Ischemic Preconditioning of One Forearm Enhances Static and Dynamic Apnea

THOMAS KJELD, MADS REINHOLDT RASMUSSEN, TIMO JATTU, HENNING BAY NIELSEN, and NIELS HENRY SECHER

The Copenhagen Muscle Research Center, Department of Anesthesia, Rigshospitalet, University of Copenhagen, Denmark

ABSTRACT

KJELD, T., M. R. RASMUSSEN, T. JATTU, H. B. NIELSEN, and N. H. SECHER. Ischemic Preconditioning of One Forearm Enhances Static and Dynamic Apnea. *Med. Sci. Sports Exerc.*, Vol. 46, No. 1, pp. 151–155, 2014. **Introduction**: Ischemic preconditioning enhances ergometer cycling and swimming performance. We evaluated whether ischemic preconditioning of one forearm (four times for 5 min) also affects static breath hold and underwater swimming, whereas the effect of similar preconditioning on ergometer rowing served as control because the warm-up for rowing regularly encompasses intense exercise and therefore reduced muscle oxygenation. **Methods**: Six divers performed a dry static breath hold, 11 divers swam underwater in an indoor pool, and 14 oarsmen rowed "1000 m" on an ergometer. **Results**: Ischemic preconditioning reduced the forearm oxygen saturation from 65% ± 7% to 19% ± 7% (mean ± SD; $P < 0.001$), determined using spatially resolved near-infrared spectroscopy. During the breath hold (315 s, range = 280–375 s), forearm oxygenation decreased to 29% ± 10%; and in preparation for rowing, right thigh oxygenation decreased from 66% ± 7% to 33% ± 14% ($P < 0.05$). Ischemic preconditioning prolonged the breath hold from 279 ± 72 to 327 ± 39 s, and the underwater swimming distance from 110 ± 16 to 119 ± 14 m ($P < 0.05$) and also the rowing time was reduced (from 186.5 ± 3.6 to 185.7 ± 3.6 s; $P < 0.05$). **Conclusions**: We conclude that while the effect of ischemic preconditioning (of one forearm) on ergometer rowing was minimal, probably because of reduced muscle oxygenation during the warm-up, ischemic preconditioning does enhance both static and dynamic apnea, supporting that muscle ischemia is an important preparation for physical activity. **Key Words:** ISCHEMIA, NEAR INFRARED SPECTROSCOPY, WARM-UP PROCEDURES, ROWING, DIVING, HEART RATE, BLOOD PRESSURE, MUSCLE OXYGENATION

Work capacity is enhanced by warm-up activities for several reasons (16). Exercise elevates body temperature in proportion to its intensity, and most biochemical processes are exposed to a Q_{10} effect expressing that its rate is doubled (within limits) for a 10°C increase (27). Furthermore, elevated body temperature affects the oxyhemoglobin dissociation curve to improve oxygen delivery to the tissue (1). Even passive warming affects performance (3), but the effect of previous physical activity is more complex than its effect on body temperature. Warm-up improves joint flexibility and prepares the athlete for the task required. Also, warm-up activities regularly involve repeated short bouts of high-intensity exercise that not only implicates body temperature but also reduces muscle oxygenation (9,32).

We considered that muscle ischemia *per se* influences work capacity. Ischemic preconditioning enhances ergometer cycling (11) and swimming (21) and may be even more important for underwater swimming and static breath hold that are not preceded by procedures that would reduce muscle oxygenation. On the contrary, divers prepare by hyperventilation and "glossopharyngeal insufflation" (37) that both aim to increase tissue oxygenation. The hypothesis of this study was that ischemic preconditioning would enhance both static and dynamic apneas. On the other hand, (ergometer) rowing is preceded by 15–30 min warm-up on the ergometer (or in the boat), likely including intense exercise and therefore desaturation of the muscles. Accordingly, we considered that ischemic preconditioning would have little, if any, impact on rowing.

