## Research

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# $\mathrm{VO}_{2}$ Kinetics during Different Forms of Cycling Exercise on Land and in Water 

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#### Abstract

Background: It is generally assumed that the physical properties of water improve aerobic metabolism by $\mathrm{O}_{2}$ utilization of the working muscle particular if a great muscle mass is recruited. This study investigated the changes in $\mathrm{VO}_{2}\left(\mathrm{VO}_{2}\right.$-work rate relationship; $\left.{ }_{\Delta} \mathrm{VO}_{2} /{ }_{\Delta} \mathrm{WR}\right)$ during increasing work rates in different exercise conditions in water immersed exercise and on land based exercise. Methods: In order to identify possible differences in $\mathrm{VO}_{2}$ required for a given work rate twelve trained cyclists performed four incremental exercise tests. The tests comprised whole body work and leg work, both in water and on land and were conducted on the same, electromagnetically braked whole body ergometer. Results: The ${ }_{\Delta} \mathrm{VO}_{2} /{ }_{\Delta}$ WR curves were found to be similar in the four exercise conditions reaching from 11.9 to $12.4 \mathrm{ml} \cdot \mathrm{W}^{-1}$ during water immersed exercise and 12.6 to $12.7 \mathrm{ml} \cdot \mathrm{W}^{-1}$ during land based exercise respectively. When coupling arms with leg exercise the ${ }_{\Delta} \mathrm{VO}_{2} /{ }_{\Delta} \mathrm{WR}$ curves shift upwards at similar work rates indicating a higher oxygen demand for an enlarged muscle mass. The extra $\mathrm{O}_{2} \operatorname{cost}\left(\mathrm{VO}_{2}\right)$ for recruited arms was lower in water immersed exercise compared to land based exercise $\left(0.057 \pm 0.0721 \cdot \mathrm{~min}^{-1}\right.$ and $0.3671 \pm 0.057 \cdot \mathrm{~min}^{-1}$, respectively; $\mathrm{p}=.000$ ). Differences exist in the rate of performing physical work above ventilatory threshold two. Work load values attained on land based exercise surpass that of water immersed exercise (204.2 watts vs. 154.0 watts for whole body work and 227.1 watts $v s .150 .0$ watts for leg work, respectively). Conclusions: Differences in ${ }_{\Delta} \mathrm{VO}_{2}$ at a given work rate are to be explained rather from a biomechanical point of view. More likely ${ }_{\Delta} \mathrm{VO}_{2}$ in water seems to be influenced by both familiarity of the task and fitness level. Exercise intensity in water need to be selected at lower levels than on land.


KEYWORDS: Whole body ergometry; $\mathrm{VO}_{2}$ kinetics; Aquatic exercise.
ABBREVIATIONS: W: Water; L: Land; NIRS: Near-infrared spectroscopy.

## INTRODUCTION

When coupling arms with leg exercise a higher oxygen demand at the same work rates is required compared with legs only. ${ }^{1-5}$ In these experimental studies on Land (L) an enlarged muscle mass requires more oxygen, ranging from 0.04 til $0.361 \cdot \mathrm{~min}^{-1}$ at equal submaximal stages. The workload- $\mathrm{VO}_{2}$ relationship curve is shifted upwards at the same work rates indicating a higher oxygen demand for whole body work than work with leg only.

Based on several studies, ${ }^{2,4,6,7}$ adding arm work to ongoing leg work leads to a higher $\mathrm{VO}_{2}$ max, ranging from 0.04 to $0.231 \cdot \mathrm{~min}^{-1}$. However, other researchers did not find significant deviations, measuring increases in $\mathrm{VO}_{2} \max$ of 0.06 to $0.121 \cdot \mathrm{~min}^{-1} .3,8-11$

It appears that oxygen transport to the exercising muscles of the whole body is mark-
edly elevated when referring to submaximum exercise stages. $\mathrm{O}_{2}$ conductance depends on muscle mass and extraction capacity can be explained by vascular muscle bed. ${ }^{12}$ In general oxygen consumption of vascular beds between the patterns of exercise is $0.2 \mathrm{l} / \mathrm{min}$ higher in whole body work. ${ }^{13}$

A slightly higher $\mathrm{VO}_{2} \max$ is expected, if a large part of active skeletal muscles is involved at relatively high work rates close to the point of fatigue. ${ }^{13}$ Adding arms to ongoing leg work increases mean arterial pressure through adrenergic vasoconstricting signals and is accompanied by a reduced cardiac output. ${ }^{14}$ These restrictions in muscle blood flow limit the ability of the exercising muscle to extract the required $\mathrm{O}_{2}$ and can be explained by the Fick relationship. The Fick equation states that $\mathrm{VO}_{2}$ equals cardiac output times.

