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Not only training but also exposure to chlorinated compounds generate a response to oxidative stimuli in swimmers

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Abstract

Mechanism of toxicity of chlorinated compounds on health were not totally identified and oxidant pathway has been proposed as mediators of their toxicity. Relations between exposure to chlorinated compounds and biological markers of response to oxidative stimuli were investigated in swimmers, taking into account the effect of training.

Twenty-two male swimmers aged 15-25 years have been surveyed twice. Prevalence of irritant symptoms and asthma, and number of hours of training were reported. Exposure to NCl_3 and blood response to oxidative stimuli (catalase, superoxide dismutase, glutathione peroxidase activities and ceruloplasmin, ferritin and total antioxidant concentrations) were measured. Univariate analysis were completed by multivariate analysis.

High prevalences of irritant symptoms and asthma were found. Multivariate analysis confirmed the results of univariate analyses and showed that Cu^{++}/Zn^{++} SOD activity was increased by exposure and by training ($p=0.01$, $p=0.0001$ respectively). Erythrocyte GSH-Px was decreased whereas plasma GSH-Px was increased by exposure ($p=0.002$, $p=0.002$). No other association was found.

Higher irritant symptoms and increases in the activities of erythrocyte Cu^{++}/Zn^{++} SOD and of plasma GSH-Px with exposure support well the hypothesis that the production of reactive oxygen species is not only related to training but also to exposure to chlorinated compounds.

Introduction

The effects of chlorinated compounds on health and the mechanism of their toxicity were not totally and clearly identified, even if chlorination is the oldest and the most widely used method of water disinfection. The use of chlorine-containing agents for the disinfection of water in swimming pool leaded to the formation of various chlorinated compounds, chloramines and halomethanes, resulting from reactions with the N and C-compounds introduced by human sweat, urine and skin (Grisham et al. 1984; Seux 1988). In animals, high doses of chlorinated compounds were well-known for having hepatotoxic and nephrotoxic effects (Hard 1998; Rossi et al. 1999; Di Consiglio 2001) and carcinogenic properties (Jorgenson et al. 1985). Increasing evidence of carcinogenic effects of chlorinated compounds and of the possible effects of these compounds on reproductive outcomes have been reported by epidemiological studies (IARC 1991; Tominaga and Midio 1999; Nieuwenhuijsen et al. 2000; Jaakkola et al. 2001). But only few studies have focused on the possible effects of exposure to chlorinated compounds on health in swimmers. Aiking et al. (1994) have studied the hepatotoxic and nephrotoxic effects of low doses of chlorinated compounds in 18 competitive swimmers and found that the β-2 microglobulin, an indicator of renal damage, was significantly higher in the urine samples of younger indoor swimmers. Helenius et al. (1998) have reported that a long-term and repeated exposure to chlorinated compounds in swimming pools during training and competition may contribute to the increased occurrence of bronchial hyperresponsiveness and airway inflammation in swimmers. Previously, we have reported that lifeguards exposed to chlorinated compounds in indoor swimming pool were at risk of developing irritant eye, nasal and throat symptoms (Massin et al. 1998). Recently, Nemery et al. (2002) have reported that chlorination may affect the respiratory health of competitive swimmers and Thickett et al. (2002) have showed that nitrogen trichloride (an

inorganic compound of chloramines) could be a cause of occupational asthma in swimming instructors and lifeguards.

Free radicals and reactive oxygen species (ROS) as hydroxyl radical, superoxide anion and hydrogen peroxide, have been proposed as mediators of the toxicity of chloroform and of various halomethanes (Tomasi et al. 1985; Rush et al. 1986; Zamora et al. 1990). Chlorinated compounds are volatile (Aggazzotti et al. 1990), resulting in high concentrations immediately above the water surface (Weiberg 1987), are long-lived (Levesque et al. 1994) and their principal routes of exposure are inhalation and dermal absorption (Lindstrom et al. 1997). The continuous production of ROS may overwhelmed antioxidant defenses and result in an oxidative stress leading to several diseases as chronic lung diseases and nephrological diseases (Sies 1985; Halliwell and Gutteridge 1989). Erythrocytes are circulating antioxidant carriers that can penetrate lung capillaries and have been shown to play a significant role in protection against lung damage due to ROS (Toth et al. 1984; Van Asbeck et al. 1984; Agar et al. 1986).

