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Reduced oxidative stress and blood lactic acidosis in trained breath-hold human divers

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Abstract

We hypothesized that the repetition of brief epochs of hypoxemia in elite human breath-hold divers could induce an adaptation of their metabolic responses, resulting in reduced blood acidosis and oxidative stress. Trained divers who had a 7–10 year experience in breath-hold diving, and were able to sustain apnea up to 440 sec at rest, were compared to control individuals who sustained apnea for 145 sec at the most. The subjects sustained apnea at rest (static apnea), and then, performed two 1-min dynamic forearm exercises whether they breathed (control exercise) or sustained apnea (dynamic apnea). We measured arterial blood gases, venous blood pH, and venous blood concentrations of lactic acid, thiobarbituric acid reactive substances (TBARS), and two endogenous anti-oxidants (reduced glutathione, GSH, and reduced ascorbic acid, RAA). In control subjects, the three experimental conditions elicited an increase in blood lactic acid concentration and an oxidative stress (increased TBARS, decreased GSH and RAA concentrations). In divers, the changes in lactic acid, TBARS, RAA, and GSH concentrations were markedly reduced after static and dynamic apnea, as well as after control exercise. Thus, human subjects involved in a long duration training programme of breath-hold diving have reduced post-apnea as well as post-exercise blood acidosis and oxidative stress, mimicking the responses of diving animals.

Keywords: Blood, lactic acid; Diving, breath-hold, hypoxia; Hypoxia, breath-hold divers; Mammals, humans; Stress, oxidative

1. Introduction

It is well known that prolonged apnea duration leads to severe hypoxemia and is responsible for blood acidosis due to the association of hypercapnia and increased lactic acid concentration (Craig and Medd, 1968a; Olsen et al., 1962; Qvist et al., 1993). Moreover, the succession of a reduced

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oxygen supply to tissues and recovery on reoxygenation may be responsible for an enhanced production of oxygen free radicals (Dhaliwal et al., 1991), resulting in lipid peroxidation and cell membrane damage. This oxidative stress is responsible for local inflammatory reactions which are suspected to impede the recovery of normal cellular metabolism (Reid, 1996; Sen, 1995; Sjödin et al., 1990; Steinberg et al., 2002). Well trained breath-hold human divers are able to sustain apnea up to 7 min at rest compared to the modest performances of untrained individuals. Thus, it is tempting to speculate that some adaptive metabolic responses might occur in trained divers, the same way as in diving animals. Diving animals, including mammals, reptiles and birds, have a high tolerance to very long apnea, which can reach more than 40 min in seals and whales (Butler and Jones, 1997). Compared to non-diving animals, their oxygen stores are larger, their blood buffers more efficient and some authors (Behrisch and Elsner, 1984) have measured a decreased activity of glycolytic enzymes in tissues poorly perfused during the dive as liver, kidney and muscle, resulting in a marked reduction of blood lactic acid concentration. The concentration of antioxidant enzymes is significantly higher at rest in the heart of resting seals than in pigs, non diving mammals (Elsner et al., 1998). We found no data in the literature on an eventual attenuation of the post-exercise oxidative stress in diving animals nor on the development of adaptive metabolic responses with their growth.

In the present study, we hypothesized that the repetition of brief epochs of hypoxemia in elite human breath-hold divers could induce an adaptation of their metabolic responses, attenuating the post-apnea and perhaps also the post-exercise blood acidosis and oxidative stress. Elite divers, who had a long experience of breath-hold diving and exhibited high apnea performances, were compared to control subjects who never practised breath-hold diving. The oxidative stress was estimated from blood measurements of thiobarbituric acid reactive substances (TBARS), in order to estimate the formation of lipid hydroperoxides, and then from measurements of plasma reduced ascorbic acid (RAA), and erythrocyte reduced glutathione (GSH), in order to measure the consumption of endogenous antioxidants

2. Materials and methods

2.1. Subjects

Nine subjects had a 7–10 year experience in breath-hold diving. When they were explored, they were all involved in the same training programme, which consisted in the repetition of breath-hold dives for a mean duration of 15 h per week. When they were explored in our laboratory, their maximal apnea duration varied from 180 to 440 sec at rest (mean $= 302 \pm 30$ sec). Divers were compared to nine control individuals, whose static apnea duration ranged from 55 to 145 sec; (mean value $= 114 \pm 10$ sec). The two groups of individuals had the same mean age (30 ± 3 years and 32 ± 3 years, respectively) and the same mean weight (74 ± 2 kg and 75 ± 4 kg). 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.

