



Effect of water-based recovery on blood lactate removal after high-intensity exercise

Francesco Lucertini¹*, Marco Gervasi¹, Giancarlo D'Amen¹, Davide Sisti², Marco Bruno Luigi Rocchi², Vilberto Stocchi¹, Piero Benelli¹

- 1 Department of Biomolecular Sciences—Division of Exercise and Health Sciences, University of Urbino Carlo Bo, Urbino, Italy, 2 Department of Biomolecular Sciences—Service of Biostatistics, University of Urbino Carlo Bo, Urbino, Italy
- * francesco.lucertini@uniurb.it



OPEN ACCESS

Citation: Lucertini F, Gervasi M, D'Amen G, Sisti D, Rocchi MBL, Stocchi V, et al. (2017) Effect of water-based recovery on blood lactate removal after high-intensity exercise. PLoS ONE 12(9): e0184240. https://doi.org/10.1371/journal.pone.0184240

Editor: Alejandro Lucía, Universidad Europea de Madrid, SPAIN

Received: December 23, 2016
Accepted: August 10, 2017
Published: September 6, 2017

Copyright: © 2017 Lucertini et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper and its Supporting Information file.

Funding: The authors received no specific funding for this work.

Competing interests: The authors have declared that no competing interests exist.

Abstract

This study assessed the effectiveness of water immersion to the shoulders in enhancing blood lactate removal during active and passive recovery after short-duration high-intensity exercise. Seventeen cyclists underwent active water- and land-based recoveries and passive water and land-based recoveries. The recovery conditions lasted 31 minutes each and started after the identification of each cyclist's blood lactate accumulation peak, induced by a 30-second all-out sprint on a cycle ergometer. Active recoveries were performed on a cycle ergometer at 70% of the oxygen consumption corresponding to the lactate threshold (the control for the intensity was oxygen consumption), while passive recoveries were performed with subjects at rest and seated on the cycle ergometer. Blood lactate concentration was measured 8 times during each recovery condition and lactate clearance was modeled over a negative exponential function using non-linear regression. Actual active recovery intensity was compared to the target intensity (one sample t-test) and passive recovery intensities were compared between environments (paired sample t-tests). Non-linear regression parameters (coefficients of the exponential decay of lactate; predicted resting lactates; predicted delta decreases in lactate) were compared between environments (linear mixed model analyses for repeated measures) separately for the active and passive recovery modes. Active recovery intensities did not differ significantly from the target oxygen consumption, whereas passive recovery resulted in a slightly lower oxygen consumption when performed while immersed in water rather than on land. The exponential decay of blood lactate was not significantly different in water- or land-based recoveries in either active or passive recovery conditions. In conclusion, water immersion at 29°C would not appear to be an effective practice for improving post-exercise lactate removal in either the active or passive recovery modes.

Introduction

Short-duration high-intensity exercise leads rapidly to muscular fatigue, which can be defined as the loss of force or power in response to physical exertion resulting in reduced performance [1]. Two of the most important mechanisms involved in exercise-induced fatigue are fibers



acidosis and depletion of ATP [2], which lead to large changes in the concentration of metabolites, such as lactate. Lactate is no longer thought to cause fiber acidosis and is believed to provide protection against this process [3]. However its exercise induced rise in the blood coincides with fiber acidosis; hence, it remains a good indirect marker for the onset of fatigue [2, 3]. The rapid removal of lactate following intense exercise remains desirable since it is taken up by both resting muscles and fibers of the same muscle working at lower intensities and used as a carbohydrate fuel source (see [4] for a comprehensive review).

In exercise and sports, blood lactate concentration ($[La]_b$) is the most widely used marker of muscular fatigue and several studies have investigated different strategies to enhance lactate removal from the blood after intense physical activity yielding mixing results [5]. Light-to-moderate intensity active recovery has clearly been shown to be a superior lactate clearance strategy compared to passive (resting) recovery [6] after short-duration high-intensity exercise.

Among such recovery strategies, water immersion is the focus of considerable attention among athletes and researchers [7]. From a physical standpoint, water immersion exerts a compressive force on the body, and there is a widely held belief among athletes and trainers that water immersion improves recovery [7]. Indeed, Wilcock *et al.* [8] have hypothesized that hydrostatic pressure, via extracellular fluid transfer to the intravascular compartment and the subsequent increase in cardiac output, may reduce the transport time of the metabolites, including lactate, which accumulate during exercise. However, only two investigations [9, 10] have found enhanced lactate clearance when the recovery from a lactate-accumulating exercise protocol was performed immersed in water rather than on land. Unfortunately, both of these studies were flawed due to their use of heart rate as the control for active recovery intensity since it has been shown that exercises performed at the same oxygen consumption yield significantly higher heart rate responses when they are performed on land compared to when they are performed while immersed at different water depths [11–13]. Therefore, the effect of water on lactate removal from intense exercise needs further investigation.

Accordingly, the aim of this study was to compare the effect of dry-land and water environments on lactate clearance from the blood after short-duration high-intensity exercise. Both active and passive recovery modes were investigated separately.