Among the few studies which have been involved in the variations of the markers of response to oxidative stimuli in swimmers, none of them have studied the effect of exposure to chlorinated compounds (Dickson et al. 1982; Lukaski 1989; Lukaski et al. 1990; Inal et al. 2001; Santos-Silva et al. 2001). Moreover, none of them have measured more than two markers of response to oxidative stimuli, their control groups were usually non-exposed and non-training groups, and age ranges and sex were not the same. Finally, they have investigated different ways of training. It's well known that physical exercise was associated with response to oxidative stimuli in two ways (Moller et al. 1996). On one hand, exercise increased oxidative metabolism and this induced oxidative stress, but on the other hand, the adaptation to regular exercise seemed to have an antioxidant protective effect.

In this study, we investigated the relations between chronic exposure to chlorinated compounds with several markers of response to oxidative stimuli in swimmers and we better understood these relations in taking into account the effect of training. Swimmers were surveyed twice: one week without exposure and one week with exposure to chlorinated compounds. The response to ROS exposure was biologically measured with specific and sensitive blood markers (catalase, Cu^{++}/Zn^{++} superoxide dismutase (Cu^{++}/Zn^{++} SOD), glutathione peroxidase (GSH-Px), total plasma antioxidants, serum ferritin and ceruloplasmin) (Halliwell and Gutteridge 1989). At each week, swimmers were examined twice before swimming in order to dismiss the acute effects of exposure or/and training. Training was assessed as swimming and other sport activities.

Material and methods

Study sample

Our study was performed in an indoor swimming pool located in Nancy (north east France). The participation was proposed to all male swimmers from the same club, training exclusively in this swimming pool during the season with the following criteria: the swimmers must be 15-25 years old and train regularly (at least once a day) without training the last week and the last weekend before examination. The participation rate was 80%. Our study sample was composed of 22 swimmers. Swimmers were examined twice the week after the draining of the swimming pool (week0 (W0), day1 (D1) and day4 (D4)) and twice 24 weeks later, before the draining of the swimming pool (week24 (W24), D1 and D4). In April 1999, works of renovation began in the swimming pool of our study. A new technique of water treatment with new pumps and the installation of "goulottes finlandaises" were realized, leading to a decrease in the concentration of chlorinated compounds (Hery et al. 1995). Among the 22 swimmers, 8 have benefited from these improvements (group 2) and 14 have

not (group 1). This study was approved by the appropriate ethical committee and a written informed consent obtained from each swimmer.

Examination and measurements of training and exposure

Each examination included a questionnaire on medical history, medication, dietary and smoking habits, alcohol consumption and sport activities. The frequency of consumption of fruits and of green or red vegetables (raw or cooked) was obtained from the questionnaire. The dietary score was the mean of the three scores obtained at each question. Training was defined as swimming and other aquatic and non aquatic sport activities. The number of years of swimming, the number of hours of swimming the last week and the last weekend before the week of examination were recorded. The number of hours of swimming between day1 and day4 and the number of hours of sports activities were also recorded.

Exposure to disinfection by-products was evaluated by the chloramine concentration in the atmosphere of the swimming pool and by the presence of trihalomethanes (THM) in the blood of the swimmers. Chloramine concentration was measured by the technique of Hery et al. (1995). A feasibility study performed in this swimming pool during the 1997-1998 season showed a difference in the atmospheric chloramine concentration between week0 and week24 (0.17 mg/m3 vs. 0.49 mg/m3). The exposure assessment campaign was conducted systematically during day1 and day4 at each week of examination. Series of 6 to 10 samples were taken at the water surface in order to be representative of the swimmers exposure to chloramines. Special care was taken to avoid the filters to be splashed. The sampling duration was from 2 to 3 hours, representative of the exposure during the swimming session and providing a sufficient analytical sensitivity.