2.2. Physiological variables and exercise protocol

The subjects were comfortably seated. The heart rate was continuously monitored (Cardiognost Hellige, Stutgart, Germany) as well as the blood oxygen saturation (Sa_{O_2},%) which was measured with a pulse oximeter (NPB 40, Nelcor Puritan Bennett, Pleasanton, CA). 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, Jaeger, Germany) computed breath-by-breath values of minute ventilation, end-tidal O_2 and CO_2 partial pressures, and O_2 uptake (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 (Pa_{O_2}) and carbon dioxide (Pa_{CO_2}) partial pressures, and arterial pH (pHa) were measured (Blood gases analyzer model 860, BAYER Diagnostics, Puteaux, France).

Dynamic forearm exercises consisted in 1-min periods of rhythmic handgrip at a rate of 30 cpm. For each trial the subject had to displace for 1 s, on a length of 3 cm, a 8.5 kg load. Then, the subject left the grip for a consecutive 1-s 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 3-min forearm exercise was 112 w. This dynamic handgrip protocol has already been used and data published (Steinberg et al., 2002). In the present study the same workload was used because measurements of the maximal handgrip strength in static condition with an appropriate strain gauge showed that all subjects developed near the same strength (40–44 kg).

2.3. Biochemical variables

On the side of the working forearm muscle, an antecubital vein was catheterized. Five milliliter 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 was measured enzymatically (lactate dehydrogenase) (BAYER Diagnostics model 860) and SEM of lactate measurements for the laboratory quality control was ± 0.05 mmol L^{-1}.

We used three blood markers of oxidative stress: first, 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), second, plasma RAA, and third, erythrocyte GSH. RAA and GSH explore 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). GSH is considered a more effective determinant in assessing oxidative stress-related changes than lipid peroxide levels (TBARS) or the consumption of ascorbate (RAA) (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 have already used in humans (Joanny et al., 2001; Steinberg et al., 2002). 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 (TCA). 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 200μl 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.

RAA concentration was estimated by spectrophotometry using the method based on the reduction of iron by ascorbic acid in the presence of orthophosphoric acid and α-α′dipyridyl (Maickel, 1960). One milliliter of plasma was deproteinized

in an equal volume of 10% TCA and vortexed. After centrifugation (2500 × *g* at 4 °C for 15 min), the supernatants were then stored at −80 °C for further biochemical analyses. In a test tube containing 500 μl of ascorbic acid extract in 5% TCA, we added 150 μl of 0.5% orthophosphoric acid, 2.5 ml of 0.5% α-α′-dipyridyl and 1 ml of 1% ferric chloride. After vortexing, the mixture was left for 10 min at room temperature in the dark. The optical density was measured at 525 nm on a spectrophotometer (Spectronic Genesys 2, Milton Roy Company, Rochester, New York). We used as a reference standard a freshly-prepared solution of 5.7 mmol L^{-1} ascorbic acid (Sigma Company), which was similarly treated.

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 × *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, 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 GSH in 5% metaphosphoric acid.

2.4. 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 to execute a 1-min dynamic handgrip exercise with the dominant arm. After a 20-min rest period, a second 1-min exercise was performed during which the subjects sustained apnea. Measurements at R20 served as control values for the following exercise trial.

2.5. Statistics

Results are expressed as mean ± SEM. A two-way analysis of variance for repeated measures was used to evaluate over time the changes in blood concentration 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. The *t*-test allowed to compare the variations of biochemical variables in the two groups of subjects. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Intergroup differences at rest

First, we searched for intergroup differences in resting individuals before they began the whole challenge. As indicated in Table 1, arterial blood gases, pHv, and venous blood concentrations of lactic acid, TBARS and RAA were not different. However, erythrocyte GSH concentration was significantly ($P < 0.001$) and markedly lower in divers than in the control group.

3.2. Consequences of static apnea

In both groups, some subjects hyperventilated before becoming apneic. However, we found no intergroup difference between pre-dive increase in minute ventilation, and no correlation between the magnitude of hypocapnia, measured by the expired end-tidal CO_2 pressure (a very sensitive index of hyperventilation), and the maximal post-dive changes in lactic acid, TBARS, RAA, and GSH.

In the control group, Table 1 clearly indicates that static apnea elicited hypoxemia plus hypercapnia with modest but significant blood acidosis (reduced pHv and increased lactic acid concentration), and also produced an oxidative stress (increased TBARS concentration and reduced concentrations of GSH and RAA).