METHODS

Twenty male and five female healthy nonsmoking subjects gave oral and written informed consent in participation in this study as approved by the Ethics Committee of the Copenhagen region (H-3-2012-058). Eleven subjects were divers (median age = 29 yr, range = 18–38 yr) and 14 were rowers (median age = 23 yr, range = 18–35 yr; Table 1). Five of the participating free divers ranked among the top 10 in the world, and one was an indoor free-diving world

---

Address for correspondence: Thomas Kjeld, M.D., Department of Anesthesia, Rigshospitalet 2041, Blegdamsvej 9, DK-2100, Copenhagen Ø, Denmark; E-mail: thomaskjeld@dadlnet.dk.
Submitted for publication October 2012.
Accepted for publication July 2013.

0195-9131/14/4601-0151/0
MEDICINE & SCIENCE IN SPORTS & EXERCISE®

DOI: 10.1249/MSS.0b013e3182a4090a

TABLE 1. Subject characteristics.

	Oarsmen	Divers
Subjects	14 (4 females)	11 (1 female)
Age (yr)	23 (18–35)	29 (18–38)
Height (cm)	184 (177–208)	186 (176–195)
Weight (kg)	79 (67–102)	82 (58–95)
Palpatory systolic blood pressure (mm Hg)	125 (112–149)	120 (105–135)

Values are presented as median (range).

champion. Five oarsmen ranked among the top 50 on the Concept2 World Record List, and eight were members of the national team (one was a world champion).

Protocol. The subjects were instructed to refrain from planned physical exercise, alcohol, and caffeine for 48 h before each day of the study. All participants prepared for the study using their usual practices, including hyperventilation and glossopharyngeal insufflation (37) for the swimmers and light to intense ergometer rowing for the oarsmen. Six divers carried out a static breath hold in a supine position and 11 divers (including those who had carried out the breath hold) swam as far as possible under water while they were video recorded and observed by a safety swimmer. The divers used the same swim or neoprene suit on the 2 d of the study, and six carried a 3.5-kg lead load to facilitate underwater swimming. For the five underwater rugby players, a snorkel was attached to their mask, but it was not used during the study, and all subjects were using their own fins. Six subjects preferred to start the swim in the water, while the others dived into the 26°C 50-m, 2-m-deep indoor pool.

A wind-braked ergometer (Concept2, Morrisville, VT) was used for a 1000-m all-out row that represents half of the distance used for competitions, and a short distance was chosen to approach the time used by the divers. Throughout the exercise, the rowers were encouraged to carry out a maximal effort. On a separate day, muscle and forehead oxygenation were assessed in six oarsmen during a 10-min warm-up row, including four sprints each lasting approximately 30 s.

The subjects were randomized to their usual preparation or, following these procedures, to be exposed to intermittent forearm ischemia through four cycles of 5-min inflation (to 40 mm Hg above palpatory systolic blood pressure) and 5-min deflation of a cuff (sphygmomanometer; Welch-Allyn Durashock, Skaneateles Falls, NY) on the nondominant arm (6). Activity started within 30 min of intermittent arm ischemia, and after at least 24 h of recovery, the protocol was repeated with the alternative procedure. However, for the static breath hold, the subjects' personal record served as control. After each trial, the subjects expressed their perceived exertion (5).

Instrumentation. On separate days, tissue oxygen saturation at rest, during muscle ischemia, during static breath hold, and during the warm-up for ergometer rowing was determined by spatially resolved near infrared spectroscopy (NIRS; Somanetics Corporation, Troy, MI) with the optodes covered to eliminate background light. The absorbance changes at two light wavelengths (730 and 810 nm) determined the ratio between oxyhemoglobin and total hemoglobin. The NIRS sensor has two light receivers placed 3 cm, respectively, 4 cm from the light-emitting diode with the signal weighted toward the "deep" tissue (30). NIRS provides a sensitive value for muscle (Sm_{O2}) (10) and cerebral oxygenation (Sc_{O2}) (29). To estimate whether NIRS would be able to reach targeted tissue, the skinfold over the muscle was measured (John Bull indicator; British Indicators Ltd., UK), and the value was divided by two.

For the static breath hold, the optodes were placed on the right vastus lateralis muscle, on the brachioradialis muscle of both arms and on the forehead to estimate Sm_{O2} and Sc_{O2} oxygenation, respectively; and for the oarsmen, they were placed over the right vastus lateralis muscle and on the forehead (29). To assess the hemodynamic response to ischemic preconditioning and static breath hold, a cuff for the determination of blood pressure (Finometer, FMS; Finapres Medical Systems BV, Amsterdam, the Netherlands) was placed on the middle part of third finger of the right hand kept at heart level and stroke volume and thus cardiac output were estimated by a nonlinear three component model of the arterial impedance (Modelflow) (4).