Trihalomethane determinations were conducted using the headspace technique with a gas chromatograph equipped with a split injector and an ^{63}Ni electron capture detector (Ambroise et al. 1995).

Biological markers of response to oxidative stimuli

At each week, blood samples were collected twice before swimming, at day1 and day4. On the same day, corresponding haemolysates, plasma and serum were prepared as described previously (Perrin et al. 1990), and stored at -80°C.

Total plasma antioxidant concentration (ascorbate, protein thiols, bilirubin, urate and α tocopherol) , Cu^{++}/Zn^{++} superoxide dismutase (Cu^{++}/Zn^{++} SOD), glutathione peroxidase (GSH-Px) and catalase activities were measured by methods previously described (Perrin et al. 1990; Nadif et al. 1998). Activities were measured in the two weeks following storage and expressed as U/g Hb (Cu^{++}/Zn^{++} SOD, GSH-Px) or k/g Hb (catalase) in erythrocytes, and U/L (GSH-Px) in plasma.

Ferritin concentration was measured at 37°C at 600 nm in a Cobas-Mira S analyser with a Roche kit (ABX, Montpellier, France). Results were expressed as ng/ml. Ceruloplasmin concentration was measured at 37°C at 340 nm in a Cobas-Mira S analyser with a Roche kit (ABX, Montpellier, France). Results were evaluated by comparing calibrators of known concentrations and were expressed as g/l.

All samples were analyzed in duplicate or triplicate and the precision (coefficient of variation) was <10%. The accuracy was checked by analyzing external reference samples together with the test samples.

Statistical analyses

Standard statistical tests were used including X^2 test (or Fisher's exact test when appropriate), McNemar's test, Student's *t* test and Pearson's correlation test (*r*). Concordance between questionnaires for variables concerning swimmer's history was quantified using Kappa coefficient for qualitative variables and intraclass correlation coefficient for quantitative variables. Swimmers were classified into subgroups according to their tertiles of the number of hours of swimming between day1 and day4 (≤4.2, from 4.2 to 9, >9).

To describe the factors associated with changes in biological markers of response to oxidative stimuli taking into account the two follow-up factors, exposure and training, mixed models were used with the MIXED procedure. Confounding factors were put into the model. Validity of the models was checked with the null model likelihood ratio test and with the number of iterations of the criteria of convergence. Although the number of swimmers included in the multivariate analyses was small (n=16), it was sufficient to achieve a power of 80%.

Significance was assessed at the 5% two-sided level. All these analyses were performed with SAS statistical software (SAS Institute, Cary, North Carolina, 2000).

Results

Characteristics of swimmers and prevalence of irritation symptoms

Among the 22 swimmers who took part in the study, 16 have returned two questionnaires. The concordance for responses of past events between W0 and W24 was acceptable (all values were higher than 0.6, Kappa or intraclass correlation coefficient). Then, only the questionnaire returned at W0 was taken into account for past events for all swimmers.

Characteristics of the swimmers at W0 were described in table 1. As expected, their mean age was between 15 and 25 years and their number of years of swimming was at least one year. Their alcohol consumption corresponded approximately to 0.25 liter of red wine per day. This consumption was 1.4 fold time lower than that of the French male consumption aged 18-24 years (Guilbert et al. 1997).

Irritant eye symptoms (red, burning or weary eyes), irritant nasal symptoms (running, burning nose or continued sneezing), throat irritations (sore throat or dry cough) and asthma were reported in table 2. In absence of control population, these values were compared with those obtained in a group of 256 male lifeguards (Massin et al. 1998) also exposed to chlorinated compounds. The prevalence of irritant nasal symptoms, throat irritation and asthma were significantly higher in swimmers.