Due to the lengthened apnea duration in trained breath-hold divers (mean apnea duration = 302 ± 30 sec), hypoxemia (measured at the breaking point of apnea) and maximal increase in lactic

Table 1
Static apneas

		Non divers ($n = 18$ static apneas)		Divers ($n = 18$ static apneas)
Apnea duration, s		114 ± 10	+++	302 ± 30
Pa_{O_2}, mmHg	Control	89 ± 3		91 ± 2
	Breaking point	62 ± 3***	++	50 ± 2***
Pa_{CO_2}, mmHg	Control	38 ± 1		38 ± 1
	Breaking point	46 ± 1***		47 ± 1***
pHv	Control	7.378 ± 0.004		7.383 ± 0.007
	Post-dive (R0)	7.367 ± 0.005*		7.375 ± 0.005
Lactic acid, mmol L^{-1}	Control	1.28 ± 0.07		1.45 ± 0.12
	Post-dive	1.59 ± 0.10*	+++	1.81 ± 0.10*
		($+0.16 \pm 0.01$ mmol L^{-1} min^{-1})		($+0.07 \pm 0.02$ mmol L^{-1} min^{-1})
TBARS, ng ml^{-1}	Control	410 ± 45		362 ± 62
	Post-dive (R0)	538 ± 34*	++	446 ± 37
		($+67 \pm 15$ μg ml^{-1} min^{-1})		($\pm$ 17 ± 3 ng ml^{-1} min^{-1})
RAA, μg ml^{-1}	Control	11 ± 2		8 ± 1
	Post-dive (R0)	6.8 ± 2.0*	+++	6.7 ± 0.5
		(-2.2 ± 0.1 μg ml^{-1} min^{-1})		(-0.3 ± 0.03 μg ml^{-1} min^{-1})
GSH, mg/100 ml erythrocytes	Control	51 ± 6	+++	16 ± 2
	Post-dive (R2)	34 ± 5*	++	11 ± 1
		(-9.0 ± 0.1 mg/100 ml min^{-1})		($-1.1 + 0.03$ mg/100 ml min^{-1})

Values are mean $\pm$SEM. Asterisks denote significant post-dive changes and crosses are used to indicate significant intergroup differences. Values in parenthesis correspond to the ratio of maximal post apnea variation to the corresponding apnea duration expressed in seconds.

acid concentration (measured at R5 in the two groups) were both significantly accentuated compared to control subjects (mean apnea duration = 114 ± 10 sec) (Table 1). However, despite the marked intergroup differences in apnea duration, hypercapnia measured at the breaking point as well as the maximal pHv reduction (found at R0) did not significantly differ in the two groups. In addition, we never measured significant post-apnea variations of the three indices of oxidative stress in our divers. These intergroup differences in the response to static apnea were markedly accentuated after dividing the maximal variation of each variable by the corresponding apnea duration (Table 1).

3.3. Effects of handgrip exercise

Because the subjects continued to breathe during the first exercise trial, they did not suffer from hypoxemia. The kinetics of post-exercise changes in lactic acid and indices of oxidative stress are shown in Fig. 1. In non divers, handgrip exercise elicited a significant increase in lactic acid and TBARS concentrations and also reduced the concentrations of GSH and RAA. In trained divers, the post-exercise increase in lactic acid ($+2.04 \pm 0.46$ mmol L^{-1}) was not significantly different from the one measured in control subjects ($+2.88 \pm 0.42$ mmol L^{-1}). However, divers had no variation of TBARS and GSH, and post-exercise RAA reduction was significantly lower than in control subjects (-2.2 ± 1.4 vs. -5.6 ± 1.7 μg ml^{-1} $P < 0.01$, respectively).

3.4. Effects of handgrip exercise during apnea (dynamic apnea)

The magnitude of hypoxemia and hypercapnia measured at the end of dynamic apnea was the