Materials and methods

Participants

Seventeen well-trained young cyclists (see Table 1 for subjects' characteristics) were recruited. The training level of each cyclist was assessed using a questionnaire [14] (average weekly training hours: 11.3±3.9). The study was approved by the Ethics Committee of the University of Urbino Carlo Bo, and the subjects signed a written informed consent form before being recruited.

Experimental design

A balanced randomized, crossover study design was used to test the effect of the recovery environment on blood lactate removal. The subjects were scheduled to undergo five experimental sessions at one-week intervals to allow them to fully recover between sessions. For each session, the participants reported to the laboratory well-rested, i.e. without having engaged in strenuous exercise in the previous 48 hours, and at least three hours after a light meal.

In session 1 the cyclists underwent anthropometric and body composition assessments, as well as maximum oxygen consumption ($\dot{V}O_2$) and lactate threshold tests (see detailed



Table 1. Participants' characteristics and baseline assessments/calculations.

	Age (years)	Height (m)	Weight (kg)	BMI (kg·m ⁻²)	FM (%)	HR _{max} (bpm)	VO _{2peak} (mL kg ⁻¹ min ⁻¹)	70% of VO _{2LT} (mL·kg ⁻¹ ·min ⁻¹)	[La] _b peak _P - (mmol⋅L ⁻¹)	[La] _b peak _A (mmol·L ⁻¹)
Mean	28.4	1.78	71.2	22.4	12.7	191.9	63.2	35.6	13.3	13.3
SD (±)	6.4	0.06	5.9	2.1	4.1	8.6	7.7	4.2	2.5	2.3

Average data calculated for seventeen subjects. Abbreviations: BMI, body mass index; FM, fat-mass; HR_{max}, maximal heart rate; \dot{V} O_{2peak}, peak oxygen consumption; 70% of \dot{V} O_{2LT}, 70% of the oxygen consumption corresponding to the lactate threshold; [La]_b peak, average peak blood lactate concentration achieved before the passive (_P) and the active (_A) recovery conditions (average value was computed for twelve subjects for passive recoveries); SD, standard deviation.

https://doi.org/10.1371/journal.pone.0184240.t001

description of assessments and tests below). Finally, participants underwent a familiarization trial of the peak anaerobic power test (Wingate Anaerobic Test—WAnT).

In sessions 2, 3, 4, and 5, [La], was measured in four different experimental recovery conditions after the WAnT (see details regarding test and measurement of [La]_b below). To raise [La]_b rapidly we used the 30-second WAnT protocol, which is commonly used for this purpose [15]. The [La]_b peak was identified as the transition from the blood lactate accumulation phase to the clearance phase. In practical terms, [La]_b was measured every minute following the completion of the WAnT, and the peak was considered as the [La]_b value followed by a measurement equal or lower than that value. The identification of the [La]_b peak was temporally out of synchrony with its actual attainment by two minutes because of the time it took for the measuring instrument to yield a result (one minute for each measurement). The actual attainment of the [La]_b peak marked the end of the accumulation phase and the beginning of the clearance phase. [La]_bs during the clearance phase were measured in all four of the following balanced randomly selected experimental conditions: 1) passive land-based recovery (PLR)—the subject remained immobile seated on the cycle ergometer used to carry out the WAnT; II) passive water-based recovery (PWR)—the subject, immersed in water up to his shoulders, remained immobile seated on an underwater cycle ergometer; III) active land-based recovery (ALR)—the subject peddled on the cycle ergometer used to carry out the WAnT; IV) active water-based recovery (AWR)—the subject, immersed in water up to his shoulders, peddled on an underwater cycle ergometer. Each of these recovery conditions lasted for 31 minutes and was always preceded by two minutes of rest, required for the preparation of the subjects and their proper positioning on the (Aqquactive Bike) underwater cycle ergometer (Aqquatix Ltd., Limena, Italy) under the water-based recovery conditions.

The ambient temperature was monitored constantly during the PLR and ALR recovery conditions. The water temperature during the PWR and AWR was set at 29°C and monitored constantly as well. The water temperature was chosen for two reasons: 1) to replicate a condition that athletes can easily find in real-life settings (29°C is the average temperature set in most swimming pools open to the public); 2) 29°C is the closest water temperature we could get to those of the studies we planned to compare our results with (i.e. 30–31°C in the study of Di Masi et al. [9] and 28–32°C in the study of Ferreira et al. [10]).

The intensity chosen for the ALR and AWR was calculated as a percentage of oxygen consumption at the blood lactate threshold $(\dot{V}O_{2LT})$, since a recovery calculated using the lactate threshold rather than the maximum $\dot{V}O_2$ has been shown to be more suitable [16]. In runners, the intensity that maximizes the clearance of lactate is between 60% and 80% of $\dot{V}O_{2LT}$ [17, 18], and it has been demonstrated that the kinetic of lactate clearance is not significantly different between runners and cyclists [19–23]. Hence, the intensity of active recovery in the present



study was fixed at 70% of $\dot{V}O_{\rm 2LT}$. On a practical level, in both active recovery conditions, oxygen consumption was constantly monitored and the subjects were instructed in real time (every two minutes) to maintain recovery intensity in the range of 65% to 75% of $\dot{V}O_{\rm 2LT}$. The subjects were told to either maintain the resistance of the ergometer and the pedaling frequency or to increase/decrease the resistance of the ergometer (by rotating the brake knob and, if necessary, by changing the pedaling frequency as well) when the intensity fell below or rose above a level that resulted in a $\pm 5\%$ difference between the actual and the target intensity, respectively. Under all the conditions, $[La]_b$ was measured at 3 minutes and then every 4 minutes (i.e. 7, 11, 15, 19, 23, 27 and 31 minutes) for a total of 8 blood draws for each recovery condition. $[La]_b$ was measured using the same procedure and instrument that was used in the maximum $\dot{V}O_2$ test (see detailed description below).

