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Breath-hold training of humans reduces oxidative stress and blood acidosis after static and dynamic apnea

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Abstract

Repeated epochs of breath-holding were superimposed to the regular training cycling program of triathletes to reproduce the adaptative responses to hypoxia, already described in elite breath-hold divers [Respir. Physiol. Neurobiol. 133 (2002) 121]. Before and after a 3-month breath-hold training program, we tested the response to static apnea and to a 1-min dynamic forearm exercise executed during apnea (dynamic apnea). The breath-hold training program did not modify the maximal performances measured during an incremental cycling exercise. After training, the duration of static apnea significantly lengthened and the associated bradycardia was accentuated; we also noted a reduction of the post-apnea decrease in venous blood pH and increase in lactic acid concentration, and the suppression of the post-apnea oxidative stress (increased concentration of thiobarbituric acid reactive substances). After dynamic apnea, the blood acidosis was reduced and the oxidative stress no more occurred. These results suggest that the practice of breath-holding improves the tolerance to hypoxemia independently from any genetic factor.

Keywords: Apnea, static, exercise; Exercise, breath-holding, triathlets; Hypoxia, adaptive response; Mammals, humans; Training, exercise

1. Introduction

In a previous study, we showed that elite breath-hold divers able to sustain apnea up to 5 min at rest presented an adaptive metabolic response to apnea (Joulia et al., 2002). Indeed, in a group of non divers, constituted by sedentary subjects and young athletes, a blood acidosis and an oxidative stress followed sustained apnea at rest (static apnea) or during handgrip exercise (dynamic apnea). On the other hand, these responses were both markedly attenuated or even absent in elite divers. However, this preliminary study did not

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allow to exclude the possibility of genetic inter-individual differences.

The aim of the present study, conducted in eight subjects who had no experience of breath-hold diving, was to search for any benefits of a 3-month training program of dynamic apneas. We hypothesized that breath-hold training could induce adaptive metabolic response to hypoxemia in the same way as that measured in elite breath-hold divers. Because breath-hold training was performed during exercise to more closely reproduce the diving conditions, we chose well trained triathletes who continued their regular training program. This allowed to only assess the benefits of repeated apneas and not of an endurance training program inaugurated in sedentary subjects which could alter their baseline redox state and also their oxidative response to exercise. Thus, we searched for the consequences of repetitive epochs of breath-holding on both the increase in venous blood concentration of lactic acid and the variations of some markers of the oxidative stress in response to static apnea and handgrip exercise with or without apnea.

2. Materials and methods

2.1. Subjects

Eight subjects were explored. They were male students who all practiced triathlon for 2–5 years. Their regular weekly training program combined 6 h of cycling, 5 h of swimming, 5 h of long-distance running, and 6 h of body-building. Their performances are shown in Table 1. When they were first explored in our laboratory, their mean apnea duration was 104 ± 14 sec, the maximal breath-hold duration being 98 sec in six of them.

2.2. Training to dynamic apnea

After determination of their physiological and metabolic responses to an incremental maximal cycling exercise and to static and dynamic apneas, the eight subjects were simultaneously involved in the same training program of breath-holding. This program consisted in the repetition of 20-sec breath-holding epochs separated by 40 sec of breathing room air during a 1-h steady state cycling exercise at 30% of their maximal oxygen uptake ($\dot{V}_{O_2,max}$). For a consecutive 3-month period, this 1-h training period of breath-holding was repeated three times a week. It was substituted to the regular triathlon cycling training program of triathletes which was limited to 3 h a week instead of 6 h. Then, the subjects were all explored during the week following the 3-month training program of dynamic apnea. The procedures involved in the study and the possible risks were explained to the subjects, whose written consents were obtained. The whole protocol was approved by the local ethics committee.