Variables are presented as mean ± SD and as median and range for the Borg scale. Wilcoxon signed test by rank was used to evaluate differences between trials involving ischemic

TABLE 2. Near infrared-determined frontal lobe (Sc_{O2}) and forearm muscle (Sm_{O2}) oxygenation (%) at rest (n = 13), during ischemia of one arm (n = 7), during reperfusion of the arm (n = 7), during a maximum breath hold (n = 7), and during a warm-up for rowing (n = 6).

		Rest	Muscle Ischemia/Warm-up	Reperfusion	Breath Hold after Muscle Ischemia	Breath Hold without Muscle Ischemia
Sc_{O2}	Divers	75 ± 9	79 ± 9	78 ± 9	42 ± 16*	58 ± 10
	Oarsmen	67 ± 5	52 ± 7*			
Sm_{O2} RF	Divers	65 ± 7	19 ± 7**	89 ± 4*	26 ± 10*	38 ± 10*
Sm_{O2} LF	Divers	71 ± 9	70 ± 10	73 ± 11	32 ± 10*	41 ± 8
Sm_{O2} AF	Divers	68 ± 8	—	—	29 ± 10*	49 ± 14
Sm_{O2} RT	Divers	80 ± 3	85 ± 6	84 ± 7	52 ± 20*	57 ± 19
	Oarsmen	66 ± 7	33 ± 14*			
S_{O2} AT	Divers	79 ± 5	63 ± 5**	81 ± 4**	38 ± 9**	45 ± 8**
	Oarsmen	67 ± 6	43 ± 10**			

Values are presented as mean ± SD.
*$P < 0.05$.
**$P < 0.001$.
RF, right forearm; LF, left forearm; AF, average both forearms; RT, right thigh; AT, average of extremities and forehead compared with rest.

TABLE 3. Hemodynamic variables at rest and during a maximal breath hold for six divers.

Hemodynamic Parameter	Rest	Breath hold
Heart rate (bpm)	64 ± 14	45 ± 14*
Mean arterial pressure (mm Hg)	84 ± 8	184 ± 12*
Stroke volume (mL per beat)	85 ± 11	41 ± 6*
Cardiac output (L·min^{-1})	5.6 ± 1.4	2.4 ± 0.8*
Systemic vascular resistance (dyn·[s·cm^{-5}]$^{-1}$)	1265 ± 295	6387 ± 2328*
Stroke volume index (mL per beat)	44 ± 4	21 ± 3*
Cardiac index (L·min^{-1}·m^{-2})	2.9 ± 0.73	1.3 ± 0.50*
Rate of change in arterial pressure, *dP/dt* (mm Hg·s^{-1})	538 ± 200	944 ± 212*

Values are presented as mean ± SD.
*$P < 0.05$.

precondition and the usual preparation for a maximal effort. A *P* value < 0.05 was considered statistically significant.

RESULTS

Ischemia of the arm reduced forearm Sm_{O2} from 65% ± 7% to 19% ± 7%; and during the pauses, forearm Sm_{O2} peaked at 89% ± 4% (Table 2; $P < 0.001$).

There were no significant changes in heart rate, blood pressure, or cardiac output during forearm ischemia.