Assessments

Anthropometry and body composition. The body mass index was calculated as the ratio between weight (kg) and height (m²), while the percentage of fat mass was calculated by the race-, age-, and sex-specific regression equation of Davidson *et al.* [24], using skinfolds of the biceps, triceps, subscapular and suprailiac as indicated by Durnin & Womerslay [25].

Maximum oxygen consumption and lactate threshold. The maximum $\dot{V}O_2$ and the lactate threshold were assessed, using the same test, on the SRM cycle ergometer (SRM Italia, Lucca, Italy) with the same settings (height and fore-aft position of the seat, height and distance of the handlebar, and length of the pedal crankarms) and the same type of bicycle pedals for each subject. The original incremental protocol to exhaustion to determine maximum VO₂ calls for a minimum of five and a maximum of nine 4-minute stages, each with resistance increments between a minimum of 20 and a maximum of 50 watts [26] and has been found to be highly reliable for the lactate threshold assessment in cyclists [27]. Although the protocol called for an intensity of 40% of maximum $\dot{V}O_2$ for the first stage, we decided to use 30% in light of the fact that to determine the lactate threshold, the blood concentration of lactate at the end of the first stage should not be significantly higher than the resting value [26]. To calculate the resistance to apply to the cycle ergometer corresponding to 30% of maximum $\dot{V}O_{2}$, the theoretical maximum oxygen consumption of each subject was estimated according to Malek et al. [28] and then converted into watts (peak) according to the regression equation of Hawley and Noakes [29]. The peak wattage was used to obtain a resistance value equal to 30%, and the difference between the two values was divided over 6 stages. Hence, we were able to determine the necessary watt increase in each stage in order to end the test hypothetically between the 5th and 9th stages (as suggested in [26]). Oxygen consumption was monitored for the duration of the trial (breath-by-breath) using the Cosmed k4b² metabolimeter (COSMED, Rome, Italy), heart rate (HR) was recorded with the Polar RS-800 heart rate monitor (POLAR, Kempele, Finland), and blood lactate was measured (before starting the test and within 30 seconds before the end of each stage) using the Lactate Pro portable blood lactate meter (Arkray, Kioto, Japan) on micro blood samples drawn from the tip of the index finger according to the manufacturer's instructions. Peak oxygen consumption ($\dot{V}O_{2peak}$) was identified as the maximum value derived from the 15-breath moving average of oxygen consumption of the entire test, as suggested by Robergs et al. [30]. The lactate threshold and the corresponding $\dot{V}O_{2LT}$ were determined using the algorithm of Bentley et al. [31], and implemented using software expressly developed by Newell et al. [32], which requires inputting the [La]_b and the steadystate oxygen consumption (as the average of the breath-by-breath measurements of the last 30s) for each stage.



Wingate Anaerobic Test. The test was conducted on the Peak-Bike cycle ergometer (Monark Exercise AB, Vansbro, Sweden) using a standard protocol [15]. Briefly: I) 12-minute warm up during which the subjects made three short maximum accelerations (for 5 seconds) without friction load at 4, 7 and 10 minutes; II) 3 minutes of recovery seated on the ergometer; III) WAnT with friction load equal to 0.098 kp·kg⁻¹ of body weight (i.e. about 10% of body weight).

Statistical analysis

The following analyses were performed using Excel (Microsoft Office, v.2010) and SPSS Statistics (IBM, v.20) software, with an α level of statistical significance of 0.05.

Peak blood lactate comparisons. The [La]_b peaks yielded before active and passive recoveries were compared using a one-way ANOVA for reaped measures.

Recovery intensity comparisons. Subjects' compliance to the target active recovery intensity (i.e., 70% of $\dot{V}O_{MT}$) was evaluated. Firstly, the difference between the target recovery intensity and the pooled (ALR and AWR) values of the actual recovery intensity (average percentage of $\dot{V}O_{OLT}$) was evaluated using a 2-tailed one sample t-test (this comparison was not made for passive recovery). Subsequently, ALR vs. AWR and PLR vs. PWR average values of the actual recovery intensities were compared separately using two 2-tailed, paired sample ttests. Individual linear regressions were performed for both the active and passive recovery conditions using the actual percentages of $\dot{V}O_{2LT}$ and the actual percentage of $\dot{V}O_{2peak}$, respectively, of the whole set of breaths of each subject for the relevant condition. Average slopes and intercepts of ALR and AWR conditions were compared to the expected values of 0 and 70% of $\dot{V}O_{21T}$, respectively, by means of two 2-tailed one sample t-tests. Average slopes and intercepts were compared between PLR and PWR using two 2-tailed paired sample t-tests. The same approach as described above was used to compare the HR responses in the active recovery condition (the percentage of HR at the lactate threshold; HR_{LT}) to those in the passive recovery condition (the percentage of the maximal HR; HR_{max}). All these comparisons were 2-tailed paired sample t-tests since no recovery intensity was planned for HR. All the t-tests were corrected according to Bonferroni's criterion.

