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Research article

Acute Cardiovascular and Metabolic Effects of Different Warm-Up Protocols on Dynamic Apnea

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Abstract
The aim of this study was to evaluate the acute physiological response to different warm-up protocols on the dynamic apnea performance. The traditional approach, including a series of short-mid dives in water (WET warm-up), was compared to a more recent strategy, consisting in exercises performed outside the water (DRY warm-up). Nine athletes were tested in two different sessions, in which the only difference was the warm-up executed before 75m of dynamic apnea. Heart rate variability, baroreflex sensitivity, hemoglobin, blood lactate and the rate of perceived exertion were recorded and analyzed. With respect to WET condition, DRY showed lower lactate level before the dive (1.93 vs. 2.60 mmol/L, p = 0.006), higher autonomic indices and lower heart rate during the subsequent dynamic apnea. A significant correlation between lactate produced during WET with the duration of the subsequent dynamic apnea, suggests that higher lactate levels could affect the dive performance (72 vs. 70 sec, p = 0.028). The hemoglobin concentration and the rate of perceived exertion did not show significant differences between conditions. The present findings partially support the claims of freediving athletes who adopt the DRY warm-up, since it induces a more pronounced diving response, avoiding higher lactate levels and reducing the dive time of a dynamic apnea.

Key words: Breath-hold diving; freediving; warm-up; diving reflex.

Introduction

Dynamic apnea is a freediving discipline in which athletes try to cover the longest possible distance, swimming in shallow water with fins (dynamic with fins - DYN) or without fins (dynamic no fins - DNF) whilst breath-holding. Three main physiological variables affect the performance in all apnea disciplines: i) total body gas storage capacity in lungs, blood and tissues, intended both as total O_2 reserve and CO_2 buffering capacity; ii) tolerance to asphyxia, that is the limit beyond which cerebral hypoxemia induces the loss of consciousness called "blackout"; iii) the metabolic rate, that is inversely correlated with apnea duration, meaning that any physical activity elevating oxygen consumption or lactate levels just before a dive would be counterproductive (Lin et al., 1974).

Breath-hold dives show a complex physiological response with respiratory, circulatory and metabolic changes (Ferretti, 2001). The cardiovascular responses to apnea, commonly known as the diving response or "diving reflex", multifaced physiologic reaction that occurs in response to water submersion, involving peripheral vasoconstriction, increase in arterial blood pressure, redistribution of blood flow from peripheral to cerebral and myocardial circulation, bradycardia and a reduced cardiac output (Gooden, 1994). Peripheral vasoconstriction causes an ischemia in muscles and skin, blood flow is directed mainly toward the brain and heart, while the rest of the organism receives a limited amount of blood, therefore working muscles quickly shift to anaerobic metabolism. In fact, higher blood lactate levels has been observed at the end of the performance in all apnea disciplines (Ferretti, 2001; Rodriguez-Zamora et al., 2018), especially in dynamic apnea (Elia et al., 2021a; Kiviniemi et al., 2012). Simultaneously, bradycardia and low cardiac output reduces the metabolic rate, leading to slower depletion of both lung and blood O_2 stores (Marabotti et al., 2009). The diving response elicited by apnea is enhanced when the face is immersed in the water due to the stimulation of cold skin receptors (Foster and Sheel, 2005; Lindholm and Lundgren, 2009). Bradycardia has been observed in all the apnea disciplines, although with different levels. The heart rate reduction is greater in resting apnea than in disciplines characterized by movement, such as dynamic apnea (Schagatay, 2010; 2011). Therefore, the energetic demands of the working muscles may result in a balance between the opposing sympathetic exercise, which increase the heart rate, and the parasympathetic diving response stimuli, leading to an optimization of the diving bradycardia (Delahoche et al., 2005; Mulder and Schagatay, 2021).

Some studies suggest that the main determinant of the diving reflex is the vagally-mediated bradycardia (Ferretti, 2001; Paton et al., 2005). Several studies applied heart rate variability (HRV) analysis for investigating the diving reflex (Christoforidi et al., 2012; Costalat et al., 2015; Hayashi et al., 1997; Kiviniemi et al., 2012; Lemaître et al., 2008). HRV describes the oscillation of the intervals between consecutive heart beats (R-R intervals), which are related to the influences of the autonomic nervous system on the sinus node (Camm et al., 1996). Kiviniemi et al. (2012) reported that during the initial phase of dynamic apnea, vagally-mediated HRV decreased, maybe due to vagal withdrawal, showing a gradual increase towards the end of apnea. The HRV indexes also revealed that the increase in the parasympathetic activity was accompanied by an increase in peripheral sympathetic activity, therefore leading to an autonomic co-activation (Costalat et al., 2015). Paton et al. (2005) suggested that simultaneous parasympathetic and sympathetic co-activation pattern evoked a greater cardiac output in comparison with the activation of the sympathetic system alone.

Another response to breath-hold diving is splenic

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contraction. Several studies conveyed that spleen contraction matched with augmented hemoglobin (Hb) concentrations after repetitive apneas (Elia et al., 2021a; 2021b; Schagatay et al., 2001; 2005). Schagatay et. al. (2001) observed that a series of resting dives spaced by a couple of minutes of recovery cause an increase of hematocrit (Hct) and Hb (6,4 and 3,3% respectively), prolonging the apnea duration. Prommer et al., (2007) reported marked spleen contractions after repeated apnea in trained divers, with no change in Hb concentrations or total red cell volume. More recently, Elia et al. (2021a) demonstrated that repeated maximal static and dynamic apneas with whole-body immersion are effective in stimulating splenic contractions in both elite and non-divers, with a greater value in dynamic with respect to static apneas in elite divers only. Moreover, hemoglobin increases were found only after the dynamic apnea, whereas hematocrit values were unchanged across groups and apneic protocols.