During the static breath hold, the average Sm_{O2} for the forearms was reduced from 68% ± 8% to 29% ± 10%, thigh Sm_{O2} from 80% ± 3% to 52% ± 20%, and Sc_{O2} from 75% ± 9% to 42% ± 16% ($P < 0.001$; Table 2). Only the right forearm Sm_{O2} was reduced significantly (to 26% ± 10%) after a breath hold preceded by muscle ischemia compared with a breath hold not preceded by muscle ischemia (38% ± 10%; $P < 0.05$). Also, during the breath hold, the mean arterial pressure increased from 84 ± 8 to 184 ± 12 mm Hg (Table 3; $P < 0.001$), whereas heart rate decreased from 64 ± 14 to 45 ± 14 bpm ($P < 0.05$). Stroke volume decreased (from 85 ± 11 to 41 ± 6 mL; $P < 0.05$) and thereby cardiac output (from 5.6 ± 1.4 to 2.4 ± 0.8 L·min^{-1}; $P < 0.05$). Thus, systemic vascular resistance increased (from 1265 ± 295 to 6387 ± 2328 dyn·[s·cm^{-5}]$^{-1}$) as did the rate of change in arterial pressure (*dP/dt*) (538 ± 200 to 944 ± 212 mm Hg·s^{-1}; $P < 0.05$). Most importantly, ischemic preconditioning improved the divers personal record from 279 ± 72 to 327 ± 39 s ($P < 0.05$). Similarly, ischemic preconditioning increased the divers' underwater swimming from 110 ± 16 to 119 ± 14 m (Fig. 1; $P < 0.05$), whereas the time spent under water did not change significantly (108 s, range = 43–198 s).

In response to the warm-up for ergometer rowing, thigh Sm_{O2} decreased from 66% ± 7% to 33% ± 14%, whereas for Sc_{O2}, the decrease was from 67% ± 5% to 52% ± 7% (Table 2; $P < 0.05$). Furthermore, the all-out ergometer row was enhanced from 186.5 ± 3.6 to 185.7 ± 3.6 s with preconditioning (Fig. 1; $P < 0.05$). Light had to penetrate 1.5 ± 0.3 mm of forearm soft tissue to reach the muscles and 3.3 ± 0.7 mm to reach the muscle of the right thigh, and on the forehead, soft tissue thickness was 2.0 ± 0.4 mm ($n = 6$). Perceived exertion was similar for the divers and oarsmen and also with and without preconditioning: 19 (17–20).

DISCUSSION

Intermittent ischemia of one forearm improved a static breath hold by ~17% and the distance covered during underwater swimming by ~8%. In contrast, the effect of previous ischemia of one forearm was minimal (~1%) for ergometer rowing. It was illustrated that arm ischemia reduces forearm muscle oxygenation to the detection limit of the NIRS apparatus and that the rowers develop similar muscle desaturation in preparation for the row. Together these observations suggest that muscle ischemia is an important preparation for maximal exercise.

This study did not address how ischemic preconditioning affects performance, but ischemic preconditioning or hypoxia appears to be cytoprotective, involving transcriptional up-regulation of protective pathways, metabolism, inflammatory cells, ion channels, and coagulation, and it preserves mitochondrial membrane integrity and function after prolonged ischemia or hypoxia in rats and mice (13,14). Also, ischemic preconditioning reduces the infarcted area of the heart, both in a porcine model and in a clinical setting (6,19,36).

Hypoxia inducible factor (HIF), a key factor in ischemic preconditioning (12,17), is a heterodimer that degrades and becomes inactive in an oxygen environment. Skeletal

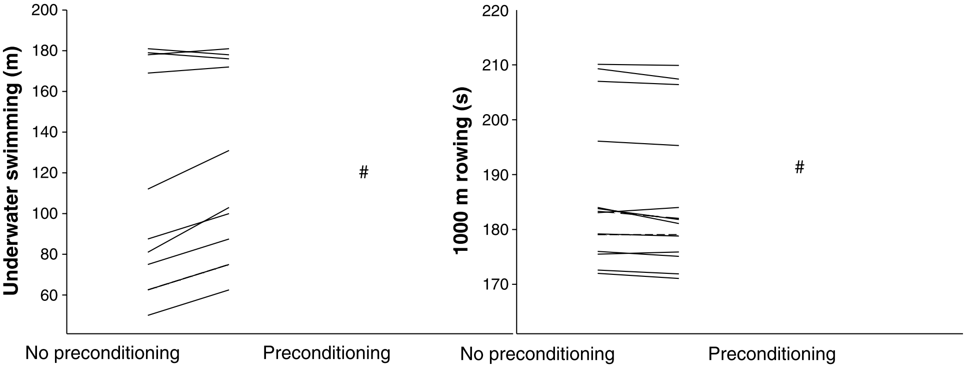

FIGURE 1—Maximal underwater swimming (left; $n = 11$) and 1000-m all-out ergometer rowing (right; $n = 14$) with and without ischemic preconditioning. #$P < 0.05$.

muscle specific HIF-1 knockout mice demonstrate that the increased expression of HIF target genes in response to exercise is HIF-1 dependent, suggesting a role for HIF in adaptation of skeletal muscle to exercise (31). HIF activation facilitates the generation of ATP by matching oxygen demand to supply (33). Thus, we suggest that HIF activation contributes to the improved performance after ischemic preconditioning.