Lactate clearance modeling. The lactate clearance kinetics of each recovery condition were modeled on a negative exponential function (as suggested in [33]) whose general form is: $y = a_0 + a_1 e^{-bx}$ where, a_0 is the predicted [La]_b at rest, a_1 is the difference between the predicted [La]_b peak and a_0 (predicted delta decrease), and b is the coefficient of the exponential decay of [La]_b. Non-linear regression (NLR) fitting was optimized by using the Levenberg-Marquardt algorithm with an initial guess made on the basis of a visual inspection of [La]_b over the time plots of each subject, for each recovery condition. A lower limit for predicted [La]_b was fixed at $a_0 \ge 0.5$ mmol·L⁻¹. The coefficient of determination (R^2) was used as a measure of goodness of fit: only regressions resulting in a high R^2 (≥ 0.8) were considered acceptable and retained for subsequent analyses.

Lactate clearance comparisons. Separate linear mixed model analyses for repeated measures were performed for active and passive recovery in order to compare the values of b, a_0 , and a_1 resulting from the lactate clearance modeling of the land- and the water-based conditions. Active and passive recovery were analyzed separately since it is widely accepted that lactate clearance varies significantly between the two conditions (e.g. see [23]).

Results

Table 1 shows the results of the anthropometric assessments and parameters measured during the maximum oxygen consumption and lactate threshold test, as well as other parameters that



were subsequently calculated. In line with Winter *et al.* [26], in all the tests the wattage increase of each stage always fell within the range of reference, and between 6 and 8 stages were always carried out.

Peak blood lactates

The [La]_b peaks did not differ significantly ($F_{(3,9)} = 1.528$; p = 0.273) before starting any recovery condition (see <u>Table 1</u> and <u>S1 File</u> for raw data of each condition).

Recovery intensities

Average ambient (PLR and ALR conditions) and water temperature (PWR and AWR conditions) remained constant at 25.3±1.4°C and 29±0.5°C, respectively. Five participants did not undergo the passive recovery conditions; therefore, the number of subjects for PLR and PWR decreased to twelve.

The α level of significance corrected according to the Bonferroni's criterion resulted in a p level of statistical significance of 0.017. Pooled ALR and AWR average recovery intensity did not differ significantly from the target recovery intensity of 70% of $\dot{V}O_{2LT}$ (p = 0.639). Average values for passive recoveries were computed for nine subjects due to technical problems we encountered in sampling oxygen consumption in three subjects. Actual average oxygen consumptions during active recovery did not differ significantly between land and water conditions (p = 0.381), and the linear regression parameters intercept and slope of both environmental conditions did not differ significantly from 70 (land: p = 0.249; water: p = 0.899) and 0 (land: p = 0.147; water: p = 0.193), respectively. Regarding HR during active recovery, average values (p = 0.004) and regression intercepts (p = 0.016) differed significantly between the environments, while regression slopes did not (p = 0.306). Average values of oxygen consumption during passive recovery were significantly different (p = 0.006) in the environments, whereas the regression parameters intercept (p = 0.069) and slope (p = 0.466) were not. HR average values (p = 0.007) and regression slopes (p = 0.002) of passive recovery differed significantly between the environments, while regression intercepts did not (p = 0.581). See Table 2 for details regarding the comparisons of the recovery intensities for both active and passive conditions.

Lactate clearance modeling and comparisons

NLRs were not acceptable (i.e. $R^2 < 0.5$ or non-plausible predicted values) in one subject for water-based recovery and in one subject for land recovery; therefore, lactate clearance comparisons were performed on sixteen and eleven subjects for active and passive recovery, respectively. The average goodness of fit for the NLRs of each condition was very high (ALR, R^2 : 0.98; AWR, R^2 : 0.99; PLR, R^2 : 0.97; PWR, R^2 : 0.96).

The environment of recovery did not affect any parameter of the negative exponential equations (b, a_0, a_1) in either active (Fig 1; $F_{(1,16)} = 0.372$, p = 0.551; Cohen's D effect size: b = 0.49; $a_0 = 0.64$; $a_1 = 0.36$) or passive (Fig 2; $F_{(1,11)} = 1.387$, p = 0.264; Cohen's D effect size: b = 0.24; $a_0 = 0.84$; $a_1 = 0.5$) recovery conditions.