During competition, athletes can perform only one attempt, therefore an optimal warm-up is critical to reach the best physiological conditioning. In the past years, the general rule was to perform a warm-up protocol including a series of dives of short-mid distance. More recently, a different approach has been adopted both by elite and amateur divers, in which water dives was avoided before the performance, switched with dry exercises executed outside the water, with breathing techniques and dry apnea (Schagatay, 2010). Nowadays, there is little consensus among divers about which kind of warm-up works better, as many divers claim to perform better with dry warm-up (Schagatay, 2010). Thus, the aim of this study was to quantify the acute effects of two different warm-up protocols, WET warm-up versus DRY warm-up, on cardiovascular, autonomic and metabolic variables following a single bout of dynamic apnea. DRY warm-up involves a diversity of breathing maneuvers and repetitive apneas executed outside the water. WET warm-up is a technique that involves repeated submaximal dives in the water. It has been suggested that the cooling effect of water on the human face may be attenuated with time spent in water due to the high dynamic sensitivity of the cutaneous thermoreceptors (Schagatay, 2010). We hypothesized that the dynamic apnea executed following DRY warm-up may result in a stronger diving reflex than after WET warm-up, with a more intense peripheral vasoconstriction that may anticipate the shift to anaerobic metabolism, thus leading to higher lactate levels after the apnea. We also hypothesized that the repetitive DRY apneas may represent a greater stimulus for splenic contraction than the submaximal dives executed in the WET warm-up, with increased release of erythrocytes from the spleen. These erythrocytes should remain in circulation for several minutes after a warm-up and contribute to oxygenation at the onset of the competition (Schagatay et al., 2005).

Methods

Participants

The experiments were performed in 9 healthy adults, who voluntarily participated to the study (Table 1). GPower 3.1.9.2 software (Heinrich Heine University, Düsseldorf, Germany) was used for the power analysis in order to establish, at 0.05, the probability of a type I error. Based on a previous article (Schagatay et al., 2005) in which authors uses a similar design, a desired power of 0.80 with 95 % confidence limits, and an expected effect size of >0.6 for the interaction between time and condition, a priori sample size of 8 participants was considered sufficient. Thus, one additional participant was included to ensure availability of data in case of a possible dropout of a test participant. Inclusion criteria were: age over 18; not being pregnant or in the secretory phase of the menstrual cycle; in possession of the freediving certification issued by a qualified diving center; in possession of a medical certificate for competitive underwater activities; regular practice of sports apnea for at least one year (at least 2 hours/week); non-smokers for at least one year; absence of chronic or acute pathologies that could be adverse for apnea tests, such as cardiac, pulmonary, splenic or neuromuscular pathologies; Hb values at baseline greater than 13 g/dL for males and 12g/dL for females. All participants received a verbal explanation of experimental procedures and informed consent was obtained before the beginning of recordings. Volunteers were asked to avoid behaviors that could alter the measurements, in particular: abstain from alcohol and avoid strenuous physical activity the day before recordings, observe an adequate sleeping period and be fasting for at least two hours before the tests because the digestive processes could recall important amounts of blood and affect the diving reflex (Schagatay, 2009). The experimental protocol was approved by the Institutional Ethic Committee of our University (Protocol number: 291696 of the 11/20/2019). The experiments were performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Table 1. Group characteristics (n = 9; 6 males, 3 females).

Age (years)	36.78 ± 9.2
Weight (kg)	72.00 ± 12.4
Height (cm)	174.56 ± 6.4
BMI (kg/m^2)	23.51 ± 2.9
Practice (years)	6.56 ± 2.1
Training (n°/week)	2.11 ± 0.3
Training (hours/week)	2.33 ± 0.7
Maximum distance underwater (m)	98.0 ± 9.7

Data are means ± SD. Abbreviations: BMI; body mass index.

Procedure

A randomized, repeated-measure cross-over design was used to compare performance (diving time, RPE), metabolic (HR, blood lactate), cardiovascular and autonomic responses (HRV, baroreflex sensitivity - BRS) in trained freedivers involved in dynamic apneas executed after two different warm-up protocols (DRY vs. WET). In two separate days and in random order, they carried out both WET and DRY warm-up before to perform 75m of dynamic apnea, with fins, in a swimming pool of 25 m in length and 1.55 m in deep. Since the diving bradycardia exhibits a diurnal variation (Konishi et al., 2016), the recording sessions were interspersed by exactly 24 hours, therefore each athlete performed the two tests at the same time of the day (9:00 - 12:00 AM). Moreover, one week

before the tests, we conducted a preliminary test session with all athletes to ensure the perfect understanding of the research protocol. Diving reflex is also influenced by the air/water temperature gradient (Foster and Sheel, 2005), therefore these data were monitored, and the day variations over the two days were very small to be considered negligible (air temperature: 23 ± 0.9 °C; water temperature: 28 ± 0.08 °C; $p > 0.05$). Athletes were asked to adopt the kicking technique and the speed normally used during competition and to perform two tests as similar as possible, therefore on both days each individual adopted the same equipment (wetsuit, fins, ballast, mask or goggles). To minimize the stress effects on diving reflex, the distance of the dynamic apnea with fins was set at 75 m, a length that allowed each participant to reach the "struggle phase" and to maintain an adequate margin of safety given that this distance was below their best performance (Table 1) (Lindholm et al., 2006).