Swimmers surveyed twice

Based on available data, comparisons of swimmers lost to follow-up (n=6) and the remainder (n=16) did not show difference regarding characteristics, training and exposure. Swimmers in whom data were available at both weeks (n=16) were 19.8 (3.8) years old, non-smokers and had a mean number of swimming of 4.4 years. Half of them currently took drugs and all of them took vitamins. Their dietary score was 2.5 (0.9) and their alcohol consumption was 13.3 g/d (14.1). Sixty seven percent never drank aperitif, 13% drank between 1 and 4 aperitifs a month and 20% drank more than 4 aperitifs a month. Comparison of sport activities between W0 and W24 in swimmers surveyed twice showed that the number of hours of swimming between D1 and D4 and the number of hours of swimming the last week were significantly higher in W24 than in W0.

Comparison of markers of response to oxidative stimuli between D1 and D4 showed several significant differences between both weeks (table 3). At W0, erythrocyte Cu^{++}/Zn^{++}

SOD activity and GSH-Px activities were significantly increased in D4. Moreover, erythrocyte GSH-Px activity at D4 was significantly and positively correlated with the number of hours of sport activities between D1 and D4 and positively correlated with the number of hours of swimming between D1 and D4 (r=0.46, p=0.04; r=0.41, p=0.09, respectively). Plasma GSH-Px activity and serum ferritin concentration at D4 were significantly and negatively correlated with the number of hours of swimming the last week (r=-0.66, p=0.007; r=-0.67, p=0.007, respectively). At W24, erythrocyte Cu^{++}/Zn^{++} SOD activity was also significantly increased in D4 but no significant increase in erythrocyte GSH-Px activity was observed. At D4, Cu^{++}/Zn^{++} SOD activity was significantly and positively related to the number of hours of non-aquatic sport activities (r=0.98, p=0.004). On the contrary, plasma GSH-Px activity and ceruloplasmin concentration were significantly lower in D4.

Among the 11 swimmers surveyed twice at W24, 80% of those with a number of hours of swimming between D1 and D4>9 had marks of THM in plasma (data not shown). Highly-exposed swimmers (marks of THM in plasma and hours >9) were younger, non-smokers, all of them were current drug users and their current drug consumption was significantly higher than lowly-exposed swimmers (no mark of THM in plasma and hours ≤9). Highly-exposed swimmers were all from the group 1. Most of them swam each day between blood collections and their number of hours of swimming the last week were higher than those of the low-exposed swimmers. As expected, highly-exposed swimmers had a number of hours of swimming between D1 and D4 two fold time significantly higher than lowly-exposed swimmers (11.6 vs. 5.4 hours, p=0.06) and plasma THM concentrations were higher in highly-exposed swimmers in D1 and in D4 (1.3 ± 2.6 µg/l vs. 0.7 ± 1.4 µg/l; 0.6 ± 0.4 µg/l vs. 0.4 ± 1.1 µg/l, in D1 and D4 respectively). No significant difference was observed in biological markers of response to oxidative stimuli between both groups of exposure at D1.

Erythrocyte catalase activity was significantly increased in D4 in highly-exposed swimmers (147 ± 27 k/g Hb vs. 175 ± 37 k/g Hb, in D1 and D4 respectively, p=0.03) and plasma GSH-Px activity was decreased in D4 in both groups with a significant difference in lowly-exposed swimmers (662 ± 69 U/l vs. 623 ± 58 U/l, in lowly-exposed swimmers in D1 and D4 respectively, p=0.05).

Multivariate analysis

Multivariate analyses were performed to study the associations between biological markers of response to oxidative stimuli and exposure, including the effect of training (table 4).

Only erythrocyte Cu^{++}/Zn^{++} SOD and GSH-Px activities were found to be significantly associated with exposure and training.

Several interactions were found. The difference in the level of erythrocyte GSH-Px activity between the group 1 and the group 2 depended on the week of examination and on the number of hours of swimming between D1 and D4. In group 2, erythrocyte GSH-Px activity decreased with the number of hours of swimming between D1 and D4 and was higher in W24. The difference in the level of plasma GSH-Px activity in W24 depended on day of measurement and on age. In W24, plasma GSH-Px activity was lower in oldest swimmers and was lower in D4 than in D1, which may partly explain the results observed at W24 in all, as in highly- and lowly-exposed swimmers.