Table 1
The mean characteristics of subjects measured before and after the 3-month breath-hold training program

	Before training	After training
Age (year)	22.6 ± 4.6 (3.9)	–
Weight (kg)	72.5 ± 6.6 (5.5)	73.2 ± 7.2 (6.3)
$\dot{V}_{O_2,max}$ (ml STPD min^{-1} kg^{-1})	63.4 ± 3.1 (2.6)	64.3 ± 2.9 (2.4)
V_{Th} (ml STPD min^{-1} kg^{-1})	51.9 ± 4.6 (3.9)	52.3 ± 3.2 (2.7)
$\dot{V}_{O_2,handgrip}$ (ml STPD min^{-1} kg^{-1})	7.2 ± 2.1 (1.7)	6.7 ± 0.8 (0.7)

$\dot{V}_{O_2,max}$, maximal oxygen uptake; V_{Th}, ventilatory threshold; $\dot{V}_{O_2,handgrip}$, plateau value of oxygen uptake measured during the control 1-min dynamic handgrip. Values are mean with standard deviation (S.D.) and 95% confidence interval (in parenthesis). No significant post-training variations were noted.

2.3. Physiological variables and exercise protocol

Before and after training to dynamic apnea, we measured $\dot{V}_{O_2,max}$ and the ventilatory threshold (V_{Th}). The subject performed an incremental exercise on an electrically braked cycloergometer (Ergometrics ER 800, Jaeger, Germany) connected to a microcomputer software. The testing protocol consisted first in a 2-min rest period, second in a 2-min 0-W work load period used to reach the 1 Hz cycling frequency, and third in a 20 W to $\dot{V}_{O_2,max}$ work period. The work period started at a work load of 20 W and the load was increased by 20 W every 1 min until $\dot{V}_{O_2,max}$ was reached. The ergometer was then unloaded and the subject continued to cycle for a 2-min recovery period.

Throughout the incremental exercise trial, the software (Oxycon Beta, Jaeger, Germany) computed breath-by-breath data of $\dot{V}_E$, $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, and the ventilatory equivalents for O_2 ($\dot{V}_E/\dot{V}_{O_2}$) and CO_2 ($\dot{V}_E/\dot{V}_{CO_2}$). V_{Th} corresponded to the $\dot{V}_{O_2}$ value at which $\dot{V}_E/\dot{V}_{O_2}$ exhibited a systematic increase without a concomitant increase in $\dot{V}_E/\dot{V}_{CO_2}$ (Davis et al., 1979). V_{Th} was expressed in absolute value of oxygen uptake related to body weight.

To explore the response to experimental conditions (static apnea, dynamic handgrip with or without apnea), the subjects were comfortably seated. The heart rate was continuously monitored (Cardiognost Hellige, Stutgart, Germany) as well as the blood oxygen saturation (SaO_2, %) which was measured with a pulse oximeter (NPB 40, Nelcor Puritan Bennett, Pleasanton CA, USA). They wore a face mask (dead space: 30 ml) forming an air-tight seal over the nose and mouth, with all the inspired and expired gas going into a volumetric rotor transducer (Triple V digital volume transducer, Jaeger, Germany), which gave measurements of minute ventilation. A side pore of the face mask was connected to fast-response differential paramagnetic O_2 and CO_2 analyzers (Jaeger: 90% response time in 100 ms). The software (Oxycon beta) computed breath-by-breath values of minute ventilation, end-tidal O_2 and CO_2 partial pressures, and $\dot{V}_{O_2}$. Prior to the experiment, the ear lobe was pretreated with a vasodilator cream. Then, it was incised to sample arterialized blood in 100 μl heparinized capillary tubes. Oxygen (PaO_2) and carbon dioxide ($PaCO_2$) partial pressures, and arterial pH (pHa) were measured (Blood gases analyzer model 860, BAYER Diagnostics, Puteaux, France).

Dynamic forearm exercises consisted in a 1-min period of rhythmic handgrip at a rate of 30 cpm. For each trial the subject had to displace for 1 sec, on a length of 3 cm, a 8.5 kg load. Then, the subject left the grip for a consecutive 1-sec period. Recordings of the load displacement revealed that each contraction developed in a saw-tooth waveform, the rate of displacement and its waveform being near constant throughout the fatigue trial in all individuals. Using a 8.5 kg work load, the power of the 1-min forearm exercise was 112 W. This dynamic handgrip protocol has already been used and data published (Steinberg et al., 2002; Joulia et al., 2002). In the present study the same workload was used in all subjects because measurements of the maximal handgrip strength in static condition with an appropriate strain gauge showed that all subjects developed similar strength before (45–52 kg) and after (44–53 kg) the training session.