At the arrival to the swimming pool, athletes were first placed in a supine position for 10 min on a comfortable bed in a quiet room, with stable temperature (22-23°C), and blood pressure (Portapres device, TNO/BMI, Amsterdam, the Netherlands) was monitored and recorded for autonomic variables analysis at baseline level. Moreover, to complete the baseline values, we took a capillary blood sample for Hb (DiaSpect hemoglobin T system, DiaSpect Medical GmbH, Sailauf, Germany) and lactate (Lactate Scout, SensLab, Leipzig, Germany) analysis. Capillary blood was sampled by the middle finger and collected according to the devices operating manuals. The finger was completely dried with a towel and cleaned with alcohol. The lactate measurements were also taken within 30 sec after the end of the warm-up and within 30 sec after the emersion from the dynamic apneas (Marongiu et al., 2015).

WET warm-up: it is the repetition of dynamic apneas, performed in the water, executed at submaximal incremental-intensity. It started with 100 m of a kicking warm-up exercise, followed by 8 repetitions of 25 m in diving interspersed by 1 minute of restart, and ended with 2 repetitions of 50 m in diving with 2 minutes of recovery (see Supplement).

DRY warm-up: it consists of breathing exercises, thoraco-pulmonary mobility and apnea conducted exclusively outside the water. It started with 5 minutes of triangular ventilation, followed by 5 minutes of a yoga exercise called uddiyana bandha (i.e., "drawing-in" or "abdominal hollowing", means that the abdomen is retracted), an ancient yoga practice that affects the core, and ended with 4 apneas after maximum expiration, interspersed by 2 minutes of restart (see Supplement).

Within 30 seconds after the end of the warm-up, we took a second capillary blood sample to analyze Hb and lactate (La) values. Then, athletes were ready for the dynamic apnea, in which the only investigated variables were the HRV parameters recorded by the waterproof heart rate monitor and its appropriate elastic chest strap worn by athletes during the test (Polar V800, Polar Electro Oy, Kempele, Finland).

The dynamic apnea started 5 minutes after the end of the warm-up, with 3 minutes of countdown, as applied during competitions. Athletes dived after maximum inhalation, while both hyperventilation and glossopharyngeal breathing were forbidden during the countdown period, due to their influence on the diving reflex and the risks associated with such maneuvers (Pollock, 2008). All athletes were videotaped during the entire dynamic apnea, in order to calculate the diving time. This one was considered as the time between immersion and emersion of the respiratory tract. Within 30 seconds after the end of the apnea, with athletes still in the water, we took a third capillary blood sample for Hb and La recovery analysis. Lastly, participants filled in the OMNI-resistance exercise scale (OMNI-RES 1-10) for collecting the rate of perceived exertion values (RPE). In brief, the OMNI-resistance exercise scale is a qualitative method that determines how hard the exercise is, according to what is perceived subjectively by the athletes (Robertson, 2004). The HRV, BRS and cardiovascular parameters were monitored during the recovery period, 15 minutes after the apnea ended, with the same methods used previously during baseline. The chronological sequence and phase durations of the various measurements is shown in Figure 1.

Data Analysis

Heart Rate Variability Analysis. Portapres device (TNO/BMI, Amsterdam, the Netherlands) was used at the baseline and at the post apnea periods to record and extract time series of RR intervals and systolic as well as diastolic blood pressures for HRV and BRS indices. Polar V800 (Polar Electro Oy, Kempele, Finland) heart rate monitor and its chest strap Polar H10 (Polar Electro Oy, Kempele, Finland) was used to record and extract time series of RR intervals during dynamic apneas, with a sampling rate of 1000 Hz. Data were analyzed with Kubios HRV software (v. 2.0, 2008, Biosignal Analysis and Medical Imaging

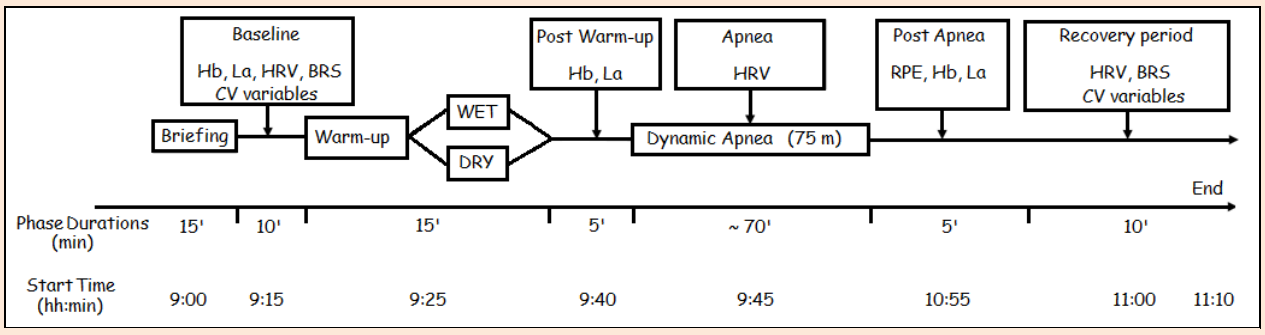

Figure 1. Graphical overview of the testing protocol with the timeline of events. Hb, blood hemoglobin; La, blood lactate; HRV, heart rate variability; BRS, baroreflex sensitivity; RPE, rate of perceived exertion.; CV, cardiovascular.

Group, University of Kuopio, Finland), where all time series were filtered to exclude artefacts (Camm et al., 1996). For the time domain analysis, the square root of the mean squared differences of successive RR intervals (RMSSD), and the standard deviation of successive RR intervals (SDRR) were investigated. Whereas, for the frequency domain analysis, we recorded low frequency (LF) ranging from 0.04 to 0.15 Hz and high frequency (HF) centered at the breathing frequency of about 12 breathes/minute following the rhythm of a metronome (0.20 - 0.25 Hz) (Piras et al., 2020). It has been shown that HF is an index of the vagal modulation, LF considers both sympathetic and vagal branches (Camm et al., 1996). Both indices (variables with skewed distributions) were log transformed (Ln).