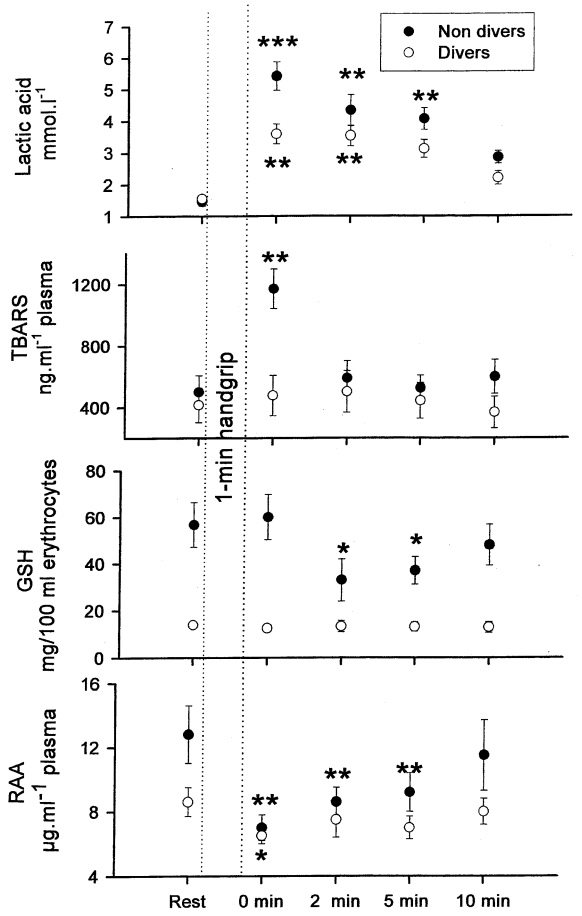

Fig. 1. One-minute handgrip exercise performed in breathing subjects. Asterisks denote the significance of post-exercise variations of lactic acid, TBARS, GSH and RAA (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

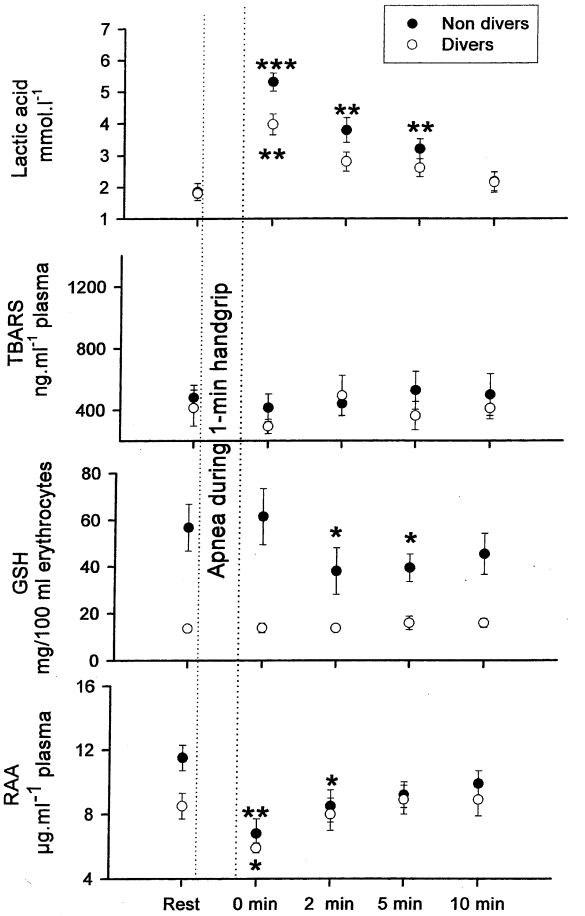

Fig. 2. One-minute handgrip exercise executed during breath-holding. Asterisks denote the significance of post-dynamic apnea variations of lactic acid, TBARS, GSH and RAA (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

same in the two groups: control subjects: $Pa_{O_2} = 68 \pm 3$ mmHg and $Pa_{CO_2} = 44 \pm 2$ mmHg; divers: $Pa_{O_2} = 63 \pm 4$ mmHg and $Pa_{CO_2} = 44 \pm 1$ mmHg.

Fig. 2 shows the changes in lactic acid and indexes of oxidative stress after dynamic apnea. In divers, the post-exercise increase in lactic acid concentration was significantly ($P < 0.01$) lower than in control subjects ($\pm 2.17 \pm 0.38$ mmol L^{-1} vs. $\pm 3.48 \pm 40$ mmol L^{-1}, respectively). In addition, there was no increase in TBARS and no reduction of GSH and the variations of RAA were always lower than in the control group. It must be pointed out that breath-holding also suppressed the post-exercise TBARS increase in control subjects.

4. Discussion

4.1. Summary and general comments

The present study shows in control subjects that static apnea elicits blood acidosis and an oxidative stress, associating increased TBARS level and consumption of endogenous anti-oxidants. These effects were majored after a 1-min handgrip

exercise during which they continued to breathe. However, despite the occurrence of hypoxemia during dynamic apnea, there was no further accentuation of blood acidosis and no further consumption of anti-oxidants. We even noted a suppression of the post-exercise TBARS increase. In well trained breath-hold divers, the changes in lactic acid, TBARS, RAA and GSH concentrations were markedly reduced after both static and dynamic apneas and also after control exercise.