2.4. Biochemical variables

On the side of the working forearm muscle, an antecubital vein was catheterized. Five ml of heparinized blood were sampled at each sequence of the protocol in order to measure all biochemical variables.

The venous blood pH (pHv) was measured with a microelectrode (BAYER Diagnostics model 860). Blood lactic acid concentration [LA] was measured enzymatically (lactate deshydrogenase) (BAYER Diagnostics model 860) and S.E.M. of lactate measurements for the laboratory quality control was ± 0.05 mmol L^{-1}.

Two blood markers of the oxidative stress were used: thiobarbituric acid reactive substances (TBARS), in order to estimate the formation of lipid hydroperoxides (Ashton et al., 1998; Bejma and Ji, 1999; Best et al., 1999; Ebbeling and Clarkson, 1989; Joanny et al., 2001; Lovlin et al., 1987; Steinberg et al., 2002), and erythrocyte reduced glutathione (GSH). GSH which explores the consumption of endogenous antioxidants (Frei et al., 1989; Glascott et al., 1996; Joanny et al., 2001; Lew and Quantanilha, 1991; Rokitzki et al., 1994; Steinberg et al., 2002) is considered a more effective determinant in assessing oxidative stress-related changes than lipid peroxide levels (TBARS) (Balakrishnan and Anuradha, 1998; Lew et al., 1985; Sen et al., 1994).

The plasma TBARS concentration was assessed according to the method by Uchiyama and Mihara (1978) that we already used in humans (Joanny et al., 2001). After centrifugation of heparinized blood samples ($1500 \times g$ at 4 °C for 10 min), ethanolic butylated hydroxytoluene (Sigma-Aldrich Co., Saint Quentin Fallavier, France) was added to the plasma to avoid the decomposition of

products that may occur during the heating step of the reaction. Then, 300 μl of plasma were deproteinized in the same volume of 10% trichloroacetic acid. After vortexing and centrifugation ($2500 \times g$ at 4 °C for 15 min), supernatants were stored at −80 °C for further analysis. In test tubes containing 200 μl aliquots, we added successively 200 μl of 8.1% sodium dodecylsulfate, 1.5 ml of 20% acetate buffer (pH 3.5), 1.5 ml of freshly prepared 0.8% thiobarbituric acid (Sigma-Aldrich Co.) and 600 μl of water. The test tubes containing glass beads were heated at 100 °C for 60 min, then cooled in tap water at room temperature. Then, we added in each tube 4 ml of n-butanol and 1 ml of water. After vortexing for 5 min, the mixture was centrifugated ($2000 \times g$ for 3 min) to obtain a rapid separation between organic and aqueous phases. The upper organic phase was pipetted and the pink pigment was measured using a spectrofluorimeter at an excitation wavelength of 515 nm and an emission wavelength of 553 nm (SHIMADZU, model RF-5000, Kyoto, Japan). A standard curve of TBARS was obtained after overnight hydrolysis at room temperature of a solution containing 720 μg ml^{-1} of tetraethoxypropane (Sigma-Aldrich Co.) in 0.1 N NHCl.

Erythrocyte GSH was assayed spectrophotometrically using a commercially available kit (GSH-400, Oxis International Inc, supplied by Biomedical Diagnostics, Marne la Vallée, France). Briefly, 200 μl of erythrocyte pellet were pipetted and extracted in 800 μl of 6.25% metaphosphoric acid. The extract was vortexed and centrifugated ($3000 \times g$ at 4 °C for 10 min).

The supernatants were maintained stable at −80 °C in metaphosphoric acid before analysis. In the test tube containing 100 μl of supernatant, we added 800 μl of dipotassium phosphate buffer (pH 7.8) containing 0.2 mmol L^{-1} diethylenetriamine-penta-acetic acid (DTPA), 0.025% lubrol, 50 μl of 1.2×10^{-2} M chromogen (4 chloro-1-methyl-7-trifluoromethyl-1-quinolinium) in 0.2 N HCl, and 50 μl of 30% NaOH. After vortexing, the solution was incubated for 10 min at 25 °C. The optical density was estimated at the wavelength of 400 nm. A standard curve was obtained with reduced glutathione in 5% metaphosphoric acid.