Baroreflex Sensitivity Analysis. Baroreflex sensitivity was computed from RR intervals and systolic arterial pressure (SAP) sequence subtracted from the finger arterial pressure waveform. These data were then used to define the oscillations in both HR and SAP measures. Beatscope version 1.1a (TNO/BMI) was used to evaluate spontaneous BRS, with a BRS add-on module that computes the time-domain cross correlation BRS (Piras et al., 2015; 2019; 2020; Piras and Gatta, 2017). In brief, this technique is based on the computer identification in the time domain of 4 or more spontaneous sequences of consecutive beats, distinguished by either a progressive increase or reduction in SAP and RR interval. The incline of the regression line between SAP and RR interval fluctuations is considered as an index of the arterial BRS of the heart, same as the laboratory method based on intravenous injection of vasoactive drugs. This technique for BRS identification has lower within-patient variance than other methods and provides more values per minute than the standard time-domain–based method (Westerhof et al., 2006).

Hemodynamic Parameters. From the blood pressure waveform, stroke volume (SV), cardiac output (CO), and total peripheral resistance (TPR) were estimated by the pulse contour method of Wesseling (the Modelflow method—software TNO/BMI) that has been validated extensively (Bos et al., 1996).

Statistical analysis

Data are present as mean ± SD. Visual inspection of model residuals showed a positive skew and heteroskedasticity that was improved by log transformation (Ln) before analysis. Paired sample t-test was used to analyze the diving time between DRY and WET conditions. Due to the different time (baseline; post warm-up; apnea; post apnea/recovery period, see Figure 1) in which dependent variables were recorded, several two-way ANOVAs were used. A repeated measures ANOVA was used to see significant differences for Hb and lactate values, in which time (baseline; post warm-up; post apnea) and condition (WET; DRY) were the within-subjects factor. A repeated measures ANOVA, in which time (baseline; apnea; post-apnea) and condition (WET; DRY) were the within-subjects factor, was used to see significant differences for heart rate variability indices. Another repeated measures ANOVA was used to see significant differences for BRS value with time (baseline; post apnea) and condition (WET; DRY) as the within-subjects factor.

The time course for changes in heart rate was determined by calculating the average for each 5-s period, beginning 60 sec before each apnea (preparation phase) until the end of each apnea. The average value calculated for the preparation phase was used as control value and compared with the first 30 and the last 30 sec of each dynamic apneas using repeated measures ANOVA, in which time (baseline; first 30 sec; last 30 sec) and condition (WET; DRY) were the within-subjects factor. Finally, the rate of perceived exertion, recorded at the end of the apnea, was analyzed with the paired sample t-test, comparing DRY with WET conditions.

The Pearson correlation coefficients were computed to identify the relations among the diving time of both apneas with the other parameters recorded in this study.

Mauchly's test was used to assess any violations of sphericity. Effect size of the repeated measure ANOVAs were expressed using partial eta-squared (η_p^2), with values of 0.01, 0.06, and 0.14 representing small, medium, and large effects respectively. Bonferroni post-hoc analysis was used for multiple comparisons, and effect sizes (Cohen's *d*) were calculated as the mean difference standardized by the between subjects standard deviation and interpreted according to the following thresholds: <0.20; small, >0.20 - 0.60; moderate, >0.60 - 1.20; large, >1.20 - 2.00; very large,>2.00 - 4.00; extremely large, >4.00 (Hopkins et al., 2009). Statistical significance was set at $p < 0.05$. Data were analyzed with SPSS v22.0 (IBM, New York, NY, USA).

Results

All athletes completed the protocol. Data regarding anthropometric and training level are presented in table 1.

First, we analyzed the diving time between experimental conditions. We found a significant difference between apnea executed after WET with respect to apnea executed after DRY. Athletes were faster during DRY (69.56 ± 5.6 sec) than WET (72.22 ± 4.8 sec) condition ($t(8) = 2.24$; $p = 0.028$; $d = 0.17$).

Lactate values showed significant differences for time ($F_{2,32} = 35.49$; $p < 0.001$; $n_p^2 = 0.69$) and time x condition interaction effect ($F_{2,32} = 3.86$; $p = 0.031$; $n_p^2 = 0.19$). Bonferroni post-hoc analysis showed a significant difference between blood lactate recorded after warm-up ($p = 0.006$; $d = 1.21$), with a greater value after WET with respect to the condition in which they performed the DRY warm-up (Table 2). In both conditions, lactate values increased from baseline to post apneas ($p < 0.05$, Table 2).

Hb values did not show significant differences, the same result was obtained for the rate of perceived exertion ($p > 0.05$; Table 2).

Time domain heart rate variability indices, such as SDNN and RMSSD showed significant interaction effect of time x condition ($F_{2,32} = 3.96$; $p = 0.038$; $n_p^2 = 0.18$ and $F_{2,32} = 4.06$; $p = 0.041$; $n_p^2 = 0.18$, respectively). Bonferroni post-hoc analysis showed a significant difference between values recorded during apnea ($p = 0.002$; $d = 0.90$ and $p < 0.001$; $d = 0.84$, respectively), with greater values after

Table 2. Hematic and fatigue indices. Data are means ± SD.