The present observations of post-handgrip oxidative stress in control subjects corroborate recent data (Steinberg et al., 2002). However, in our previous study dynamic handgrip lasted 3 min and thus the post-exercise variations of TBARS, GSH and RAA were greater and their peak changes occurred earlier.

4.2. Specific influences of apnea

Our observations suggest the existence of a specific mechanism restricted to apnea, this phenomenon being markedly accentuated in trained human divers. First, in the two groups of subjects, the peak value of lactic acid was similar after the handgrip exercise and after the dynamic apnea, despite the fact that hypoxemia should rather increase the anaerobic glycolysis. Second, breath-holding during handgrip markedly reduced the post-exercise oxidative stress in control individuals. Thus, apnea itself seems to exert specific effects on cell metabolism. The explanation could be the occurrence of an apnea-induced reduction of muscle blood flow. Indeed, an apnea-induced reflex vasospasm in limb muscles is well documented (Craig and Medd, 1968b; Sterba and Lundgren, 1988). This phenomenon should reduce glucose and oxygen supplies to tissues, limiting the production of lactate and perhaps also of oxygen free radicals. However, the specific effects of breath-holding on muscle metabolism can not result from the sole reflex changes in muscle perfusion which do not explain the overall metabolic differences here reported between divers and non divers when they continued to breathe during handgrip trials. Indeed, dynamic handgrip exercise is rather responsible for a marked increase in muscle blood flow.

4.3. Metabolic consequences of repeated hypoxemia during breath-holding

As already said, there are no human studies to explain the high tolerance to hypoxia of elite breath-hold divers. Most of the previous studies in breath-hold divers were limited to measurements of the alveolar gas exchange at the breaking point of apnea (Courteix et al., 1993; Craig and Harley, 1968; Craig and Medd, 1968b; Hill, 1973; Lanphier and Rahn, 1963; Liner and Linnarsson, 1994, 1995; Linnarsson et al., 1993; Mithoefer, 1959), and the changes in blood pH and lactic acid concentration were documented in very few studies (Craig and Medd, 1968a; Olsen et al., 1962; Qvist et al., 1993). In the Korean ama, who breath-hold dived more than 100 times a day, Qvist et al. (1993) measured arterial blood gases and pH in blood samples taken immediately before diving, then at depth during continued breath-holding, and finally after breath-holding ended. During these experiments breath-hold dives maximally lasted 95 s (mean $= 62 \pm 4$ sec) and the arterial pH only decreased to 7.35. However, there was no comparison between these trained divers and control subjects.

In diving mammals (Weddell seals), Kooyman et al. (1981) clearly showed a modest post-apnea increase in lactic acid which was even absent when apnea duration was inferior to 20 min. No comparative studies were performed between adult, young, and newborn seals. Thus, there may be the possibility of the existence of adaptive mechanisms accentuating the genetic metabolic differences between diving and non-diving animal species. Hochachka et al. (1996) proposed a unifying theory of hypoxia tolerance mainly based on data in a highly anoxia tolerant aquatic turtle. After a defense phase, adaptation to hypoxia is expressed in a rescue phase in which hypoxia sensing and signal transduction result in preferential regulating expression of several proteins with significant gene-based metabolic reprogramming, maintaining a down-regulation of energy demand throughout the hypoxic period.

The reduced post-apnea increase in blood lactic acid concentration in seals and also in our trained breath-hold human divers could result from sev-

eral mechanisms. 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 can not separate the production from the catabolism of lactic acid. Thus, the present observations of reduced blood acidosis in well trained divers may signify a reduced production and/or an increased catabolism.

The present observations of attenuated post-exercise blood acidosis in trained divers may be compared to data collected in humans acclimated to hypoxia. It is well known that the blood lactic acid concentration at a given exercise intensity is greater in acute hypoxemia than in normoxemia (Knight et al., 1996) whereas, after acclimation to high altitude, 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). In chronic hypoxemic subjects, Bender et al. (1989) have evidenced a decrease in net lactic acid release from exercising leg but the mechanism of this phenomenon is not fully understood. It may result from a reduced production and/or an increased removal of lactic acid by exercising hypoxic muscles. Some studies suggest that chronic exposure to hypoxia increases the mobilization and use of free fatty acids during exercise, resulting in sparing muscle glycogen, and thus, in reduced lactic acid production (Jones et al., 1972; Young et al., 1982). We did not find data in the literature on similar observations of reduced blood lactic variations in humans repetitively exposed to short periods of hypoxemia, a circumstance produced by breath-hold diving.