Discussion

To our knowledge, this study is the first to describe associations between biological markers of response to oxidative stimuli not only with training but also with exposure to chlorinated compounds in swimmers. In this study, we found that erythrocyte Cu^{++}/Zn^{++} SOD

activity was related to training and exposure to chlorinated compounds and that GSH-Px activities were related to exposure to chlorinated compounds.

We found no change in catalase activity and ceruloplasmin concentration with exposure nor with training. Our results are consistent with those of other studies (Buchman et al. 1988; Ji 1993; Marzatico et al. 1997; Inal et al. 2001; Miyazaki et al. 2001). We found a weak increase in ferritin concentration after training, as previously described by Lukaski et al. (1990) and Dickson et al. (1982). We also reported a weak decrease in total plasma antioxidant concentration with exposure. In contrast, Santos-Silva et al. 2001 reported an increase in total plasma antioxidant concentration in swimmers. Our results were not confirmed by multivariate analysis and difference in design with study previously mentioned did not allow to discuss the results.

The increase in Cu^{++}/Zn^{++} SOD activity we found in D4 at each week is in agreement with other studies. Miyazaki et al. (2001) have reported an increase in Cu^{++}/Zn^{++} SOD activity in 9 healthy, untrained and 19-21 years old male subjects after a 12 week running program. Marzatico et al. (1997) have reported that basal Cu^{++}/Zn^{++} SOD activity was higher in athletes (6 marathon runners aged 26.8 years and 6 national sprint-trained athletes aged 25.2 years) as compared to 6 healthy males aged 28.5 years and normally physically active. They also found that Cu^{++}/Zn^{++} SOD activity was significantly increased in the runners and in the sprinters after a training session and suggested that this result could reflect a training effect. Finally, they concluded that a potential oxidative stress was generated by daily training sessions. Only one follow-up study has investigated the effect of training on markers of response to oxidative stimuli in swimmers (Lukaski et al. 1990). The authors found an increase in erythrocyte Cu^{++}/Zn^{++} SOD activity in 13 male swimmers after 6 months of training. They also found that before and after the training season, Cu^{++}/Zn^{++} SOD activity was higher in swimmers than in age-matched untrained controls and concluded to a training effect. We found that Cu^{++}/Zn^{++}

SOD activity was also increased by exposure. In spite of no information on the exposure to chlorinated compounds in the swimming pool of the study of Lulaski et al.(1990) (draining, chlorinate rate, etc.), we may not exclude that the "training effect" they observed was in fact a combined effect of training and exposure. Moreover, they didn't perform any multivariate analyses taking separately into account the effect of exposure and the effect of training. In our study, the relation between Cu^{++}/Zn^{++} SOD activity and exposure was reinforced by the fact that Cu^{++}/Zn^{++} SOD activity was higher in group 1 than in group 2. One may not explain the difference between group 1 and group 2 by a difference in one or several characteristics of the swimmers or in their ways of training because these two groups were totally similar regarding to these covariables and the only difference we know was the water treatment. The difference we observed between the groups could be partly due to a difference in exposure to chlorinated compounds. All these results well supported the hypothesis that exposure and training were both involved in the increase of the Cu^{++}/Zn^{++} SOD activity.

We found several variations in GSH-Px activities, mostly with exposure. Inal et al. (2001) previously reported that erythrocyte GSH-Px activity was higher one minute after both long-distance and short-distance swimming in 19 male or female aged 15-21 years than at rest. Marzatico et al. (1997) and Ji (1993) also observed an increase in erythrocyte GSH-Px activity after training with a concomitant increase in erythrocyte Cu^{++}/Zn^{++} SOD activity. We showed that Cu^{++}/Zn^{++} SOD activity was related to training. Our explanation is that the increase in erythrocyte GSH-Px activity we observed in D4, as those observed in the studies of Marzatico et al. (1997) and of Ji (1993) was in fact a response to an increase in erythrocyte Cu^{++}/Zn^{++} SOD activity. The increase in Cu^{++}/Zn^{++} SOD activity leads to a production of H_2O_2 following by an increase in GSH-Px activity (Halliwell and Gutteridge 1989). This is underlined by the positive correlation we found between erythrocyte Cu^{++}/Zn^{++} SOD and GSH-Px activities in W0 ($r=0.39$, $p=0.01$). In W24, we found a decrease in erythrocyte GSH-