	WET			DRY		
	Baseline	**Post Warm-up**	**Post Apnea**	**Baseline**	**Post Warm-up**	**Post Apnea**
Hb (g/dL)	14.26 ± 1.2	14.17 ± 1.6	13.98 ± 1.8	14.05 ± 1.7	14.01 ± 1.6	14.50 ± 1.6
La (mmol/L)	1.67 ± 0.2	2.60 ± 0.6*#	3.26 ± 0.7#	1.96 ± 0.5	1.93 ± 0.4	3.33 ± 0.7#
RPE (OMNI-RES 1-10)	/	/	3.44 ± 1.0	/	/	3.77 ± 1.2

Hb; hemoglobin, La; lactate, RPE; rate of perceived exertion. * represent significant differences between conditions at $p < 0.05$; # represent significant differences across time at $p < 0.05$;

DRY with respect to the condition in which they did the WET warm-up (Figure 2). Similar results were obtained for low and high frequency domain analysis of HRV indices. Both values, log transformed (Ln) before analysis due to skewed distribution, showed significant interaction effect of time x condition ($F_{2,32} = 4.01$; $p = 0.040$; $n_p^2 = 0.18$ and $F_{2,32} = 4.02$; $p = 0.041$; $n_p^2 = 0.18$, respectively). Bonferroni post-hoc analysis showed a significant difference between values recorded during apnea ($p < 0.001$; $d = 0.70$ and $p = 0.002$; $d = 0.64$, respectively), with greater values after DRY in comparison to the condition in which they did the WET warm-up (Figure 3).

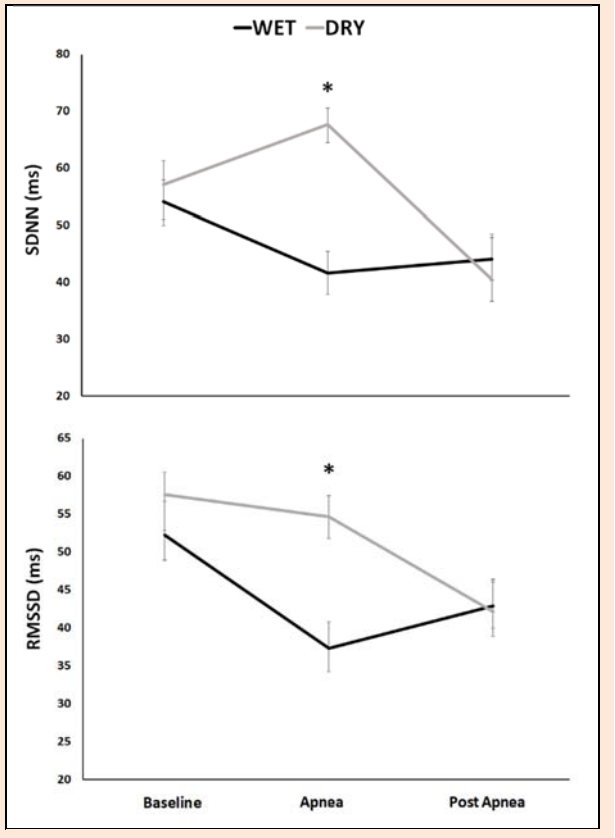

Figure 2. Time domain of heart rate variability (HRV) data (mean ± SD) at baseline, during apnea, and during the post apnea period in WET (black line) and DRY (grey line) condition. RMSSD, root mean square of successive standard deviations; SDNN, standard deviation of successive NN intervals. * represent significant differences between conditions at p < 0.05.

Several cardiovascular indices, such as diastolic and mean arterial pressure, stroke volume, cardiac output and total peripheral resistances showed significant differences for time main effect ($F_{1,16} = 12.41$; $p = 0.003$; $n_p^2 = 0.44$; $F_{1,16} = 9.49$; $p = 0.007$; $n_p^2 = 0.37$; $F_{1,16} = 9.70$; $p = 0.007$; $n_p^2 = 0.38$; $F_{1,16} = 6.75$; $p = 0.019$; $n_p^2 = 0.30$; $F_{1,16} = 10.23$; $p = 0.006$; $n_p^2 = 0.39$, respectively). The other cardiovascular indices, such as systolic arterial pressure, heart rate, interbeat interval and baroreflex sensitivity did not show any significant differences (Table 3).

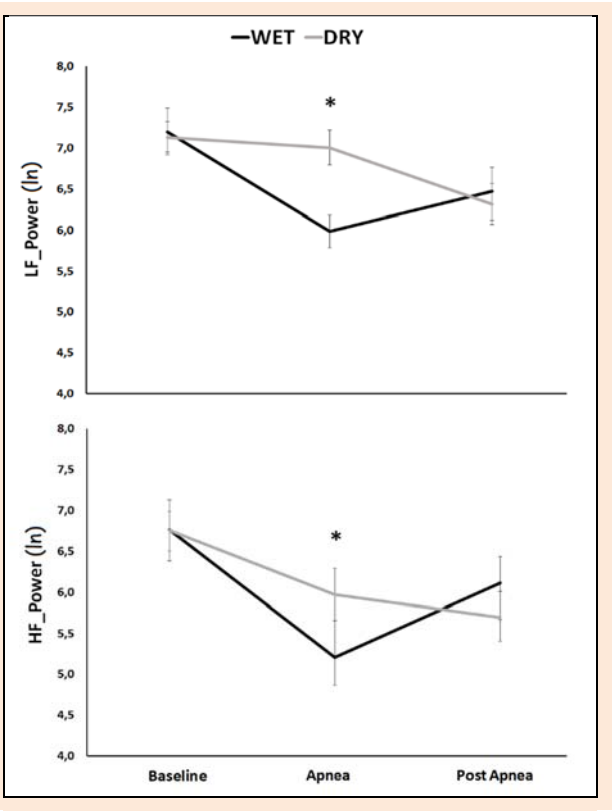

Figure 3. HRV high- and low-frequency (HF and LF) spectral band (mean ± SD) at baseline, during apnea, and during the post apnea period in WET (black line) and DRY (grey line) condition. Data are log-transformed (ln) (see methods). * represent significant differences between conditions at p < 0.05.