Discussion and conclusions

The results of the present investigation clearly show that immersion to the shoulder in 29°C water does not improve lactate clearance in active or passive recovery. These results are not in agreement with the only two studies on this specific topic in literature, which showed an overall positive effect of the water environment in the clearance of blood lactate [9, 10]. These conflicting results can probably be attributed to the substantial differences in the experimental



			Active re	covery	Passive recovery	
			VO _{2LT}	HR _{LT}	VO _{2peak}	HR _{max}
Actual intensities						
	Land					
		Mean % (±SD)	70.1 (3.9)	83.8 (8.9) *	12.6 (3.1) *	47.4 (3.6) *
	Water					
		Mean % (±SD)	69 (3)	77.5 (6.4) *	13.9 (3.2) *	43.1 (3.9) *
Linear regressions						
	Land					
		Intercept (±SD)	71.1 (3.8)	83.2 (8.5)	15.1 (4.2)	50.3 (3.9)
		Slope (±SD)	-0.0 (0.0)	0.0 (0.0)	-0.0 (0.0)	-0.0 (0.0) *
	Water					
		Intercept (±SD)	70.2 (5.1)	77.9 (6.7) *	16. (4.3)	49.6 (3.9)
		Slope (±SD)	-0.0 (0.0)	-0.0 (0.0)	-0.0 (0.0)	-0.0 (0.0) *

Average data were calculated for seventeen and nine subjects for active and passive recovery, respectively. Linear regression parameters were calculated for sixteen and eleven subjects for active and passive recovery, respectively. Abbreviations: $\dot{V}O_{2LT}$, oxygen consumption at the lactate threshold; $\dot{V}O_{2peak}$, peak oxygen consumption; HR_{max} , maximal heart rate; SD, standard deviation;

https://doi.org/10.1371/journal.pone.0184240.t002

design of the studies, in particular, the modalities of intensity control in active recovery and the identification of the starting point of the recovery phase.

In the study by Di Masi *et al.* [9], the subjects recovered actively in both land-based and water-based conditions at an intensity equal to 65% of the age-predicted HR (calculated with the 220-age formula), whereas in the study by Ferreira *et al.* [10], the subjects recovered actively in land-based and water-based conditions pedaling at 85% of the HR corresponding to the ventilatory threshold. Hence, both studies used HR to equalize the intensity levels of active recovery in the two different environmental conditions, even though it is known that during

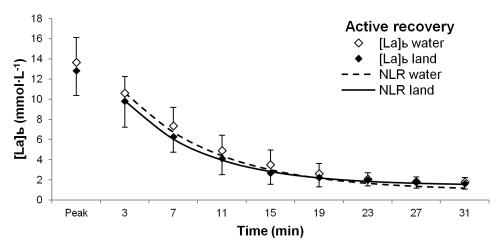


Fig 1. Blood lactate removal during active recovery. Blood lactate concentration decays during water-based (white diamonds) and land-based (black diamonds) active recovery conditions following a 30-second all-out bout of cycling. Non-linear regression curves are also shown for water-based (dashed line) and land-based (solid line) recovery. Abbreviations: [La]_b, blood lactate concentration; NLR, non-linear regression.

https://doi.org/10.1371/journal.pone.0184240.g001

^{*,} significantly different from the other recovery environment.

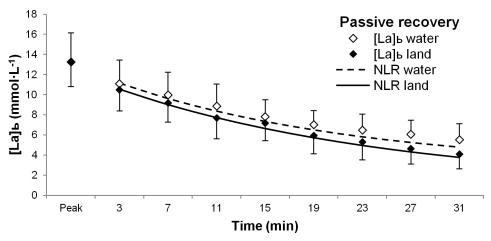


Fig 2. Blood lactate removal during passive recovery. Blood lactate concentration decays during water-based (white diamonds) and land-based (black diamonds) passive recovery conditions following a 30-second all-out bout of cycling. Non-linear regression curves are also shown for water-based (dashed line) and land-based (solid line) recovery. Abbreviations: [La]_b, blood lactate concentration; NLR, non-linear regression.

https://doi.org/10.1371/journal.pone.0184240.g002

submaximal exercise, oxygen consumption being equal, HR in water is lower than it is on land by approximately 10-15 bpm [11, 13, 34-36]. Although both Di Masi et al. [9] and Ferreira et al. [10] correctly chose percentages of HR that theoretically determine workloads under the lactate threshold [16], the use of HR implies that subjects in both investigations made an active recovery that varied in terms of metabolic intensity according the conditions (land-based or water-based). Hence, the measured [La]_b levels could not be compared because the intensity of the active recovery has a considerable effect on blood lactate clearance capacity [18]. On the other hand, in the present study, the intensity of the active recovery was monitored in both environmental conditions on the basis of the true oxygen consumption, which guarantees the comparability of intensity in the various experimental conditions. Moreover, we had the subjects recover actively at an intensity that was undoubtedly lower than the lactate threshold (70% of $\dot{V}O_{MT}$), under continuous monitoring by members of our research team, who informed the participants every time intensity fell below or exceeded the target level. The absence of a statistically significant difference in the intensities of oxygen consumption between the environments of recovery in the active condition points to the suitability of our experimental design. On the contrary, HR comparisons revealed a significantly lower average active recovery intensity (about -6%) in water compared to land conditions, which is in line with the above-mentioned studies and further supports our choice of using oxygen consumption as the control for recovery intensity. Unfortunately, passive recovery average values of oxygen consumption were found to be slightly (about +1.3% of $\dot{V}O_{2peak}$) but significantly higher in water than on land. This result is in line with other studies (e.g. see Park et al. [34]) carried out under similar conditions of water temperature and resting duration while seated on the cycle ergometer. Under those water-based conditions, the average skin temperature reduces significantly [34] and oxygen consumption and metabolism increase to maintain core temperature [8]. In support of this view, five participants did not well tolerate the water environment during passive recovery and after about 20 minutes started to feel too cold to conclude the 31-minute recovery and voluntarily interrupted the experiment. However, since both the regression intercept and slope of passive recovery oxygen consumption did not differ significantly between the environments, we are confident that comparisons of passive recovery lactate clearance between land and water-based conditions can still be drawn. That would not have been the case if HR



had been used as the control for passive recovery intensities since average values were found to be significantly higher (about +4.3% of HR_{max}) on land than in water and regression slopes differed significantly between the environments.