2.5. Experimental protocol

First, we asked the subject to perform two apneas at rest (static apneas), separated by a 20-min period of recovery (R). Then they performed a 1-min control dynamic handgrip exercise with the dominant arm during which they continued to breathe room air. After a 20-min rest period, a second 1-min exercise was performed during which the subjects sustained apnea (dynamic apnea). Measurements at R20 served as control values for the following exercise trial. The entire protocol was repeated after the 3-months training program to dynamic apnea.

2.6. Statistics

Results are expressed as mean ± S.E.M. A two-way analysis of variance(ANOVA) for repeated measures was used to evaluate over time the changes in blood concentrations of lactic acid, TBARS, GSH and RAA. When significant changes were identified over time, Bonferroni's t-test was used to determine which time points were different. ANOVA was also used to compare the variations of biochemical variables after a training session in the same individuals. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Exercise performances before and after breath-hold training

As shown in Table 1, breath-hold training did not modify $\dot{V}_{O_2,max}$ and V_{Th}, and the response to the 1-min control dynamic handgrip exercise. Indeed, the plateau $\dot{V}_{O_2}$ value as well as the heart rate increase during handgrip did not vary (HR increase = +32 ± 3% before training vs. +29 ± 4% after) and we measured no variation of the maximal post-handgrip increase in [LA] (before: +2.80 ± 0.34 mmol L^{-1}; after: +2.26 ± 0.45 mmol L^{-1}). The 1-min handgrip exercise never elicited an oxidative stress (no TBARS increase and no GSH consumption) in these triathletes, even before the training program.

3.2. Consequences of breath-hold training on the response to static apnea

The subjects were not accustomed to perform breath-holding, thus none of them hyperventilated before becoming apneic. We asked them to refrain from changing this practice after the training program. Breath-by-breath measurement of end-tidal expired CO_2 concentration allowed us to verify the absence of hyperventilation before static apnea. Before training, static apnea elicited hypoxemia, hypercapnia and blood acidosis (decreased pHv and increased [LA]) (Table 2). Static apnea also produced a modest but significant increase in TBARS concentration and a consumption of GSH. As shown in Table 2, the training program significantly increased in all subjects the mean static apnea duration. Then, five of them were able to sustain apnea for more than 3 min (maximal apnea duration = 275 sec). Training significantly accentuated the diving reflex assessed by the maximal heart rate decrease during static apnea (Fig. 1). Because the training program significantly lengthened the apnea duration, it also accentuated the changes in arterial blood gases. However, the apnea-induced blood acidosis (pHv reduction and [LA] increase), the [TBARS] increases, and the [GSH] decrease disappeared after training. We also noted a significant reduction of the resting [TBARS] after breath-hold training whereas the resting [GSH] was not modified.

3.3. Effects of breath-hold training on handgrip exercise with apnea

Before breath-hold training, apnea sustained during the 1-min handgrip significantly reduced the maximal increase in lactic acid concentration compared with that measured after the control 1-min handgrip trial ($+1.99 \pm 0.22$ vs. $+2.80 \pm 0.34$ mmol L^{-1}, respectively) but a significant oxidative

Table 2
Static apneas

	Before training	After training
Apnea duration (sec)	104 ± 14§§	155 ± 15
Pa_{O_2} (*mmHg*)		
Control	89 ± 2	90 ± 2
Breaking point	65 ± 3***§	60 ± 3***
Pa_{CO_2} (*mmHg*)		
Control	40 ± 1	40 ± 1
Breaking point	45 ± 2***§	49 ± 1***
pHv		
Control	7.389 ± 0.007	7.375 ± 0.008
Post-dive (R0)	7.376 ± 0.009*	7.350 ± 0.008
Lactic acid (*mmol* L^{-1})		
Control	1.35 ± 0.11	1.23 ± 0.09
Post-dive	1.51 ± 0.12* (+0.09 ± 0.02 mmol L^{-1} min^{-1})§§	1.31 ± 0.08 (+0.03 ± 0.01 mmol L^{-1} min^{-1})
TBARS (*ng* ml^{-1})		
Control	162 ± 22§	102 ± 24
Post-dive (R0)	223 ± 24* (+35 ± 10 ng ml^{-1} min^{-1})§§§	132 ± 13 (+12 ± 4 ng ml^{-1} min^{-1})
GSH (*mg/100 ml erythrocytes*)		
Control	19 ± 2	24 ± 3
Post-dive (R2)	14 ± 2* (−2.82 ± 0.40 mg/100 ml min^{-1})§§	21 ± 1 (−1.16 ± 0.30 mg/100 ml min^{-1})