HR was also analyzed 60 sec before (preparation phase) and 70 sec during apneas. Time course analyses showed no significant differences between conditions in the preparatory phase ($p = 0.77$; $d = 0.13$). During the last 20 sec before the dynamic apnea, the heart rate increased in both conditions. During the first 30 sec of the apnea, the heart rate has increased of 10.4% with respect to the preparation phase in the WET condition ($p = 0.001$; $d = 1.54$). Whereas, gradually diminished of 7.5% in the DRY condition ($p = 0.03$; $d = 0.71$). During the last 30 sec of apneas, in both conditions, the heart rate continued to decrease, with a more marked reduction after DRY (-57.5%; $p < 0.001$; $d = 6.57$) than WET (-24.7%; $p = 0.004$; $d = 1.88$) with respect to the preparation phase (Figure 4). A significant correlation was found between lactate

produced after WET warm-up and the duration of the subsequent apnea (Pearson = 0.71; p = 0.015). Whereas, the lactate produced after DRY warm-up was not significantly correlated with its subsequent apnea duration (Pearson = 0.29; p = 0.23). It seems that, the more lactate is produced after warm-up the more time is needed for doing 75m of dynamic apnea.

Moreover, we found a significant negative correlation between the diving time and the lactate produced during apnea executed after DRY warm-up (Pearson = -0.60; p = 0.045). It is plausible that the faster they go during apnea, the more lactate they produce, and the less time they take to complete the track. This hypothesis is supported by a further analysis, which showed a significant correlation between apnea duration and the cardiac autonomic nervous system parameters recorded at the end of the apnea (BRS = 0.62; p = 0.003, RMSSD = 0.47; p = 0.025, SDNN = 0.46; p = 0.032, and HF = 0.45; p = 0.030), suggesting that the slower the apnea is executed, the faster is the recovery process.

Table 3. Cardiovascular and autonomic variables. Data are means ± SD.

		SAP (mmHg)	DAP (mmHg)	MAP (mmHg)	HR (bpm)	SV (ml)	CO (L/min)	TPR (mmHg.s/ml)	IBI (sec)	BRS (mmHg/ms)
WET	**Baseline**	129.37 ± 4.9	65.76 ± 2.9	84.42 ± 3.4	72.69 ± 3.7	95.54 ± 4.1	6.94 ± 0.6	0.78 ± 0.2	0.85 ± 0.05	12.98 ± 1.6
	Post Apnea	137.03 ± 4.5	73.03 ± 2.7#	92.03 ± 3.1#	72.97 ± 12.3	86.08 ± 5.9#	6.25 ± 0.4#	1.29 ± 0.2#	0.85 ± 0.05	12.44 ± 1.8
DRY	**Baseline**	130.43 ± 4.9	68.31 ± 2.9	86.97 ± 3.7	70.58 ± 3.1	93.28 ± 5.9	6.61 ± 0.5	0.85 ± 0.3	0.86 ± 0.04	13.75 ± 1.5
	Post Apnea	134.38 ± 4.5	74.60 ± 2.7#	93.09 ± 3.3#	73.08 ± 4.6	83.25 ± 4.5#	6.04 ± 0.4#	1.36 ± 0.2#	0.84 ± 0.05	12.51 ± 1.9

SAP; systolic arterial pressure, DAP; diastolic arterial pressure, MAP; mean arterial pressure, HR; heart rate, SV; stroke volume, CO; cardiac output, TPR; total peripheral resistance, IBI; Interbeat interval, BRS; baroreflex sensitivity. # represent significant differences across times at $p < 0.05$.

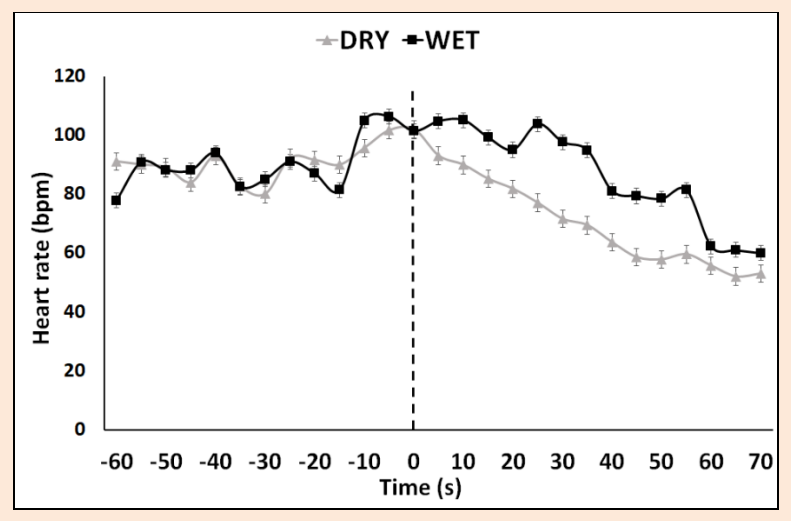

Figure 4. Heart rate before and during dynamic apneas. Vertical dashed line separates the two phases, preparation (left) and apnea (right). Each point is a 5-s average from 9 athletes. Black squares represent apneas after WET warm-up; grey triangles represent apneas after DRY warm-up.

Discussion

A wide number of athletes who execute breathing exercises and dry apneas before the performance declare to have advantageous physiological responses with respect to short-mid dives of the wet warm-up. Empirical evidence is required to identify and experimentally manipulate such factors under controlled laboratory conditions. We hypothesized that, executing the warm-up outside the water may result in a greater spleen contraction and a more pronounced diving reflex. This would allow an increase of total body gas storage and a decrease of the metabolic rate, involving a prolongation of the dive. The present research showed a greater autonomic regulation on heart during dynamic apnea when preceded by the DRY warm-up, as demonstrated through the higher heart rate variability and the different trend exhibited by the heart rate during the immersion. Athletes were faster during DRY than WET condition, maybe because blood lactate was lower after DRY than WET warm-up. Hemoglobin concentration did not show significant differences; the same result was obtained for the rate of perceived exertion.