The effect of ischemic preconditioning lasts for 1–2 h, but there also seems to be a second window lasting 1–3 d (12,18,39). Furthermore, repetitive hypoxic preconditioning is protective for the retina of mice and that lasts for weeks (40). The effect of limb (24) ischemic preconditioning is, however, blocked if the limb is de-innervated (24).

Five minutes of ischemia is needed for affecting the body, whereas longer periods (7–10 min) do not seem to have an additional effect (7,8), and similarly, there seems to be little if any advantage of including more than three cycles of ischemia (39). Whether more extended ischemia, for example, of two or all four limbs would be beneficial remains to be established. For rowing, both arms and legs are intensively involved, and therefore there developed a vast reduction in muscle oxygenation.

Free divers typically prepare for apnea by hyperventilating and hyperinflating their lungs using glossopharyngeal insufflation, hereby increasing the air available for pressure equilibration and oxygen storage in the tissue (13,37). Thus, the preparation for apnea aims to increase rather than to decrease tissue oxygenation, explaining why the effect of preconditioning affected underwater swimming (~8%) more than ergometer rowing (1%), albeit the values are not directly comparable since the units are different. Even with the short warm-up used for the present evaluation, the oarsmen demonstrated a decrease in Sm_{O2} that was comparable with that developed during ischemic preconditioning, suggesting that muscle ischemia is an important element in the warm-up procedures carried out in the preparation for maximal exercise.

Trained divers demonstrate a strong diving response as confirmed by a 20-bpm decrease in HR and a 100-mm Hg increase in MAP and *dP/dt* also increased despite a 60% decrease in CO during the breath hold. Also, trained divers demonstrate reduced postapnea acidosis and oxidative stress, and they develop reduced sensitivity to hypoxia and hypercapnia (15,35). As expected, at the end of a dry breath hold, there is a large increase in the middle cerebral artery mean flow velocity (34), and the increase is larger than would be expected by the concomitant increase in the arterial carbon dioxide tension ($PaCO_2$) (23). During a breath hold, Sm_{O2} decreases (35), and we add that divers tolerate a reduction in Sc_{O2} by 33%.

The soft tissue above the sites recorded by NIRS was only 1 mm thick in the investigated subjects, indicating no limitation for light to reach the targeted organs because the penetration debt is considered to be approximately one third of the interoptode distance (3 and 4 cm for the Invos apparatus).

Free divers are at risk of "shallow water blackout" (loss of consciousness) that seems independent of the increase in $PaCO_2$ and likely caused by hypoxia (25). Cerebral blood flow increases during exercise (20), suggesting an increase in blood flow, independent of the concomitant increase blood pressure (22). The exercise-induced increase in cerebral blood flow is modified by $PaCO_2$ (26,28), and hypoxia enhances blood flow especially to phylogenetically older regions of the brain (2). Reduced cerebral oxygenation may limit work capacity (38), and we speculate that ischemic preconditioning postpones a cerebral limitation to exercise and, potentially reducing the risk of loss of consciousness. It may be that ischemic preconditioning affects not only the working muscles and the heart but also the brain.

In conclusion, ischemic preconditioning increases both time for apnea and distance for underwater swimming. In contrast, the effect of ischemic preconditioning of one arm was minimal for rowing may be because of the reduction in muscle oxygenation developed during the warm-up. It remains to be established whether ischemic preconditioning would have a similar effect on (ergometer) rowing as we demonstrated for static and dynamic apnea, provided that the warm-up did not precede the maximal effort. However, we suggest that muscle ischemia is an important element in the preparation for maximal physical activity.

Professor T. T. Nielsen, Department of Cardiology, Skejby Hospital, Aarhus, Denmark, inspired this study, but he passed away on August 21, 2011.

This study was supported by The Copenhagen Muscle Research Center.