The experimental protocol of this investigation called for a high number of blood samples during the recovery phase (8 blood draws in 31 minutes). This allowed us to model the $[La]_b$ decay over time and to make more accurate comparisons between the two environmental conditions compared to previous investigations [9, 10], hence obtaining a more complete and detailed picture of the kinetics of lactate clearance.

In addition, the clear identification of the onset of the blood lactate clearance phase is indispensable to correctly assess its kinetics. In the studies by Di Masi et al. [9] and Ferreira et al. [10], a comparison was drawn among the [La]_b values obtained from blood draws made starting at a time chosen arbitrarily after the completion of the trial used for [La]_b accumulation. In particular, in the investigation of Di Masi et al. [9], the onset of the recovery phase was established one minute after the termination of the anaerobic exercise, whereas in the Ferreira et al. protocol [10], the onset of the recovery phase was established at five minutes after the termination of the exercise. It has been shown that following a short bout of maximal physical exertion, the moment of peak blood lactate is rather unpredictable [33, 37]. Hence, considering the low frequency of the blood draws in these investigations, values that actually belong to the lactate accumulation phase immediately following the end of the maximal exercise test, may have been included among the values used to establish the average rate of blood lactate clearance identified by Di Masi et al. [9] and Ferreira et al. [10] for the two environmental conditions. This may account for the differences between the results of the above-mentioned investigations and the results obtained in the present study, which only used [La], values that clearly belonged to the clearance phase after the maximal trial because the experimental design called for the identification of the peak [La]_b. In line with the interindividual variability regarding the time taken to reach [La]_b peak values reported in literature [33, 37], in the present study the [La]_b peak was recorded on average after 3.6±1.2 minutes from the end of the WAnT, despite the [La]_b peaks were not significantly different before starting any of the recovery conditions.

The main limitation of our investigation lies in the delay between the end of the WAnT and the identification of the $[La]_b$ peak and between the identification of the peak and the beginning of the selected recovery condition. In the first case, the limitation is due to the lactate meter used in this study, which takes one minute to yield a measurement; hence, the $[La]_b$ peak was always identified after two minutes from the onset of the clearance phase. In the second case, we elected to delay the start of all the recovery conditions by two minutes to allow for the correct positioning of the subjects on the cycle in the water-based recovery conditions. Hence, the $[La]_b$ of the first four minutes of recovery following the peak were never recorded. Nevertheless, since the kinetic of lactate clearance follows a well-known negative exponential pattern [33], it can be hypothesized that the pattern of lactate clearance from the fourth minute onwards is representative of the kinetics of the first four minutes. In any case, the aim of this investigation was to assess clearance differences attributable to the various conditions. Therefore, on a practical level, the short delay more accurately reflects the reality of recovery for athletes, who after a maximal exertion take a few minutes before starting their recovery immersed in water.

In conclusion, our main finding suggests that, contrary to what has been postulated in literature to date, the water environment does not produce different effects than the land environment on the kinetics of blood lactate clearance during active recovery from intense exercise. Hence, immersion in 29°C water would not appear to be a practice that can speed up post-exercise lactate removal in either the active or passive mode, and is therefore not advisable under the specific conditions investigated in the present study.



Additional research on this topic is needed and future studies should take into account possible variations in aquatic environmental conditions (water temperature, exercise type and mode, depth of immersion, etc.) to further investigate the potential of the water environment in modulating blood lactate clearance after intense exercise.

Supporting information

S1 File. Raw data. (XLSX)

Acknowledgments

The authors wish to thank the Aqquatix Ltd company (Limena, Italy), which provided the Aqquactive Bike underwater cycle ergometer free of charge. We also would like to thank Sara Antonella Me for her help in supervising the experimental sessions and Eugenio Grassi for his technical assistance. Finally, we wish to thank Timothy C. Bloom for his linguistic revision of the paper.

Author Contributions

Conceptualization: Francesco Lucertini, Marco Gervasi, Vilberto Stocchi, Piero Benelli.

Data curation: Francesco Lucertini, Marco Gervasi, Giancarlo D'Amen, Davide Sisti, Marco Bruno Luigi Rocchi.

Formal analysis: Francesco Lucertini, Marco Gervasi, Davide Sisti, Marco Bruno Luigi Rocchi.

Investigation: Francesco Lucertini, Marco Gervasi, Giancarlo D'Amen, Piero Benelli.

Methodology: Francesco Lucertini, Marco Gervasi, Davide Sisti, Marco Bruno Luigi Rocchi, Piero Benelli.

Project administration: Francesco Lucertini, Vilberto Stocchi, Piero Benelli.

Resources: Vilberto Stocchi, Piero Benelli.

Supervision: Francesco Lucertini, Vilberto Stocchi, Piero Benelli.