Values are mean + S.E.M. ($n = 32$ apneas in eight individuals). Asterisks denote significant post-dive changes and symbol § was used to indicate significant differences between pre- and post-training sessions (§, $P < 0.05$; §§, $P < 0.01$; §§§, $P < 0.001$). Values in parenthesis correspond to the ratio of maximal post apnea variation to the corresponding apnea duration expressed in min.

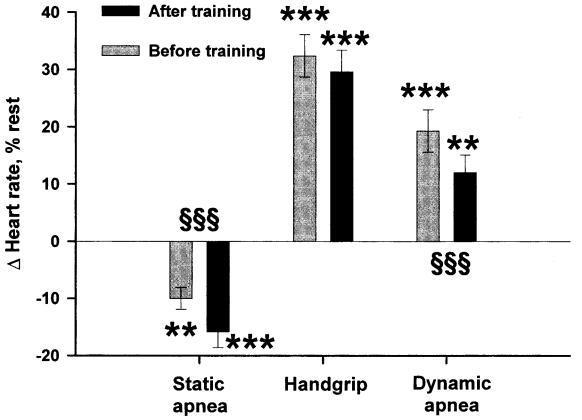

Fig. 1. Heart rate changes, expressed in percentage of corresponding rest values, in response to static or dynamic apnea, and to control handgrip exercise before and after the 3-month breath-hold training program. Asterisks indicate that heart rate changes are significant and symbols § show that heart variations are significantly modified after training (§§, $P < 0.01$; §§§, $P < 0.001$).

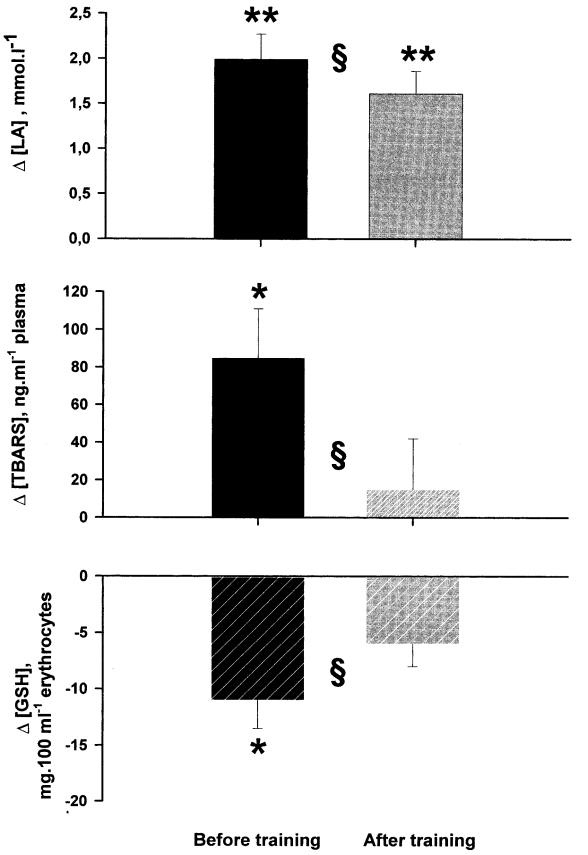

Fig. 2. 1-min handgrip exercise executed during breath-holding (dynamic apnea) before then after the 3-month breath-hold training program in the same subjects. Asterisks denote the significance of post-dynamic apnea variations of lactic acid (LA), thiobarbituric acid reactive substances (TBARS), and reduced gluthatione (GSH) (*, $P < 0.05$; **, $P < 0.01$) and symbols § show that the changes in biochemical variables are significantly reduced after training (§, $P < 0.05$).