The authors declare no conflict of interest.

REFERENCES

1. Astrup P, Engel K, Severinghaus JW, Munson E. The influence of temperature and pH on the dissociation curve of oxyhemoglobin of human blood. *Scand J Clin Lab Invest*. 1965;17:515–23.
2. Binks AP, Cunningham VJ, Adams L, Banzett RB. Gray matter blood flow change is unevenly distributed during moderate isocapnic hypoxia in humans. *J App Physiol*. 2008;104:212–7.
3. Bishop D. Warm up I: potential mechanisms and the effects of passive warm up on exercise performance. *Sports Med*. 2003; 33:439–54.
4. Bogert LW, Van Lieshout JJ. Non-invasive pulsatile arterial pressure and stroke volume changes from the human finger. *Exp Physiol*. 2005;90:437–46.
5. Borg GA. Psychophysical bases of perceived exertion. *Med Sci Sports Exerc*. 1982;14(5):377–81.
6. Botker HE, Kharbanda R, Schmidt MR, et al. Remote ischaemic conditioning before hospital admission, as a complement to angioplasty, and effect on myocardial salvage in patients with acute myocardial infarction: a randomised trial. *Lancet*. 2010;375:727–4.

7. Bushell AJ, Klenerman L, Davies H, Grierson I, McArdle A, Jackson MJ. Ischaemic preconditioning of skeletal muscle 2. Investigation of the potential mechanisms involved. *J Bone Joint Surg Br*. 2002;84:1189–93.
8. Bushell AJ, Klenerman L, Taylor S, Davies H, Grierson I, Helliwell TR, Jackson MJ. Ischaemic preconditioning of skeletal muscle. 1. Protection against the structural changes induced by ischaemia/reperfusion injury. *J Bone Joint Surg Br*. 2002;84:1184–8.
9. Chance B, Dait MT, Zhang C, Hamaoka T, Hagerman F. Recovery from exercise-induced desaturation in the quadriceps muscles of elite competitive rowers. *Am J Physiol*. 1992;262: C766–75.
10. Cole AL, Herman RA, Heimlich JB Jr, Ahsan S, Freedman BA, Shuler MS. Ability of near infrared spectroscopy to measure oxygenation in isolated upper extremity muscle compartments. *J Hand Surg Am*. 2012;37:297–302.
11. de Groot PC, Thijssen DH, Sanchez M, Ellenkamp R, Hopman MT. Ischemic preconditioning improves maximal performance in humans. *Eur J Appl Physiol*. 2010;108:141–6.
12. Dirnagl U, Becker K, Meisel A. Preconditioning and tolerance against cerebral ischaemia: from experimental strategies to clinical use. *Lancet Neurol*. 2009;8:398–412.
13. Eichinger M, Walterspacher S, Scholz T, et al. Lung hyperinflation: foe or friend? *Eur Respir J*. 2008;32:1113–6.
14. Endres M, Meisel A, Biniszkiewicz D, et al. DNA methyltransferase contributes to delayed ischemic brain injury. *J Neurosci*. 2000;20:3175–81.
15. Foster GE, Sheel AW. The human diving response, its function, and its control. *Scand J Med Sci Sports*. 2005;15:3–12.
16. Fradkin AJ, Zazryn TR, Smoliga JM. Effects of warming-up on physical performance: a systematic review with meta-analysis. *J Strength Cond Res*. 2010;24:140–8.
17. Fukuda R, Zhang H, Kim JW, Shimoda L, Dang CV, Semenza GL. HIF-1 regulates cytochrome oxidase subunits to optimize efficiency of respiration in hypoxic cells. *Cell*. 2007;129:111–22.
18. Halestrap AP, Clarke SJ, Khaliulin I. The role of mitochondria in protection of the heart by preconditioning. *Biochim Biophys Acta*. 2007;1767:1007–31.
19. Hoole SP, Heck PM, Sharples L, et al. Cardiac Remote Ischemic Preconditioning in Coronary Stenting (CRISP Stent) Study: a prospective, randomized control trial. *Circulation*. 2009;119:820–7.
20. Ide K, Secher NH. Cerebral blood flow and metabolism during exercise. *Prog Neurobiol*. 2000;61:397–414.
