing of HR, up to five beats per minute, recorded in a 41-year-old man during apnea combined with immersion of the face in water at a temperature of 2 °C [26]. In this study, MIN\_div was  $52.66 \pm 8.16 \text{ min}^{-1}$ . The low temperature of the water in which the subjects immersed their faces potentially had a significant impact on the cardiac response, which was associated with the enhancement of the cardiodepressive response evoked by the trigemocardial reflex [27].

The LF band was originally thought to be an approximate indicator of cardiovascular sympathetic activity. The research related to the spectral analysis during the orthostatic tests shows the reduction in the HF band with a simultaneous increase in the LF component in the power spectrum. It is associated with an increase in the activity of the sympathetic nervous system in response to the decompression of baroreceptors [28]. Additionally, an increase in the amplitude of the LF band is observed during sympathetic arousal under conditions of mental stress or exercise [29]. However, the assumption of a simple relationship between the activity of intracardiac sympathetic activity and the variability of the heart rate in the low frequency range was not confirmed in further studies. It was shown that activity of intracardiac parasympathetic fibres may also contribute to the LF component. This is evidenced by studies related to the blockade of muscarinic receptors,  $M_1$ , which leads not only to the reduction in the HF band, but also to the reduction in the low-frequency component of HRV [30]. It is also suggested that the LF oscillations are an expression of the activity of the efferents of the sympathetic and parasympathetic systems, modulated by changes in arterial pressure in the arterial baroreceptor reflex [31]. The influence of the ANS on heart rate variability in the low frequency range is unquestionable, while the interpretation of the “physiological correlates” of the LF band encounter difficulties due to the complexity of the regulatory mechanism of heart rhythm.

In our study, the investigated population consisted of healthy medical students. We did not measure  $\text{VO}_2$ , so we are not able to provide their detailed training capacity, but volunteers did not declare practising any particular sport, and the students most likely represented the typical population of students of average physical activity. In a few cases of arrhythmia (five times), the simulated diving test was stopped. Since arrhythmias appeared at the end of the diving, there were no reasons to exclude these participants from the study.

## 5. Conclusions

In everyday life, there are situations in which there is a temporary apnea with simultaneous cooling of the face. This can trigger a hemodynamic response with an increase in blood pressure and a slow HR in the mechanism of the diving reflex. Sudden cooling of the face, especially in the nasal vestibule, associated with temporary inhibition of expiration or apnea, may trigger a reflex cardiodepressive response.

The results of present study indicate that selected indicators of the neurogenic regulation of HR at rest can be used to approximate the course of the cardiac response during diving and could open the field for practical applications. The development of warning algorithms against the unfavourable course of the cardiodepressive response to diving could be used to prevent their unfavourable course. Prospectively, new diagnostic algorithms could be incorporated as part of the software for sensors such as a heart rate monitor, which are increasingly used during recreational and sports physical activity, as well as when performing professional work in conditions similar to those that occur while diving.

The previously cited studies suggest a different regulatory profile of the heart rhythm in athletes. Normally, increased parasympathetic activity could predispose them to greater HR reduction during diving. Additionally, apnea time, depending on training and on physical predispositions (especially on total lung capacity), can also affect a different cardiac response to diving. The above premises may be the subject of further studies examining the

impact of training and fitness on the cardiac response to diving and its relationship with resting HRV. Moreover, interesting studies could refer to an explanation of the detailed impact of the trigeminal and arterial chemoreceptors reflex on the cardiodepressive response to diving.

The cardiac response in the simulated dive test is dependent on the intracardiac influences of the ANS. The varying strength of the parasympathetic and sympathetic nervous systems leads to different cardiac responses during diving, including potential arrhythmias. Further research could also focus on the relationship between HRV, illustrating the influence of ANS on the heart rhythm, and spontaneously occurring arrhythmias.

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