4.4. Oxidative stress

Compared to control individuals, we measured in trained breath-hold divers, who were repeatedly exposed to hypoxia, lower resting level of GSH whereas TBARS and RAA concentrations were not affected. GSH being considered a powerful endogenous antioxidant (Lew et al., 1985; Sen et al., 1994) and also a more effective determinant in assessing oxidative stress than TBARS (Balakrishnan and Anuradha, 1998), we may suppose that trained divers had either a permanent increased consumption of GSH, due to the repetition of apnea-induced oxidative stress, or, on the contrary, that their resting antioxidant blood activity was lower, because of a reduced production of oxygen free radicals in response to hypoxia-reoxygenation. Our observations agreed with those by Singh et al. (2001) who reported a significant decrease in GSH content in skeletal muscles and blood of rats repeatedly exposed to hypoxia (6 h. day^{-1}) and by Radak et al. (1997) who found in the same species that high altitude (4000 m) training did not induce significant increase in TBARS content in white and red muscles. All the aforementioned data oppose the observations by Elsner et al. (1998) who measured a higher concentration of antioxidant enzymes at rest in the heart of resting seals than in pigs, non diving mammals.

Exhaustive exercise is associated with an enhanced formation of oxygen free radicals (Davies et al., 1982; Ebbeling and Clarkson, 1989; Sen, 1995; Sjödin et al., 1990; Steinberg et al., 2002; Suzuki et al., 1996). It results in membrane lipid peroxidation which triggers a post-exercise inflammatory reaction. Numerous human studies indicate that the changes in blood markers of the oxidative stress are not present during an exhaustive exercise, i.e. a period of unbalance between oxygen supply and demand in contracting muscle, but during the recovery on normal muscle reoxygenation (Sen, 1995; Sjödin et al., 1990; Steinberg et al., 2002). We observed that the succession of apnea and recovery on reoxygenation induced an oxidative stress in control subjects, whereas in trained divers this oxidative stress was absent after static apnea or markedly reduced after a handgrip exercise, whether the subjects breathed or sustained apnea. We also reported that dynamic apnea abolished the post-exercise TBARS increase in control subjects but did not affect the consumption of endogenous antioxidants. Thus, it seems that acute hypoxemia does not exacerbate membrane lipid peroxidation but rather attenuates it. Sjödin et al. (1990) first hypothesized that a reduced oxygen supply to muscle should decrease the oxygen free radical formation. In healthy subjects, a brief period of hypoxemia produced by the inhalation of a hypoxic gas mixture

abolished the post-handgrip increase in TBARS (Dousset et al., 2002). Apart from the direct effects of hypoxemia on cellular metabolism and production of oxygen free radicals, apnea is also responsible for a reflex vasospasm in limb muscles. This reflex response could lower the muscular oxygen stores, explaining the significant reduction of the oxidative stress in divers and also the absence of TBARS variations in control subjects after they sustained apnea when exercising.

4.5. Conclusions

Our study indicates that the post-apnea and also the post-exercise blood acidosis and the production of oxygen free radicals are attenuated in trained breath-hold divers. This may be considered an adaptive mechanism to repeated apnea because both acidosis and oxidative stress have noxious consequences on cellular and organ functions. At this time, one may only speculate on the progressive development of an adaptive metabolic response to repetitive apneas, which should be explored throughout longitudinal studies in subjects involved in a training programme of diving.

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References

Ashton, T., Rowlands, C.C., Jones, E., Young, I.S., Jackson, S.K., Davies, B., Peters, J.R., 1998. Electron spin resonance spectroscopic detection of oxygen-centred radicals in human serum following exhaustive exercise. Eur. J. Appl. Physiol. 77, 498–502.

Balakrishnan, S.D., Anuradha, C.V., 1998. Exercise, depletion of antioxidants and antioxidant manipulation. Cell Biochem. Funct. 16, 269–275.

Behrisch, H.W., Elsner, R., 1984. Enzymatic adaptations to asphyxia in the harbor seal and dog. Respir. Physiol. 55, 239–254.

Bejma, J., Ji, L.L., 1999. Aging and acute exercise enhance free radical generation in rat skeletal muscle. J. Appl. Physiol. 87, 465–470.

Bender, P.R., Groves, B.M., McCullough, R.E., McCullough, R.S., Trad, L., Young, A.J., Cymerman, A., Reeves, T.J., 1989. Decreased exercise muscle lactate release after high altitude acclimatization. J. Appl. Physiol. 67, 1456–1462.