21. Jean-St-Michel E, Manlhiot C, Li J, et al. Remote preconditioning improves maximal performance in highly trained athletes. *Med Sci Sports Exerc*. 2011;43(7):1280–6.
22. Jorgensen LG, Perko G, Secher NH. Regional cerebral artery mean flow velocity and blood flow during dynamic exercise in humans. *J Appl Physiol*. 1992;73:1825–30.
23. Kjeld T, Pott FC, Secher NH. Facial immersion in cold water enhances cerebral blood velocity during breathhold exercise in humans. *J Appl Physiol*. 2009;106:1243–8.
24. Lim SY, Yellon DM, Hausenloy DJ. The neural and humoral pathways in remote limb ischemic preconditioning. *Basic Res Cardiol*. 2010;105:651–5.
25. Lindholm P. Loss of motor control and/or loss of consciousness during breath-hold competitions. *Int J Sports Med*. 2007;28:295–9.
26. Linkis P, Jorgensen LG, Olesen HL, Madsen PL, Lassen NA Secher NH. Dynamic exercise enhances regional cerebral artery mean flow velocity. *J Appl Physiol*. 1995;78:12–6.
27. Lowitzsch K, Hopf HC, Galland J. Changes of sensory conduction velocity and refractory periods with decreasing tissue temperature in man. *J Neurol*. 1977;216:181–8.
28. Madden JA. The effect of carbon dioxide on cerebral arteries. *Pharmacol Ther*. 1993;59:229–50.
29. Madsen PL, Secher NH. Near-infrared oximetry of the brain. *Prog Neurobiol*. 1999;58:541–60.
30. Mancini DM, Bolinger L, Li H, Kendrick K, Chance B, Wilson JR. Validation of near-infrared spectroscopy in humans. *J Appl Physiol*. 1994;77:2740–7.
31. Mason SD, Howlett RA, Kim MJ, et al. Loss of skeletal muscle HIF-1alpha results in altered exercise endurance. *PLoS Biol*. 2004; 2:e288. doi: 10.1371/journal.pbio.0020288.
32. Nielsen HB, Boushel R, Madsen P, Secher NH. Cerebral desaturation during exercise reversed by O2 supplementation. *Am J Physiol*. 1999;277:H1045–52.
33. O'Hagan KA, Cocchiglia S, Zhdanov AV, et al. PGC-1alpha is coupled to HIF-1alpha-dependent gene expression by increasing mitochondrial oxygen consumption in skeletal muscle cells. *Proc Natl Acad Sci U S A*. 2009;106:2188–93.
34. Palada I, Obad A, Bakovic D, Valic Z, Ivancev V, Dujic Z. Cerebral and peripheral hemodynamics and oxygenation during maximal dry breath-holds. *Respir Physiol Neurobiol*. 2007;157:374–81.
35. Schagatay E, van Kampen M, Emanuelsson S, Holm B. Effects of physical and apnea training on apneic time and the diving response in humans. *Eur J Appl Physiol*. 2000;82:161–9.
36. Schmidt MR, Smerup M, Konstantinov IE, et al. Intermittent peripheral tissue ischemia during coronary ischemia reduces myocardial infarction through a KATP-dependent mechanism: first demonstration of remote ischemic perconditioning. *Am J Physiol*. 2007;292:H1883–90.
37. Seccombe LM, Rogers PG, Mai N, Wong CK, Kritharides L, Jenkins CR. Features of glossopharyngeal breathing in breath-hold divers. *J Appl Physiol*. 2006;101:799–801.
38. Seifert T, Rasmussen P, Secher NH, Nielsen HB. Cerebral oxygenation decreases during exercise in humans with beta-adrenergic blockade. *Acta Physiol (Oxf)*. 2009;196:295–302.
39. Yellon DM, Downey JM. Preconditioning the myocardium: from cellular physiology to clinical cardiology. *Physiol Rev*. 2003;83: 1113–51.
40. Zhu Y, Zhang Y, Ojwang BA, Brantley MA Jr, Gidday JM. Long-term tolerance to retinal ischemia by repetitive hypoxic preconditioning: role of HIF-1alpha and heme oxygenase-1. *Invest Ophthalmol Vis Sci*. 2007;48:1735–43.