To date no scientific basis is provided for change in the dynamics of $\mathrm{VO}_{2}$ for this setting in Water (W) immersed, in particular there are uncertainties with regard to the additional $\mathrm{O}_{2}$-cost for whole body work. The physical properties of water change hemodynamic and metabolic responses: stroke volume increases by $30-50 \%$ and cardiac output at a given work load also increases about $25 \%$ through the Frank Starling mechanism. ${ }^{15,16}$ From increased cardiac output as a result of increased cardiac filling pressure and lowered total peripheral resistance ${ }^{17}$ it can be assumed that $\mathrm{O}_{2}$ extraction in working muscles is more efficient in W than on L. However at present the effect is discussed controversial. Blood flow to oxidative muscles can also be affected if muscle pump generates a greater pressure gradient across the capillary bed and increases blood flow. ${ }^{18}$ MacDonald, et al. ${ }^{18}$ observed a slower response of both $\mathrm{VO}_{2}$ and leg blood flow compared to the same work stages in supine position ergometry (which is similar to water immersed exercise) compared to an upright position during leg exercise at light to moderate intensity.

Hence in this study oxygen uptake and delivery to active muscle mass in different exercise conditions were determined. Rates of gas exchange reflect the relationship between $\mathrm{O}_{2}$ delivery and $\mathrm{O}_{2}$ uptake. They allow for an indirect assessment of the relationship of muscle blood flow and muscle oxygen uptake. ${ }^{19}$ The present study tested the hypothesis, that coupling arm with leg exercise would increase metabolic load and the extra $\mathrm{O}_{2}$-cost $\left({ }_{\Delta} \mathrm{VO}_{2}\right)$ in W would be similar to L ; furthermore hemodynamic changes in W would improve $\mathrm{O}_{2}$-extraction capacity. This is the case if the onset of anaerobiosis - determined by the gas exchange method (excess $\mathrm{CO}_{2}$ above ventilatory threshold two; VT2) - for the particular workload occurs at a higher $\mathrm{VO}_{2}$. The gas exchange measurements are relevant regarding energy generated from aerobic and anaerobic sources and energy metabolism. Aquatic exercise is a common and alternative method to land based exercise. Persons suffering from dysfunctions of the skeletal mucles and/or obesity will benefit from a change in the dynamics of $\mathrm{VO}_{2}$ depending on a beneficial distribution of the blood flow in the muscles.

## METHODS

## Participants

Twelve trained male volunteers (age $35.1 \pm 5.4$ years; body weight $79.4 \pm 11.4 \mathrm{~kg} ; \mathrm{VO}_{2}$ peak $3.89 \pm 0.651 \cdot \mathrm{~min}^{-1}$ ) gave written and informed consent for participation. The study protocol was approved by the regional Ethics Committee (EKLU 11007).

## Study Protocol

All subjects performed whole body work and leg work both in W and on L using the same whole body ergometer. This newly developed device allows for power output measurements as well as pedal arm forces (Reha-Aquabike, Swissrehamed, Chur, Switzerland, Figure 1). Prior to this study the new ergometer was validated for cardiopulmonary stress tests by determining its accuracy in W and on L . Before testing each subject underwent one practice session comprising both exercise patterns. The tests were conducted in a thermoneutral laboratory in W (water temperature $27-28^{\circ} \mathrm{C}$ ) and on L (air temperature 22-24 ${ }^{\circ} \mathrm{C}$ ) at intervals $\geq 48 \mathrm{~h}$ and within 14 days. Work was varied by two minute adjustments in the workload (WHO-protocol: increment 25 watts, starting with 50 watts, ${ }^{20}$ constant pedalling frequency at 70 revolutions per minute). The relative contribution of arm work to total work was set at $20 \%$ to strain the cardiovascular system close to maximum. ${ }^{5}$ In order to avoid bias, a randomised cross over design study was conducted. All subjects were randomly allocated into two groups. Order of testing was assigned first in W - whole body and leg work - then on L. The other group started in reversed order.