Best, T.M., Fiebig, R., Corr, D.T., Brickson, S., Ji, L.L., 1999. Free radical activity, antioxidant enzymes, and glutathione changes with muscle stretch injury in rabbits. J. Appl. Physiol. 87, 74–82.

Butler, P.J., Jones, D.R., 1997. Physiology of diving of birds and mammals. Physiol. Rev. 77, 837–899.

Courteix, D., Bedu, M., Fellmann, N., Heraud, M.C., Coudert, J., 1993. Chemical and nonchemical stimuli during breath holding in divers are not dependent. J. Appl. Physiol. 75, 2022–2027.

Craig, A.B., Harley, A.D., 1968. Alveolar gas exchanges during breath-hold dives. J. Appl. Physiol. 24, 183–185.

Craig, A.B., Jr, Medd, W.L., 1968a. Oxygen consumption and carbon dioxide production during breath-hold diving. J. Appl. Physiol. 24, 190–202.

Craig, A.B., Jr, Medd, W.L., 1968b. Man's responses to breath-hold exercise in air and water. J. Appl. Physiol. 24, 773–777.

Davies, J.A., Quintanilha, A.T., Brooks, G.A., Packer, L., 1982. Free radicals and tissue damage produced by exercise. Biochem. Biophys. Commun. 107, 1198–1205.

Dhaliwal, H., Kirshenbaum, L.A., Randhawa, A.K., Singal, P.K., 1991. Correlation between antioxidant changes during hypoxia and recovery on reoxygenation. Am. J. Physiol. 261, H632–H638.

Dousset, E., Steinberg, J.G., Faucher, M., Jammes, Y., 2002. Acute hypoxemia does not increase the oxidative stress in resting and contracting muscle in humans. Free Radical Res. 36, 701–704.

Ebbeling, C.B., Clarkson, P.M., 1989. Exercise-induced muscle damage and adaptation. Sports Med. 7, 207–234.

Edwards, H.T., 1936. Lactic acid in rest and work at high altitude. J. Physiol. London 116, 367–375.

Elsner, R., Oyasaeter, S., Almaas, R., Saugstad, O.D., 1998. Diving seals, ischemia-reperfusion and oxygen radicals. Comp. Biochem. Physiol. A 119, 975–980.

Frei, B., England, L., Ames, B., 1989. Ascorbate is an outstanding antioxidant in human blood plasma. Proc. Nat. Acad. Sci. USA 86, 6377–6381.

Glascott, P.A., Tsyganskaya, M., Gilfor, E., Zern, M.A., Farber, J.L., 1996. The antioxidant function of the physiological content of vitamin C. Mol. Pharmacol. 50, 994–999.

Hill, P.McN., 1973. Hyperventilation, breath holding and alveolar oxygen tensions at the breaking point. Respir. Physiol. 19, 201–209.

Hochachka, P.W., Buck, L.T., Doll, C.J., Land, C.S., 1996. Unifying theory of hypoxia tolerance: Molecular/metabolic defense and rescue mechanisms for surviving oxygen lack. Proc. Natl. Acad. Sci. USA 03, 9493–9498.

Joanny, P., Steinberg, J.G., Robach, P., Richalet, J.P., Gardette, B., Jammes, Y., 2001. Operation Everest III (Comex'97): The effect of simulated severe hypobaric hypoxia on lipid peroxidation and antioxidant defence systems in

human blood at rest and after maximal exercise. Resuscitation 49, 311–319.

Jones, N.L., Robertson, D.G., Kane, J.W., Hart, R.A., 1972. Effect of hypoxia on free fatty acid metabolism during exercise. J. Appl. Physiol. 33, 733–738.

Knight, D.R., Poole, D.C., Hogan, M.C., Bebout, D.E., Wagner, P.D., 1996. Effect of inspired oxygen concentration on leg lactate release during incremental exercise. J. Appl. Physiol. 81, 246–251.

Kooyman, G.L., Castellini, M.A., Davis, R.W., 1981. Physiology of diving in marine animals. Ann. Rev. Physiol. 43, 343–356.

Lanphier, E.H., Rahn, H., 1963. Alveolar gas exchange during breath hold diving. J. Appl. Physiol. 18, 471–477.

Lew, H., Pyke, S., Quintanilha, A., 1985. Changes in glutathione status of plasma, liver and muscle following exhaustive exercise in rats. FEBS 185, 262–266.

Lew, H., Quantanilha, A., 1991. Effects of endurance training and exercise on tissue antioxidative capacity and acetaminophen detoxification. Eur. J. Drug. Metab. Pharmacokinet. 16, 59–68.