stress (increased [TBARS] and reduced [GSH]) followed dynamic apnea (Fig. 2). After breath-hold training, the magnitude of hypoxemia and hypercapnia measured before the breaking point of the 1-min dynamic apnea significantly ($P < 0.05$) decreased (before training: $Pa_{O_2} = 72 \pm 3$ mmHg and $Pa_{CO_2} = 46 \pm 1$ mmHg; after training: $Pa_{O_2} = 79 \pm 3$ mmHg and $Pa_{CO_2} = 42 \pm 1$ mmHg). Breath-hold training significantly reduced the heart rate increase in response to dynamic apnea (Fig. 1). As shown in Fig. 2, training modestly but significantly reduced the changes in [LA] and suppressed the associated increase in [TBARS] and GSH consumption.

4. Discussion

4.1. General comments

This study shows that a 3-month breath-hold training program, superimposed to the regular cycling training program of triathletes, significantly lengthened the duration of static apnea whereas it reduced the blood acidosis (decreased pHv and increased lactic acid concentration) and suppressed the oxidative stress, both following static and dynamic apneas. We also noted a significant reduction of the resting TBARS concentration after breath-hold training.

The present observations can be analyzed in terms of the specific influence of the breath-hold training program and not of any benefits of a 3-month prolongation of the regular training program of triathletes, which in fact was minimized (3 h of cycling a week instead of 6 h). Indeed, the physiological and biochemical measurements confirmed the absence of a further improvement of the maximal performance (no increase in $\dot{V}_{O_2,max}$ and

V_{Th}) and also of any change in the response to the 1-min control dynamic handgrip (no variation of the plateau $\dot{V}_{O_2}$ value, heart rate increase, and peak lactic acid concentration).

4.2. Metabolic consequences of breath-hold training

We already reported in elite divers (Joulia et al., 2002) that, despite their lengthened duration of static apnea (5 min and more) which should increased asphyxia (hypoxemia plus hypercapnia), there were no significant accentuation of hypercapnia, and no further blood acidosis, compared with data obtained in control subjects. In the present study, the adaptative mechanisms, which reduce the production of CO_2 and lactic acid in elite divers were partly found after the breath-hold training of subjects who had no experience of breath-hold diving. Indeed, breath-hold training prolonged the static apnea duration, accentuating both hypoxemia and hypercapnia, but did not induce a significant blood acidosis. Breath-hold training also reduced the blood acidosis and hypercapnia measured at the breaking point of the 1-min apnea associated with handgrip. Several observations converge to show up the existence of a specific mechanism restricted to apnea, with a marked accentuation of this phenomenon after practice of breath-holding. First, before breath-hold training the maximal post-handgrip increase in lactic acid concentration was significantly lower when subjects sustained apnea ($+1.99 \pm 0.28$ mmol L^{-1}) than when they continued to breathe during the handgrip trial ($+2.88 \pm 0.42$ mmol L^{-1}). Second, after breath-hold training in the present study as well as in "elite" divers in our previous work, we noted no reduction of the blood acidosis following the control handgrip trials whereas an attenuated [LA] increase occurred when the subjects sustained apnea during the handgrip. The well documented apnea-induced reflex bradycardia and vasospasm in limb muscles in humans (Craig and Medd, 1968b; Sterba and Lundgren, 1988) and the harbor seal (Behrisch and Elsner, 1984) reduces the blood supply to muscles and should lower their demand for glycolytic metabolism limiting the lactate production. The peripheral vasospasm during the diving reflex is probably simply due to an asphyxia-induced increase in sympathetic nerve activity as demonstrated in humans exposed to intermittent asphyxia while they continued to breathe (Xie et al., 2000). This recent study also showed that a short-term exposure to intermittent asphyxia, as produced during breath-holding, causes a sympathetic activation that persists after the removal of the chemical stimuli. This key observation could explain why we found that the diving reflex was significantly accentuated after breath-hold training.