Heart Rate Variability and the cardiovascular indices

The heart rate variability was investigated by the analysis of time domain indexes (SDNN and RMSSD) and frequency domain indexes (LF power and HF power). The SDNN and the LF power reflect the overall activity of the autonomic nervous system, while the RMSSD and the HF power are associated with the activity of the parasympathetic branch alone (Camm et al., 1996). To our knowledge, no study has investigated the effects of different warm-up protocols on heart rate variability before, during and after a dynamic apnea. The results showed no significant differences before and after apnea, whereas during dynamic apnea HRV indices were higher in the DRY than in the WET condition, suggesting a greater response of the cardiac autonomic nervous system. Bradycardia was observed in both conditions (Figure 4). During the first 30 sec of the apnea, the heart rate has increased of 10.4% with respect to the preparation phase in the WET condition, while gradually diminished of 7.5% in

the DRY condition. During the last 30 sec of apnea, the heart rate continued to decrease, with a more marked reduction after DRY (-57.5%) than WET (-24.7%) condition.

The effect of face immersion may have affected the cardiovascular response, influenced by the different air and water temperatures. It has been suggested that cold skin receptors response depends on rapid temperature change and that vasodilatation before the apnea, as with warm air temperature, will result in a more pronounced vasoconstriction and, via the baroreflex, a larger bradycardia (Schagatay and Holm, 1996). Accordingly, it has been hypothesized that the cooling effect of water on the face may be lost with time spent in water (Schagatay, 2010). In the present study the WET warm-up lasted about 15 minutes on the water and may resulted in loss of body heat, with the vasoconstriction enhanced by the diving responses of the repetitive warm-up dives, and with a reduced activity of the cold skin receptors response. Conversely, the DRY warm-up allowed the athletes to start the dynamic apnea in a condition of vasodilatation and a preserved cold skin receptors response. In the present study the water temperature was quite warm (28°C), however we decided not to change it in order to reproduce the real conditions that athletes face during competitions, since this is a normal temperature for swimming pools in which training sessions and dynamic apnea competitions took place. This may have reduced the effect of facial chilling in our athletes, nevertheless this effect has been observed by Schagatay and Holm (1996) with air temperature of 20°C and the water temperature of 30°C, that are values quite similar to those of our tests (air: 23°C; water: 28°C). Schagatay and Holm (1996) monitored the heart rate during resting apnea, while our athletes performed dynamic apnea, therefore less bradycardia, due to muscular movements, may be expected. On the other hand, they tested subjects without apnea diving experience while we tested competitive amateur divers, who may present a larger diving response due to training adaptation (Schagatay et al., 2000).

A significant correlation has been found between apnea duration and the autonomic nervous system parameters recorded at the end of the apnea, suggesting that the slower the dynamic apnea is executed (higher apnea duration), the faster is the recovery process (higher cardiac autonomic values after apnea). Cardiovascular variables showed some significant changes with respect to the baseline in both WET and DRY condition. Diastolic and mean arterial pressure were increased, while stroke volume and cardiac output were reduced. This is primarily ascribable to the significant strong raise of the total peripheral resistance induced by the diving response.

Hemoglobin

The Hb concentration did not show significant differences between conditions nor during the transition from baseline to post warm-up and to post-apnea. These results agree with those of Prommer et al. (2007) who did not observe any variations in Hb concentration in trained divers after a series of 5 apneas in a heated pool (28°C), despite a spleen size reduction. Conversely, other studies reported Hb increases from 2,7% (Richardson et al., 2005) to 4% (Schagatay et al., 2007) in trained apneic divers. In these studies, the protocol consisted in a series of maximal resting apneas (from 3 to 5) spaced by 2 minutes of recovery. Hb increases even when the protocol was executed outside the water. These conditions are similar to our DRY warm-up protocol, which showed an increment, but not significantly different ($p = 0.17$; $d = 0.26$), from the baseline to the post-apnea of about 3.2%, greater than that reported by Richardson et al (2005). The level of training does not seem to play a role, since even non-divers showed a significant increase (Richardson et al., 2005; Schagatay et al., 2001). A possible explanation may be the source of blood collection since most of the other researches collected venous blood samples, and the intragroup variability, 4 of 9 showed a decrement from baseline to post-apnea.

Blood lactate

At the baseline, the blood lactate values were 1.67 and 1.96 mmol/L (WET and DRY respectively), considering that the concentration of blood lactate is usually 1-2 mmol/L at rest (Biagi et al., 2012). These data are in accordance with those of Schagatay (2011), who reported values between a range of 1-2.5 mmol/L before the dives. Higher blood lactate level during apnea is a consequence of the muscle ischemia induced by the peripheral vasoconstriction (Ferretti, 2001) and has been observed in all the apnea disciplines (Schagatay, 2009; 2010; 2011). In the current study, no changes were found after the DRY warm-up (1.93 mmol/L), conversely, an increase occurred after the WET warm-up (2.60 mmol/L). This difference is attributable to the muscle activity and to the facial immersion implied in the short dynamic apneas of the WET warm-up protocol. A further increase occurred at the end of the 75 meters dynamic apnea in both conditions, showing similar final values (3.26 vs. 3.33 mmol/L). These values are lower than those measured after dynamic apnea competition (~10 mmol/L) during a world championship (Schagatay, 2010). The difference may be due to the sub-maximal distance performed by our athletes, to their lower level of training and to the lack of competitive stress. Considering that lactate levels were different before the dynamic apneas but similar at the end of it, it seems that DRY warm-up induced a higher lactate level during the subsequent dive. Since the distance covered in the two trials was the same, it is conceivable that a greater lactate level matches with a higher vasoconstriction, therefore an anticipated shift to anaerobic metabolism. This is supported by the lower HR time course and higher HRV indexes, which show a greater activity of both sympathetic and parasympathetic branches during the dynamic apnea after DRY warm-up.