Figure 1: Photograph of the electromagnetically braked, whole body "rehaaquabike", measuring power output.

The backrest of the recumbent cycle ergometer was inclined at an angle of $110^{\circ}$, the height adjustable seat ensured immersion to the xiphoid process. The participants were tested after a 3 h abstinence from food following a standardized meal and refrained from any physical exercise on the day before testing. All subjects abstained from taking any medication. Heart rate and electrocardiographic activity were continuously monitored using Medilog Darwin Holter System AR12 Plus; Schiller, Dietikon, Switzerland. Prior to the tests the gas analyzer for $\mathrm{VO}_{2}, \mathrm{VCO}_{2}$ and ventilatory parameters (K4b2, Cosmed, Rome, Italy) was calibrated due to Wasserman et al. ${ }^{21}$ and pulmonary gas exchange and expired ventilation were measured breath by

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breath throughout the test. Values for oxygen consumption were referred to a given workload during the last 30 s and maximum capacity during the last 30 s with constant pedalling frequency. ${ }^{22}$ To determine the onset of metabolic acidosis the $\mathrm{CO}_{2}$-excess associated with hyperventilation was assessed (alveolar ventilatory threshold two; VT2) according to Wasserman et al. ${ }^{21}$

## Statistical Analysis

To calculate the maximum sample size we examined the differences between whole body and leg work exercise in terms of $\mathrm{VO}_{2} \mathrm{max}$. Gutin et al. ${ }^{3}$ gave examples, which differ by an effect size of about $\delta=91, \alpha=0.05$, power $\beta=0.8$ for a onetailed test. Comparing it to the effect sizes which are common and using GPower 3.1. - Cohen describes an effect size of 0.8 as large - equating a sample size of twelve subjects.

Data are normally distributed and variances are equal (Shapiro-Wilk test). Differences in individual $\mathrm{VO}_{2}$ at VT2 and peak power between trials ( $\mathrm{p} \leq 0.05$ ) were analysed using oneway analysis of variance (ANOVA) with Tukey`s post-hoc test (SPSS 17.0; SPSS Inc, Chicago, IL, USA) to discern differences between groups. Values are expressed as means $\pm$ SE.

## RESULTS

Twelve subjects were available for all of the four consecutive examinations. The contribution to the total power output from both arms showed no differences ( $\mathrm{p}>0.05$ ). In four tests $\mathrm{VO}_{2}$ increased linearly ( $\mathrm{r}>0.90 ; \mathrm{p}>0.090$ ) with increasing work rate up to the ventilatory threshold two (VT2) thus providing
proof that the ergometer was accurately calibrated.
The increase in work rate related to the increase in $\mathrm{VO}_{2}$ $\left({ }_{\Delta} \mathrm{VO}_{2} / \Delta \mathrm{WR}\right)$ was similar ( $\mathrm{p}>0.05$ ) in the four exercise conditions and reached values from $11.9-12.4 \mathrm{ml} \cdot \mathrm{min} \cdot \mathrm{W}^{-1}$ during water immersed exercise and $12.6-12.7 \mathrm{ml} \cdot \mathrm{min} \cdot \mathrm{W}^{-1}$ during land based exercise (Table 1).

During whole body work a greater amount of oxygen in terms of $\mathrm{VO}_{2}\left(\mathrm{ml} \cdot \mathrm{min}^{-1}\right)$ was used at a given work load compared to leg work only. However, $\mathrm{VO}_{2}$ responses were not significantly different ( $\mathrm{p}>0.05$ ). $\mathrm{VO}_{2}$ curves were shifted upwards linearly for whole body work (Figure 2), both in W and for L each at similar power output levels during incremental work stages.