Px activity with exposure and a negative correlation between erythrocyte Cu^{++}/Zn^{++} SOD and GSH-Px activities (r=-0.41, p=0.03) and we found that erythrocyte Cu^{++}/Zn^{++} SOD activity was positively related to exposure and training. The capacity of Cu^{++}/Zn^{++} SOD activity to be overwhelmed by the high production of $O_2^{\cdot -}$, and the excess of $O_2^{\cdot -}$ able to inactivate GSH-Px activity (Blum and Fridovich 1985) could explain our results. A similar inactivation of GSH-Px activity was not observed in plasma and no correlation between erythrocyte Cu^{++}/Zn^{++} SOD and plasma GSH-Px activities was observed in W0 nor in W24. A difference in the homology between plasma and cytosolic GSH-Px and the fact that both biological compartments are distinct, may explain these results (Harris 1992). Nevertheless, we cannot exclude that the positive association between plasma GSH-Px and exposure found in the multivariate analysis could reflect in part a compensatory mechanism of the lack of increase in erythrocyte GSH-Px activity. As we are the only study dealing with the relation between exposure and plasma GSH-Px, this is open to debate.

Another interesting finding that indirectly supported the hypothesis of a production of reactive species from exposure to chlorinated compounds was the higher prevalence of eye, nasal and throat irritant symptoms observed in swimmers than in lifeguards. Among lifeguards, the prevalence tended to increase with increasing exposure to nitrogen trichloride (NCl_3) (Massin et al. 1998). The lifeguards worked essentially outside the water, in the surrounding area of the pool and the swimmers were directly exposed to chlorinated compounds on the pool water surface. Exposure to higher concentration of NCl_3 could explain higher prevalence reported in swimmers. Zwick et al. (1990) studied 14 competitive swimmers and 14 matched control subjects. Conjonctival or respiratory symptoms (rhinitis, laryngitis and bronchitis) were founded in 11 swimmers and in 3 controls. They concluded that this high prevalence could be due to repeated exposure to chlorine in swimming pools. We also found a high prevalence of asthma in swimmers. Nystad et al. (2000) studied asthma

among elite athletes included all Norwegian athletes of the national junior and senior teams in 1997 (n=1620) and a random sample from the general population (n=1680). Their results indicated that the prevalence of asthma was greater among athletes (10%) as compared to the general population (6.9%) and that the risk of asthma was the highest in sport requiring strength and endurance. Helenius and Haahtela (2000) supported the same judgment after reporting that asthma is most commonly found in endurance events, such as cycling, swimming, or long-distance running. But they also found that the risk of asthma was especially increased among competitive swimmers of which 36% to 79% show bronchial responsiveness to methacholine or histamine. High or intensive training but also exposure to chlorinated compounds could explain this high prevalence of asthma.

In conclusion, results reported in this study are in good agreement with the hypothesis that the production of reactive oxygen species is not only related to training but also related to exposure to chlorinated compounds, and that ROS may partly explain the toxicity of chlorinated compounds.

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Table 1 Characteristics of swimmers at week0 (n=22)

Age (y, mean (SD))	20.2 (3.8)
Current smokers (n (%))	1 (4)
Cigarette smoking (cigarettes/day, n)	25
Sport activity (n (%))	
Water-Polo	10 (45)
Tri-athlete	6 (27)
Only swimming	5 (23)
Other	1 (5)
Number of years of swimming (y, geometric mean)	4.1
Current medications (n (%))	
Drugs	11 (55)
Multivitamin supplement	22 (100)
Dietary score (mean (SD))*	2.6 (0.8)
Alcohol consumption (g/day) (mean (SD))	13.8 (14.2)
Aperitif consumption (n (%))	
Never	13 (65)
1 to 4 drinks / month	4 (20)
> 4 drinks / month	13 (65)

*: means of the three scores of dietary habits ranging from 1 (never) to 4 (at least once a day).