The time needed to complete the dynamic apnea was ~72 s for the WET condition and ~70 s for the DRY one. We found a significant correlation between lactate produced during WET warm-up and the subsequent apnea duration. These data suggest that the more lactate is produced during the warm-up the more time is needed for doing 75m in dynamic apnea. Despite the short-mid dives were well below their maximum distance underwater,

WET warm-up induced higher lactate level that have affected the underwater fin swimming technique and its speed. Conversely, the absence of the changes in the lactate level from the baseline to the end of the DRY warm-up could have allowed the athletes to perform a better underwater technique. The data then confirm that apnea duration is inversely correlated to the metabolic rate, so any physical exercise that elevates lactate levels just before a dive would be counterproductive.

Rate of perceived exertion

To our knowledge, the perceived exertion rate has not yet been investigated in the apnea disciplines and no scale has been validated to assess subjective intensity of dynamic apnea exercise. Therefore, we decided to adopt the OMNI-resistance exercise scale (OMNI-RES 1-10), with a response range from 0 to 10 (Robertson, 2004). The OMNI-RES has been validated both for resistance and aerobic exercises such as walking, running and cycling. No significant differences emerged between the conditions tested. The 75m dynamic apneas requested in the present study represent the 76,5% of the mean maximal performance of our athletes (see Table 1), but the rating was ~3 for the WET condition and ~4 for the DRY one. Thus, there is no correspondence between the distance achieved and the ratings provided by the athletes. Collecting the feedbacks from the athletes, we have formulated some hypotheses to explain this phenomenon. A prolonged apnea can be divided into two phases by the "physiological breaking point", which is reached when the elevated $PaCO_2$ triggers involuntary breathing movements. Before the physiological breaking point, the subject feels no urge to breathe, therefore this period has been named the "easy-going phase". After the physiological breaking point, the subject has to withstand an increasing urge to breathe, including increasing involuntary breathing movements, also called "struggle phase" (Schagatay et al., 2001). It is plausible that the perception of effort is based primarily on the bad feelings associated with hypercapnia and hypoxia and that these do not show a linear trend with respect to the distance or duration of the dive. Consequently, the effort could be irrelevant during the easy-going phase, modest during the transition to the struggle phase, but it can grow suddenly while reaching the personal limit, similarly to an exponential trend. Another hypothesis is that stress and fear of blackout can generate anticipatory anxiety that initially leads the athlete to overestimate the difficulty of the exercise and, subsequently, to underestimate the effort after surfacing, when the actual difficulty is less than expected.

Study limitations

Within the design of the current study, we acknowledge the following limitations. Capillary blood was collected from the finger to avoid the application of a venous catheter and to allow normal mobility to the athletes during dynamic apnea. It is possible that capillary blood collection does not represent a reliable method for Hb measurements due to the diving reflex and consequent peripheral vasoconstriction, since poor circulation in the fingers may lead to incorrect Hb results (Neufeld et al., 2002; Whitehead et al., 2019). On the other hand, fingertip capillary blood samples for the measurement and analysis of Hb is extensively utilized (Engan et al., 2020; Holmström et al., 2021; Lodin-Sundström et al., 2021).

We have not measured end-tidal PO_2, PCO_2, neither hemoglobin O_2 saturation parameters that could have helped in interpreting results more clearly. Nevertheless, our study showed the differences between the two warm-up protocols, supporting the claims of freediving athletes who adopt the DRY warm-up because it induces a more pronounced diving response, avoiding higher lactate level at the end of the warm-up and reducing the dive time of the subsequent dynamic apnea.

Conclusion

Before the dynamic apnea performance, the use of the DRY warm-up seems to be a better strategy with respect to the WET warm-up since it induces a more pronounced diving response, avoids higher lactate level pre-apnea, and reduces the time needed to complete the dive. We are aware that our findings must be corroborated by investigating the effects of different warm-up strategies during maximal performances and competitions, by testing elite athletes and compare gender differences. Moreover, the results of the present study raise some questions, and further research are needed to assess if capillary blood collection represent a valid method for Hb measurement in apnea and for better understanding the role of spleen contraction in different warm-up strategies. Finally, the rating of perceived exertion, widely used in many sports, should be well examined and appropriate scales for apnea disciplines should be developed.

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Key points

- DRY warm-up showed lower lactate level with respect to the WET warm-up
- DRY warm-up seems to induce a more pronounced diving response
- When the dynamic apnoea is preceded by the DRY warm-up we found a greater cardiac autonomic regulation

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SUPPLEMENT

This supplementary material has the intention to describe some terms used to identify the exercises suggested in the two warm-up protocols:

- *Kicking warm-up exercise*: this is a moderate warm-up exercise intensity that consists of making 4-5 kicks during diving, alternating with a single breath in surface;
- *Restart*: represents the time interval at which each subsequent repetition must start, therefore it includes both the apnea phase and the recovery phase;
- *Triangular ventilation*: it is a ventilation characterized by lower ventilatory rate and higher tidal volume with respect to the eupneic ventilation.
- *Uddhyana bandha*: it is a yoga exercise that involves performing one maximum exhalation, close the vocal cords, dilate the rib cage using inspiratory muscles and at the same time relax the diaphragm as much as possible. This allows you to stretch this muscle by lifting it cranially. During the warm-up, this exercise was performed in the supine position, with the lower limbs flexed and the upper limbs extended.