Validation: Francesco Lucertini, Marco Gervasi, Giancarlo D'Amen, Davide Sisti, Marco Bruno Luigi Rocchi.

Visualization: Francesco Lucertini, Marco Gervasi, Davide Sisti, Marco Bruno Luigi Rocchi.

Writing - original draft: Francesco Lucertini, Marco Gervasi, Davide Sisti.

Writing - review & editing: Francesco Lucertini, Marco Gervasi, Davide Sisti.

References

- Swain DP, American College of Sports Medicine, editors. ACSM's resource manual for Guidelines for exercise testing and prescription. 7th ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins: 2014.
- Finsterer J. Biomarkers of peripheral muscle fatigue during exercise. BMC musculoskeletal disorders. 2012; 13:218. https://doi.org/10.1186/1471-2474-13-218 PMID: 23136874; PubMed Central PMCID: PMC3534479.
- Robergs RA, Ghiasvand F, Parker D. Biochemistry of exercise-induced metabolic acidosis. American
 journal of physiology Regulatory, integrative and comparative physiology. 2004; 287(3):R502–16. Epub
 2004/08/17. https://doi.org/10.1152/ajpregu.00114.2004 PMID: 15308499.



- Adeva-Andany M, Lopez-Ojen M, Funcasta-Calderon R, Ameneiros-Rodriguez E, Donapetry-Garcia C, Vila-Altesor M, et al. Comprehensive review on lactate metabolism in human health. Mitochondrion. 2014; 17:76–100. https://doi.org/10.1016/j.mito.2014.05.007 PMID: 24929216.
- Barnett A. Using recovery modalities between training sessions in elite athletes: does it help? Sports Med. 2006; 36(9):781–96. Epub 2006/08/30. PMID: 16937953.
- van Hall G. Lactate kinetics in human tissues at rest and during exercise. Acta physiologica (Oxford, England). 2010; 199(4):499–508. https://doi.org/10.1111/j.1748-1716.2010.02122.x PMID: 20345411.
- Versey NG, Halson SL, Dawson BT. Water Immersion Recovery for Athletes: Effect on Exercise Performance and Practical Recommendations. Sports Med. 2013; 43(11):1101–30. https://doi.org/10.1007/s40279-013-0063-8 PMID: 23743793
- Wilcock IM, Cronin JB, Hing WA. Physiological response to water immersion: a method for sport recovery? Sports Med. 2006; 36(9):747–65. PMID: 16937951.
- Di Masi F, De Souza Vale RG, Dantas EHM, Barreto AC, da Silva Novaes J, Reis VM. Is blood lactate removal during water immersed cycling faster than during cycling on land? Journal of sports science & medicine. 2007; 6(2):188–92. PMID: 24149328; PubMed Central PMCID: PMC3786239.
- Ferreira JD, Carvalho RGD, Barroso TM, Szmuchrowski LA, Śledziewski D. Effect of different types of recovery on blood lactate removal after maximum exercise. Pol J Sport Tourism. 2011; 18:105–11.
- 11. Barbosa TM, Garrido MF, Bragada J. Physiological adaptations to head-out aquatic exercises with different levels of body immersion. Journal of strength and conditioning research / National Strength & Conditioning Association. 2007; 21(4):1255–9. https://doi.org/10.1519/R-20896.1 PMID: 18076241.
- Benelli P, Colasanti F, Ditroilo M, Cuesta-Vargas A, Gatta G, Giacomini F, et al. Physiological and biomechanical responses to walking underwater on a non-motorised treadmill: effects of different exercise intensities and depths in middle-aged healthy women. Journal of sports sciences. 2014; 32(3):268–77. https://doi.org/10.1080/02640414.2013.824601 PMID: 24015764.
- Benelli P, Ditroilo M, De Vito G. Physiological responses to fitness activities: a comparison between land-based and water aerobics exercise. Journal of strength and conditioning research / National Strength & Conditioning Association. 2004; 18(4):719–22. https://doi.org/10.1519/14703.1 PMID: 15574073.
- Edwards LM, Jobson SA, George SR, Day SH, Nevill AM. Whole-body efficiency is negatively correlated with minimum torque per duty cycle in trained cyclists. Journal of sports sciences. 2009; 27 (4):319–25. Epub 2009/01/22. https://doi.org/10.1080/02640410802526916 PMID: 19156562.
- **15.** Bar-Or O. The Wingate anaerobic test. An update on methodology, reliability and validity. Sports Med. 1987; 4(6):381–94. PMID: 3324256.
- Meyer T, Gabriel HH, Kindermann W. Is determination of exercise intensities as percentages of VO2max or HRmax adequate? Medicine and science in sports and exercise. 1999; 31(9):1342–5. PMID: 10487378.
- Devlin J, Paton B, Poole L, Sun W, Ferguson C, Wilson J, et al. Blood lactate clearance after maximal exercise depends on active recovery intensity. The Journal of sports medicine and physical fitness. 2014; 54(3):271–8. PMID: 24739289.
- Menzies P, Menzies C, McIntyre L, Paterson P, Wilson J, Kemi OJ. Blood lactate clearance during active recovery after an intense running bout depends on the intensity of the active recovery. Journal of sports sciences. 2010; 28(9):975–82. Epub 2010/06/15. https://doi.org/10.1080/02640414.2010. 481721 PMID: 20544484.
- Baldari C, Videira M, Madeira F, Sergio J, Guidetti L. Blood lactate removal during recovery at various intensities below the individual anaerobic threshold in triathletes. The Journal of sports medicine and physical fitness. 2005; 45(4):460–6. Epub 2006/02/01. PMID: 16446676.
- **20.** Belcastro AN, Bonen A. Lactic acid removal rates during controlled and uncontrolled recovery exercise. Journal of applied physiology. 1975; 39(6):932–6. Epub 1975/12/01. PMID: 765313.
- Boileau RA, Misner JE, Dykstra GL, Spitzer TA. Blood lactic acid removal during treadmill and bicycle exercise at various intensities. The Journal of sports medicine and physical fitness. 1983; 23(2):159–67. Epub 1983/06/01. PMID: 6632854.
- Millet GP, Vleck VE, Bentley DJ. Physiological differences between cycling and running. Lessons from triathletes. Sports Med. 2009; 39(3):179–206. ISI:000265002300002. https://doi.org/10.2165/ 00007256-200939030-00002 PMID: 19290675
- Monedero J, Donne B. Effect of recovery interventions on lactate removal and subsequent performance. International journal of sports medicine. 2000; 21(8):593–7. Epub 2001/01/13. https://doi.org/10.1055/s-2000-8488 PMID: 11156281.
- 24. Davidson LE, Wang J, Thornton JC, Kaleem Z, Silva-Palacios F, Pierson RN, et al. Predicting fat percent by skinfolds in racial groups: Durnin and Womersley revisited. Medicine and science in sports and