The adaptive metabolic mechanisms to asphyxia reported in elite human divers, and also in "naïve" humans trained in breath-holding may be compared with data collected in animals and humans acclimated to hypoxia. When they compared the activities of glycolytic enzymes in heart, liver, kidney and cerebral cortex from the harbor seal and the adult and newborn dog, Behrisch and Elsner (1984) already showed that the organs which were rendered ischemic (reduced blood supply) in the diving seal or asphyxiated in the newborn dog had a marked reduction of these enzymatic activities compared with the adult dog. It is well known in humans acclimated to high altitude, that the post-exercise increase in blood lactic acid concentration returns to values measured at sea level (Bender et al., 1989; Edwards, 1936; Maher et al., 1974). The mechanism of this phenomenon is not understood because opposite data are reported. Some studies suggest that both chronic and repeated exposure to hypoxia increase the mobilization and use of free fatty acids during exercise, resulting in sparing of muscle glycogen and a reduced lactic acid production (Jones et al., 1972; Young et al., 1982). By contrast, in rats acclimated to a 4300 m simulated altitude Kennedy et al. (2001) did not confirm the sparing of the glycolytic pathway in a white muscle and the heart. We took care to avoid interpreting the changes in blood lactic acid concentration in terms of the sole variations of its cellular production. Indeed, venous blood measurements cannot distinguish the production from catabolism of lactic acid. Thus, the present observations of reduced blood acidosis in subjects trained in breath-holding may signify a reduced production by exercising

muscles and/or an increased catabolism by other tissues.

4.3. The consequences of breath-hold training on the oxidative stress

After breath-hold training the resting TBARS level was significantly reduced in our subjects. Our previous comparative study between elite breath-hold divers and "naïve" sedentary subjects and also well trained athletes (Joulia et al., 2002) indicated a lower resting level of GSH in elite divers whereas their blood concentrations of TBARS and reduced ascorbic acid concentrations were not affected. This suggests that repeated epochs of asphyxia during breath-hold training has attenuated the oxidative stress and/or potentiated the mechanisms protecting against membrane lipid peroxidation. There are some similarities between our observations and those reported by authors who studied the consequences of chronic hypoxemia on the oxidative stress. Indeed, Singh et al. (2001) found a significant decrease in GSH content in muscles and blood of rats repeatedly exposed to hypoxia (6 h a day). Moreover, Radak et al. (1997) found in the same species that high altitude (4000 m) training did not induce any significant increase in TBARS content in white and red muscles. The changes in resting levels of TBARS and endogenous antioxidant were not found in humans acutely exposed to hypoxemia (Dousset et al., 2002). Thus, the adaptive mechanism, which protects the tissues against deleterious effects of an enhanced formation of reactive oxygen species at rest needs a prolonged exposure to hypoxemia.

We confirmed that the succession of apnea and recovery on reoxygenation induced an oxidative stress in our triathletes when they sustrained apnea at rest or during the 1-min handgrip (dynamic apnea). This oxidative stress in response to breath-holding was also measured in the population of "naïve" sedentary subjects and trained athletes of our previous study (Joulia et al., 2002). After breath-hold training this oxidative stress was markedly reduced after static apnea or even absent after dynamic apnea. Thus, it seems that asphyxia during breath-holding does not exacerbate but rather attenuates the membrane lipid peroxidation. We recently published that a brief period of hypoxemia produced by the inhalation of a hypoxic gas mixture in normal subjects abolishes the post-handgrip increase in TBARS (Dousset et al., 2002). As we discussed above, apnea is also responsible for a reflex vasospasm in limb muscles which was accentuated after the 3-month breath-hold training program. This reduction of the blood supply to muscles may lower their oxygen stores, explaining the significant reduction of the oxidative stress here reported after training and also in elite divers (Joulia et al., 2002). The aforementioned observations strongly suggest that the muscle response to hypoxemia alone or to asphyxia (breath-holding) is characterized by a reduced and not an enhanced oxidative stress. Sjödin et al. (1990) already hypothesized that a reduced oxygen supply to muscle should decrease the oxygen free radical formation.

The present longitudinal study clearly shows the development of an adaptive metabolic response to repetitive periods of asphyxia. The post-training attenuation of lethal cellular consequences of blood acidosis and production of oxygen free radicals is probably one explanation for the tolerance to a lengthened apnea duration.

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