Figure 2: $\mathrm{O}_{2}$-uptake at increasing work load in 4 replicated tests.

|  |  | L_leg | L_whole | W_leg | W_whole | p |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| ${ }_{\Delta} \mathrm{VO}_{2} /{ }_{\Delta} \mathrm{WR}$ | M | 12.7 | 12.6 | 12.4 | 11.9 | $\geq 0.582$ |
| $\left(\mathrm{ml} \cdot \mathrm{min}^{-1} \cdot\right.$ watts $\left.^{-1}\right)$ | SE | 1.2 | 1.2 | 1.7 | 1.9 |  |
| $\mathrm{VO}_{2}$ peak | M | 3890.5 | 3884.6 | 3639.2 | 3728.7 | $\geq 0.669$ |
| $\left(\mathrm{ml} \cdot \mathrm{min}^{-1}\right)$ | SE | 649.8 | 613.2 | 601.0 | 485.5 |  |
| load_max | M | 268.8^ | 254.7 ${ }^{\text {\# }}$ | 196.3 | 195.1 | $=0.000 / 0.002$ |
| (watts) | SE | 36.1 | 38.6 | 37.8 | 34.7 |  |
| HR_peak | M | 175.9 | 176.1 | 170.2 | 173.5 | $\geq 0.665$ |
| (b. $\mathrm{min}^{-1}$ ) | SE | 12.3 | 12.6 | 13.1 | 12.4 |  |
| RER_peak | M | 1.02" | 1.03 | 1.12 | 1.09 |  |
|  | SE | 0.07 | 0.10 | 0.11 | 0.07 |  |
| $\mathbf{V O} \mathbf{2}^{-} \mathbf{V T 2}$ | M | 3199 | 3290 | 3086 | 3128 | $\geq 0.704$ |
| $\left(\mathrm{ml} \cdot \mathrm{min}^{-1}\right)$ | SE | 385 | 475 | 435 | 426 |  |
| load_VT2 | M | 227.1^ | 204.2* | 150.0 | 154.0 | $=0.000$ |
| (watts) | SE | 32.0 | 29.8 | 13.4 | 11.8 |  |
| VO ${ }_{2}$ VT2/load | M | $14.1^{\wedge}$ | $16.1^{*}$ | 20.6 | 20.3 | $=0.000$ |
| $\left(\mathrm{ml} \cdot\right.$ watts $^{-1}$ ) | SE | 0.8 | 1.6 | 3.0 | 3.2 |  |
| HR_VT2 | M | 161.0 | 159.0 | 150.1 | 152.8 | $\geq 0.096$ |
| (b. $\mathrm{min}^{-1}$ ) | SE | 11.3 | 12.1 | 11.3 | 10.7 |  |
| RER_VT2 | M | 0.94 | 0.97 | 1.01 | 1.00 |  |
|  | SE | 0.07 | 0.09 | 0.11 | 0.05 |  |

Table 1: Values are means (SD). ${ }^{\wedge} p=0.000$ compared with $W$ _leg; ${ }^{*} p=0.000$ compared with $W$ _whole. " $p=0.002$ compared with $W$ _ whole.

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Comparison of $\mathrm{O}_{2}$-uptake at increasing work load (regression lines) in combined arm with whole (com) and leg ergometry (leg) in Water environment (W) and on Land environment (L). The $\mathrm{VO}_{2}$ kinetic of all curves shows linearity over load stages but work in W displaces the curves upward from water resistance. Power output (workload in watts) displays external load on pedalling system but does not calculate demanded aerobic power from water resistance. $\mathrm{VO}_{2}$-work rate relation from whole curves parallels that of leg and is displaced upward. The additional aerobic demand for recruited arms is represented at defined power output by the difference of the slopes from combined and leg- $\mathrm{VO}_{2}$. Filled circles show the corresponding values at ventilatory threshold two.

The extra $\mathrm{O}_{2}$ cost for recruited arms $\left({ }_{\Delta} \mathrm{VO}_{2}\right)$ was lower during water immersed exercise compared to land based exercise $\left(0.057 \pm 0.0721 \cdot \mathrm{~min}^{-1}\right.$ and $0.3671 \pm 0.057 \cdot \mathrm{~min}^{-1}$, respectively; $\mathrm{p}=.000$ ).

The magnitude of $\mathrm{O}_{2}$ changes in W compared to L at work loads for the two work patterns influences the responses at the anaerobic threshold and at maximum effort: if exercise capacity is expressed as work load reduced work capacities at VT2 occur in W (leg work: 227.1 watts vs. 150.0 watts; whole body work: 204.2 watts $v s .154 .0$ watts) (Figure 2).

No statistically significant differences at VT2 were found when exercise patterns were matched for their $\mathrm{VO}_{2}$ response. Moreover, no statistical significance could be reached for the relationship of $\mathrm{O}_{2}$ requirements to maximum $\mathrm{VO}_{2}$.