- exercise. 2011; 43(3):542–9. Epub 2010/08/07. https://doi.org/10.1249/MSS.0b013e3181ef3f07 PMID: 20689462; PubMed Central PMCID: PMC3308342.
- 25. Durnin JV, Womersley J. Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. The British journal of nutrition. 1974; 32(1):77–97. Epub 1974/07/01. PMID: 4843734.
- 26. Winter EM, Jones AM, Davison RC, Bromley PD, Mercer TH, British Association of Sport and Exercise Sciences, editors. Sport and exercise physiology testing guidelines. The British Association of Sport and Exercise Sciences guide. 1st ed. New York, NY: Routledge; 2007.
- 27. Hoefelmann CP, Diefenthaeler F, Costa VP, de Lucas RD, Shambrook P, Guglielmo LG. Test-retest reliability of second lactate turnpoint using two different criteria in competitive cyclists. Eur J Sport Sci. 2015; 15(4):265–70. https://doi.org/10.1080/17461391.2014.944874 PMID: 25135192.
- Malek MH, Housh TJ, Berger DE, Coburn JW, Beck TW. A new non-exercise-based Vo2max prediction equation for aerobically trained men. Journal of strength and conditioning research / National Strength & Conditioning Association. 2005; 19(3):559–65. https://doi.org/10.1519/1533-4287(2005)19[559: ANNOPEI2.0.CO:2 PMID: 16095416.
- Hawley JA, Noakes TD. Peak power output predicts maximal oxygen uptake and performance time in trained cyclists. European journal of applied physiology and occupational physiology. 1992; 65(1):79– 83. Epub 1992/01/01. PMID: 1505544.
- Robergs RA, Dwyer D, Astorino T. Recommendations for improved data processing from expired gas analysis indirect calorimetry. Sports Med. 2010; 40(2):95–111. https://doi.org/10.2165/11319670-00000000-00000 PMID: 20092364.
- Bentley DJ, Newell J, Bishop D. Incremental exercise test design and analysis: implications for performance diagnostics in endurance athletes. Sports Med. 2007; 37(7):575–86. Epub 2007/06/28. PMID: 17595153.
- Newell J, Higgins D, Madden N, Cruickshank J, Einbeck J, McMillan K, et al. Software for calculating blood lactate endurance markers. Journal of sports sciences. 2007; 25(12):1403–9. https://doi.org/10. 1080/02640410601128922 PMID: 17786693.
- 33. Beneke R, Jumah MD, Leithäuser RM. Modelling the lactate response to short-term all out exercise. Dynamic medicine. 2007; 6(10):1–7. https://doi.org/10.1186/1476-5918-6-10 PMID: 17996077
- **34.** Park KS, Choi JK, Park YS. Cardiovascular regulation during water immersion. Applied human science: journal of physiological anthropology. 1999; 18(6):233–41. PMID: 10675972.
- 35. Christie JL, Sheldahl LM, Tristani FE, Wann LS, Sagar KB, Levandoski SG, et al. Cardiovascular regulation during head-out water immersion exercise. Journal of applied physiology. 1990; 69(2):657–64. PMID: 2228879.
- Connelly TP, Sheldahl LM, Tristani FE, Levandoski SG, Kalkhoff RK, Hoffman MD, et al. Effect of increased central blood volume with water immersion on plasma catecholamines during exercise. Journal of applied physiology. 1990; 69(2):651–6. PMID: 2228878.
- Weinstein Y, Bediz C, Dotan R, Falk B. Reliability of peak-lactate, heart rate, and plasma volume following the Wingate test. Medicine and science in sports and exercise. 1998; 30(9):1456–60. PMID: 9741617.