Table 2 Comparison of current irritation symptoms and asthma between swimmers at week0 and lifeguards

	Swimmers (n=22)	Lifeguards (n=256)*	p Value
Irritation symptoms:			
Eye	16 (73)	167 (65)	0.5
Nose	17 (77)	87 (34)	<0.0001
Throat	12 (54)	64 (25)	0.003
Asthma	3 (14)	7 (3)	0.04

Results are expressed as n (%).

*: data obtained from Massin et al. (1998) using same questions.

Table 3 Comparisons of biological markers of oxidative stress between D1 and D4 at each week in swimmers surveyed twice

	W0				W24			
	D1 (n=20)	D4 (n=18)	D4-D1 (n=17)	p Value	D1 (n=15)	D4 (n=12)	D4-D1 (n=11)	p Value
Chloramine concentration (mg/m^3)								
In group 1 / 2	0.27/0.25	0.27/0.24	—	—	0.32/0.30	0.19/0.30	—	—
In erythrocytes:								
Catalase (k/g Hb)	160 (37)	173 (31)	3.11 (42.50)	0.8	157 (27)	161 (28)	3.60 (27.18)	0.7
Cu^{++}/Zn^{++} SOD (U/g Hb)	152 (18)	176 (19)	21.6 (17.5)	0.0001	173 (22)	187 (25)	21.7 (23.6)	0.01
GSH-Px (U/g Hb)	40.9 (9.4)	42.7 (10.3)	2.36 (3.68)	0.02	33.8 (7.3)	34.0 (6.7)	0.38 (2.75)	0.7
In plasma:								
GSH-Px (U/L)	567 (119)	631 (101)	61.5 (77.1)	0.005	653 (83)	635 (57)	-46.5 (48.5)	0.01
Total antioxidant concentration (mmol/L)	1.40 (0.07)	1.38 (0.07)	-0.007 (0.061)	0.6	1.37 (0.11)	1.34 (0.10)	-0.01 (0.10)	0.6
In serum:								
Ferritin (ng/mL)	55.3	56.2	0.11 (0.22)	0.06	36.3	46.5	0.11 (0.45)	0.5
Ceruloplasmin (g/L)	0.19 (0.02)	0.19 (0.03)	0.003 (0.017)	0.6	0.19 (0.02)	0.18 (0.02)	-0.007 (0.008)	0.03

Results are expressed as mean (SD) except for ferritin (geometric mean).

Table 4 Factors associated with biological markers of oxidative stress in swimmers surveyed twice

	Erythrocyte Cu^{++}/Zn^{++} SOD (U/g Hb)		Erythrocyte GSH-Px (U/g Hb)		Plasma GSH-Px (U/L)		Total antioxidant concentration (mmol/L)	
	Coefficient (SD)	*p Value*	*Coefficient (SD)*	*p Value*	*Coefficient (SD)*	*p Value*	*Coefficient (SD)*	*p Value*
Exposure: *W24 vs. W0*	16.1 (5.28)	0.01	-15.3 (1.37)	0.002	246 (52.7)	0.002	-0.038 (0.021)	0.09
Training: *D4 vs. D1*	19.3 (3.49)	0.0001	-0.11 (1.17)	0.9	71.0 (27.3)	0.3	-0.014 (0.021)	0.5
Group: *2 vs. 1*	-25.8 (4.53)	< 0.0001	-8.29 (4.19)	0.9	-4.44 (25.7)	0.9	-0.003 (0.032)	0.9
Hours of swimming between D1 and D4			0.24 (0.33)	0.15				
Hours of swimming between D1 and D4 * group 2			-1.47 (0.58)	0.06				
Exposure * group 2			15.5 (2.38)	0.0009				
Age					14.2 (4.42)	0.01		
Exposure * age					-6.99 (2.69)	0.02		
Exposure * D4					-94.0 (16.2)	< 0.0001		