## DISCUSSION

This study investigated oxygen uptake $\left(\mathrm{VO}_{2}\right)$ during four cycling exercises in W/L both for whole body and leg work.

During increasing work load the linearity of the $\mathrm{VO}_{2}$ work relationships $\left({ }_{\Delta} \mathrm{VO}_{2} /{ }_{\Delta} \mathrm{WR}\right)$ were found to be nearly the same in the four exercise patterns.
$\mathrm{VO}_{2}$ responses tend to result in higher values at a given work load during whole body work compared to leg work, both indicating additional oxygen consumption $\left({ }_{\Delta} \mathrm{VO}_{2}\right)$ in both W and L .

The observed shift of the $\mathrm{VO}_{2}$ curves show that the extra cost for recruited arms $\left({ }_{\Delta} \mathrm{VO}_{2}\right)$ during land based exercise is more pronounced than that of water immersed exercise.

Differences at VT2 and related to maximum effort between land based exercise and water immersed exercise occur when exercise intensity is expressed in work load (watts). Leg work levels at VT2 attained 227.1 watts on L vs. 150.0 watts in W. Whole body work levels reached 204.2 watts on L vs. 154.0 watts in W.

However, $\mathrm{VO}_{2}$ levels were unchanged when anaerobic
threshold determined by the gas exchange method was related to their $\mathrm{VO}_{2}$ responses.

The extent to which ${ }_{\Delta} \mathrm{VO}_{2}$ increases is not only attributed to usage of muscle mass. Research has confirmed that $\mathrm{O}_{2}$-cost during arm work and whole body work is related to higher percentage of type-II-fibers in arm muscles ${ }^{23,24}$ and that the amount of external work is greater. ${ }^{19}$ Van Hall, et al. ${ }^{25}$ and Jensen-Urstad, et al. ${ }^{26}$ provided evidence that during whole body work more carbohydrates are utilized and more lactate is released than during leg. Moreover, extra oxygen is needed to overcome the forces of gravity. This is the case, when the pedaling axis during arm cranking is elevated above the horizontal position ${ }^{27}$ or if the distance to arm crank axis is different. ${ }^{28}$ Bergh, et al. ${ }^{7}$ and Billat, et al. ${ }^{11}$ linked a portion of the extra $\mathrm{O}_{2}$-cost to posture and body position during whole body work.

The remarkable finding in the present study was that ${ }_{\Delta} \mathrm{VO}_{2}$ responses for recruited arms to a given workload is lower for W than for L . The following controversial issues are to consider:

- In W the oxygen demand of the working muscles is met more efficiently than on L due to improved hemodynamics in W.
- Biomechanical properties define the relation of $\mathrm{VO}_{2}$ uptake and power output.

Studies reporting elevated oxygen uptake on L adress the main determinants: stroke volume and Mean Arterial Pressure (MAP). The latter is considered as a balance between local vasodilation and general sympathetic activity. ${ }^{13}$ It is known that sympathetic activity to the vessels (vasoconstrictor signal) is opposed by baroreflex through increased blood volume and by vasoactive metabolites. ${ }^{29}$ The feedback signal from the local tissue milieu regulates the demand of the muscles by adjusting blood pressure precisely. ${ }^{30}$

Studies which adress arm contribution give evidence that sympathetic nerve activity regulates blood pressure at the expense of flow. ${ }^{13,14}$ When adding arm exercise to on-going leg exercise the Cardiac Output (CO) level can be restricted. MAP regulated by peripheral vasoconstriction can be a disadvantage with regard to muscle blood flow and oxygenation. If intense arm work is associated with a large MAP response arm vascular conductance and blood flow in working legs is reduced by $10 \% .{ }^{6}$ Vice versa when leg work is added to ongoing arm exercise vascular conductance and arm muscle oxygenation in the upper extremities decreases by $5 \% .{ }^{31}$ Such reductions in regional blood flow are mainly attributed to peripheral vasoconstriction to support the prevailing blood pressure. ${ }^{13}$