Liner, M.H., Linnarsson, D., 1994. Tissue oxygen and carbon dioxide stores and breath-hold diving in humans. J. Appl. Physiol. 77, 542–547.

Liner, M.H., Linnarsson, D., 1995. Intrapulmonary distribution of alveolar gas exchange during breath-hold diving in humans. J. Appl. Physiol. 78, 410–416.

Linnarsson, D., Ornhagen, H., Gennser, M., Berg, H., 1993. Breathing volumes and gas exchange during simulated rapid free ascent from 100 msw. J. Appl. Physiol. 74, 1293–1298.

Lovlin, R., Cottle, W., Pyke, I., Kavanagh, M., Belcastro, A.N., 1987. Are indices of free radical damage related to exercise intensity? Eur. J. Appl. Physiol. 56, 313–316.

Maher, J.T., Jones, L.G., Hartley, L.H., 1974. Effects of high-altitude exposure on submaximal endurance capacity of men. J. Appl. Physiol. 37, 895–898.

Maickel, R.P., 1960. A rapid procedure for the determination of adrenal ascorbic acid: application of the Sullivan and Clarke method to tissues. Anal. Biochem. 1, 498–501.

Mithoefer, J.C., 1959. Mechanism of pulmonary gas exchange and CO_2 transport during breath holding. J. Appl. Physiol. 14, 706–710.

Olsen, C.R., Fanestil, D.D., Scholander, P.F., 1962. Some effects of apneic underwater diving on blood gases, lactate and pressure in man. J. Appl. Physiol. 17, 938–942.

Qvist, J., Hurford, W.E., Park, Y.S., Radermacher, P., Falke, K.J., Ahn, D.W.A., Guyton, G.P., Stanek, K.S., Hong, S.K., Weber, R.E., Zapol, W.M., 1993. Arterial blood gas tensions during breath-hold diving in the Korean ama. J. Appl. Physiol. 75, 285–293.

Radak, Z., Asano, K., Lee, K., Ohno, H., Nakamura, A., Nakamoto, H., Goto, S., 1997. High altitude training increases reactive carbonyl derivates but not lipid peroxidation in skeletal muscle of rats. Free Rad. Biol. Med. 22, 1109–1114.

Reid, M.B., 1996. Reactive oxygen and nitric oxide in skeletal muscle. News Physiol. Sc. 11, 114–119.

Rokitzki, L., Logemann, E., Sagredos, A.N., Murphy, M., Wetzel-Roth, W., Keul, J., 1994. Lipid peroxidation and antioxidative vitamins under extreme endurance stress. Acta Physiol. Scand. 151, 149–158.

Sen, C.K., Atalay, M., Hänninen, O., 1994. Exercise-induced oxidative stress: glutathione supplementation and deficiency. J. Appl. Physiol. 77, 2177–2187.

Sen, C.K., 1995. Oxidants and antioxidants in exercise. J. Appl. Physiol. 79, 675–686.

Singh, S.N., Vats, P., Kumria, M.M.L., Ranganathan, S., Shyam, R., Arora, M.P., Jain, C.L., Sridharan, K., 2001. Effect of high altitude (7620 m) exposure on glutathione and related metabolism in rats. Eur. J. Appl. Physiol. 84, 233–237.

Sjödin, B., Westing, Y.N., Apple, F., 1990. Biochemical mechanisms for oxygen free radical formation during exercise. Sports Med. 10, 236–254.

Steinberg, J.G., Gainnier, M., Michel, F., Faucher, M., Arnaud, C., Jammes, Y., 2002. The post-exercise oxidative stress is depressed by acetylsalicylic acid. Respir. Physiol. Neurobiol. 130, 189–199.

Sterba, J.A., Lundgren, C.E.G., 1988. Breath-hold duration in man and the diving response induced by face immersion. Undersea Biomed. Res. 15, 361–375.

Suzuki, K., Sato, S., Kikuchi, T., Abe, T., Nakaji, S., Sugawara, T., Totsuka, M., Sato, K., Yamaya, K., 1996. Capacity of circulating neutrophils to produce reactive oxygen species after exhaustive exercise. J. Appl. Physiol. 81, 1213–1222.

Uchiyama, M., Mihara, M., 1978. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal. Biochem. 86, 271–278.

Young, A.J., Evans, W.J., Cymerman, A., Pandolf, K.B., Knapik, J.J., Maher, J.T., 1982. Sparing effect of chronic high altitude exposure on muscle glycogen utilization. J. Appl. Physiol. 52, 857–862.