In W the local vasodilatation and general sympathetic activity seem to be proficiently balanced: from cardiac filling pressure through central blood shift ANP (Atrial Natriuretic Peptide) concentrations are elevated up to 2-3-fold. ${ }^{32}$ The well-
characterized ANP pathway regulates vascular tone - which is under sympathetic nervous system control - and renal sodium handling. ANP acts as a vasodilator via endothelial cells and promotes baroreflex-mediated activation. The pronounced sympatholytic effect of ANP leads to a reduced vasoconstriction in W compared to L. ${ }^{17}$ The sympathicolysis corresponds well with lowered plasma noradrenalin concentrations. ${ }^{33,34}$ Furthermore, larger ANP blood concentrations constrain the RAA-system by reducing the release of renine and aldosterone. The hor-mone-driven actions modify the fluid resistance within vessels thereby improving blood perfusion and $\mathrm{O}_{2}$-extraction. Data derived from animal models also provided evidence of improved blood perfusion in regional vessels of the musculature. ${ }^{35,36}$

In this paper the hypothesis was tested that, depending on $\mathrm{O}_{2}$ flow in the working muscle, $\mathrm{O}_{2}$ supply will meet the $\mathrm{O}_{2}$ demand of the working muscle more efficiently. The metabolic acidosis in a graded exercise test would occur later reflecting a proficient availability of $\mathrm{O}_{2}$ for the muscles. Therefore $\mathrm{VO}_{2}$ rate at VT2 and maximum effort were monitored. In fact $\mathrm{VO}_{2}$ levels showed equal proportions of aerobic and anaerobic potential in all cycling exercises. Thus the hypothesis is to reject that the metabolic conditions of cycling exercises in water are advantageous. It can therefore be assumed that $\mathrm{O}_{2}$ extractions are similar both on L and in W. Probably the contribution of the processor reflex provides different but adequate mechanisms for the interdependent regulation of the cardiac output and the perfusion of the working muscle.

Differences in ${ }_{\Delta} \mathrm{VO}_{2}$ can be rather explained from a biomechanical point of view, the transfer of metabolic energy into physical work. The total power output depends on the surface resistivity of air or water. Due to buoyancy arm power is better preserved than that of the leg because of the contractile properties and the content of myosin of the arm muscles. ${ }^{37}$ Last but not least the extra $\mathrm{O}_{2}$-cost can be explained by the isometric exercise component required for the stabilization of the exercising body.

The ${ }_{\Delta} \mathrm{VO}_{2}$ values can be explained with the much higher relative percentage of $\mathrm{VO}_{2}$ max in W reached by well-trained. At higher work load the ${ }_{\Delta} \mathrm{VO}_{2}$ are a result of the participant's familiarisation with whole body exercise. ${ }^{38}$ Recommendations regarding performance improvements normally include information on appropriate training load. If the maximum workload reached on L is transferred to exercise in W , it is to consider that the resistance that water provides acts on moving limbs and require adaptations for safe working especially for those with medical conditions. Based on the present study aquatic exercise shall be performed with reduced mechanical workloads if the oxygen uptake in water immersed exercise is to be the same as on land based exercise.

For years, the scientific community has been responding to the lively question of oxygen delivery to superimposing arm exercise with leg exercise. ${ }^{39}$ We addressed the metabolic responses of added arm work to ongoing leg work in W in particu-
lar. We only measured respiratory responses but did not assess the distribution of the regional blood flow in arms and legs. The use of Near-infrared spectroscopy (NIRS) would give insight into blood flow redistribution.

## CONCLUSION

This study illustrates the importance of the selection of the correct exercise intensity in W . The application of exercise intensities assessed on L leads to an overload in W. Our results suggest a reduced workload in W of 50.2 watts for whole body work and by 77.1 watts for leg work. This estimate applies to 70 revolutions per minute in water cycling.

Predicting $\mathrm{VO}_{2}$ based on work load can lead to an overestimation of energy expenditure, if threshold patterns are not taken into account. Thus, the oxygen requirements of whole body work in W at a high intensity steady state will not exceed those of leg only. Furthermore the influence of W on $\mathrm{VO}_{2}$ supply to the exercising muscles is only marginal. The cardiovascular system seems to regulate its $\mathrm{O}_{2}$-supply via modulations of vascular conductance by MAP and a differential contribution of cardiac output similar to exercise on $L$.

## CONFLICTS OF INTEREST

The authors declare that the main outcome obtained for the regulation of the intensity in water is not influenced by competing interests.

## AUTHOR'S CONTRIBUTIONS

BV, JG designed the study, MF and JG participated in the data collection. MF, KK and CS drafted the manuscript. CS, BV and JG gave critical comments on the manuscript. All authors checked and approved the final version before submission.

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