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Original paper

Redox, iron, and nutritional status of children during swimming training

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Abstract

Effects of exercise training on important determinants of children's long-term health, such as redox and iron status, have not been adequately investigated. The aim of the present study was to examine changes in markers of the redox, iron and nutritional status of boy and girl swimmers during a prolonged period of training. 11 boys and 13 girls, aged 10–11 years, were members of a swimming club. They were assessed at the beginning of the training season, at 13 weeks and at 23 weeks through blood sampling and recording of the diet. Reduced glutathione increased at 13 and 23 weeks, whereas oxidised glutathione decreased at 13 weeks, resulting in an increase of the reduced/oxidised glutathione ratio at 13 and 23 weeks. Total antioxidant capacity, catalase, thiobarbituric acid-reactive substances, hemoglobin, transferrin saturation and ferritin did not change significantly. Carbohydrate intake was below 50% of energy and fat intake was above 40% of energy. Intakes of saturated fatty acids and cholesterol were excessive. Iron intake was adequate but intakes of folate, vitamin E, calcium and magnesium did not meet the recommended daily allowances. No significant differences were found between sexes in any of the parameters measured. In conclusion, child swimmers improved the redox status of glutathione during training, although the intake of antioxidant nutrients did not change. The iron status was not impaired by training. Suboptimal intake of several nutrients suggests the need for nutritional monitoring and education of children athletes.

Keywords: Antioxidants; Glutathione; Micronutrients; Oxidative stress

1. Introduction

Oxidative stress has been implicated in a variety of physiological and pathological conditions.[1] Although regular exercise is known to enhance antioxidant defenses in adults,[1] few data exist concerning its effect on the redox status of children.[2] This issue merits attention, as participation in several sports including swimming, commences in childhood. We know of only two studies that examined the effect of training on the antioxidant capacity of children.[3,4] Their duration was rather short (1 month), and findings were equivocal. Additionally, acute swimming increased oxidative stress in children,[5] and child swimmers exhibited higher oxidative stress and lower antioxidant capacity compared to untrained counterparts at rest.[6]

Data on the iron status of children athletes are scarce too. Besides being important for many physiological functions, iron is related to the redox status because of its involvement in free radical formation.[7] Although some studies have monitored the iron status of adolescent and adult athletes during training,[8,9] to our knowledge, only one study[10] has focused on the iron status of children athletes.

It is well established that nutrition affects the redox and iron status,[11] thus being critical for health and physical performance. Nevertheless, recent reports show suboptimal dietary habits in adult swimmers.[12,13] Numerous studies have focused on the nutrition of nonathletic children (e.g., ref. [14])

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or adult athletes (e.g., ref. [15]) but few have examined children athletes[16,17] and none have examined child swimmers during a training period.

Considering the metabolic and physiologic differences between children and adults, as well as between sexes, in their responses to exercise,[18] one would expect to find more studies investigating the effect of training on the redox status of boys and girls. Methodological difficulties in studying children (consent, compliance, etc.) may explain this dearth of information. Thus, the aim of the present study was to examine changes in the redox, iron, and nutritional status of boy and girl swimmers during a prolonged period of training.

2. Methods

16 boys and 16 girls, aged 10–11, participated initially in the study but data from 5 boys and 3 girls were excluded because they did not train regularly and/or missed a programmed blood sampling because of illness. All participants were members of a swimming club and had been training for at least 1 year. They were at Tanner stages 1–2, based on pubic hair development estimated by self-determination with the aid of pictures, and did not change Tanner stage throughout the study. No girl had experienced menarche. Parents and children provided written informed consent. Procedures were in accordance with the Helsinki declaration and were approved by the Institutional Review Board.

The children were subjected to blood sampling, dietary assessment, anthropometric measurements, and performance measurements at three time points: at the beginning of the training season (baseline), after 13 weeks of training, and at 23 weeks of training. Each training session lasted 75–90 min, the distance swum was 2687 ± 547 m, and the children attended at least 3 sessions per week. The design of training was according to the training contents for these ages.[19,20] The children participated in regional swimming competitions and attended physical education classes at school twice weekly.

Participants provided 6 mL of venous blood into a plain evacuated tube and 2 mL into an EDTA tube. Practical reasons dictated that sampling take place in the afternoon. To minimise possible effects of the last meal and exercise session, the children took a light meal at least 3 h and abstained from exercise for 24–26 h before sampling. 1.5 mL of blood from the first tube was handled for glutathione analysis as described[5]; the remaining was used to prepare serum for all other biochemical analyses. The EDTA blood was used for hematology analysis.

Hematocrit, hemoglobin, erythrocyte count and leukocyte count were measured in a Coulter Microdiff autoanalyser (Miami, FL, USA). Reduced glutathione (GSH), oxidised glutathione (GSSG), total antioxidant capacity (TAC), catalase and thiobarbituric acid-reactive substances (TBARS) were assayed as described.[5] Iron was determined spectrophotometrically through a reagent kit from Biosis (Athens, Greece). Total iron-binding capacity (TIBC) was determined likewise after saturation of transferrin with Fe^{3+} and precipitation of the excess Fe^{3+} with a kit from Elitech (Sees, France). Transferrin saturation was calculated as iron/TIBC $\times$ 100. Ferritin and cortisol were assayed by enzyme immunoassay (DRG, Marburg, Germany). Creatine kinase (CK) was determined spectrophotometrically (Dialab, Vienna, Austria).

Participants recorded food intake for 3 days (2 weekdays and 1 weekend day) before each blood sampling with the aid of their parents according to oral and written instructions. Dietary records were analysed in Microsoft® Access through a food database created on the basis of published data.[21] Children did not use nutritional supplements. Body weight was measured by an electronic balance and height was measured by a stadiometer. To assess performance, a 100 m individual medley test[20] was performed in a 25 m pool. Time was recorded manually with a digital stopwatch.

Data are reported as means (95% CI). All parameters were analysed by two-way (sex $\times$ time) ANOVA with repeated measures on time, followed by simple contrast analysis. The level of statistical significance was set at $\alpha = 0.05$.

3. Results

Data regarding the parameters of the redox status are presented in Fig. 1. No significant difference between sexes or sex-by-time interaction was found. Regarding time, a main effect was found on GSH, GSSG, and GSH/GSSG ($p = 0.020$, 0.045, and 0.002, respectively). GSH and GSH/GSSG were significantly higher at 13 and 23 weeks compared to baseline, whereas GSSG decreased from baseline to 13 week.

Data on the iron status parameters are shown in Table 1. Only the main effect of time on hematocrit was significant ($p = 0.032$) and was located in a decrease from 13 to 23 weeks. No differences were found in leukocyte count or leukocyte subpopulations (Table 2). There were also no differences in serum CK. This parameter was within the reference interval for the general population, except for the girls at the final measurement. Serum cortisol decreased at 13 and 23 weeks compared to baseline ($p = 0.002$) and was within the reference interval at all times.

Nutrient intake remained relatively stable during the study, with few significant main effects of time. Thus, and for the sake of clarity, we have pooled the corresponding data (Supplemental files 1 and 2). Regarding macronutrients (Supplemental file 1) and according to recommendations for children athletes,[23] protein intake was adequate, exceeding the recommended 12–15% of total energy. Carbohydrate intake was below the recommended minimum of 50%, whereas fat intake exceeded the recommended 25–30%, with a major contribution from saturated fatty acids. Regarding micronutrients (Supplemental file 2), the participants did not meet the recommended daily allowances (RDA) for folate, vitamin E (both related to the antioxidant capacity), vitamin

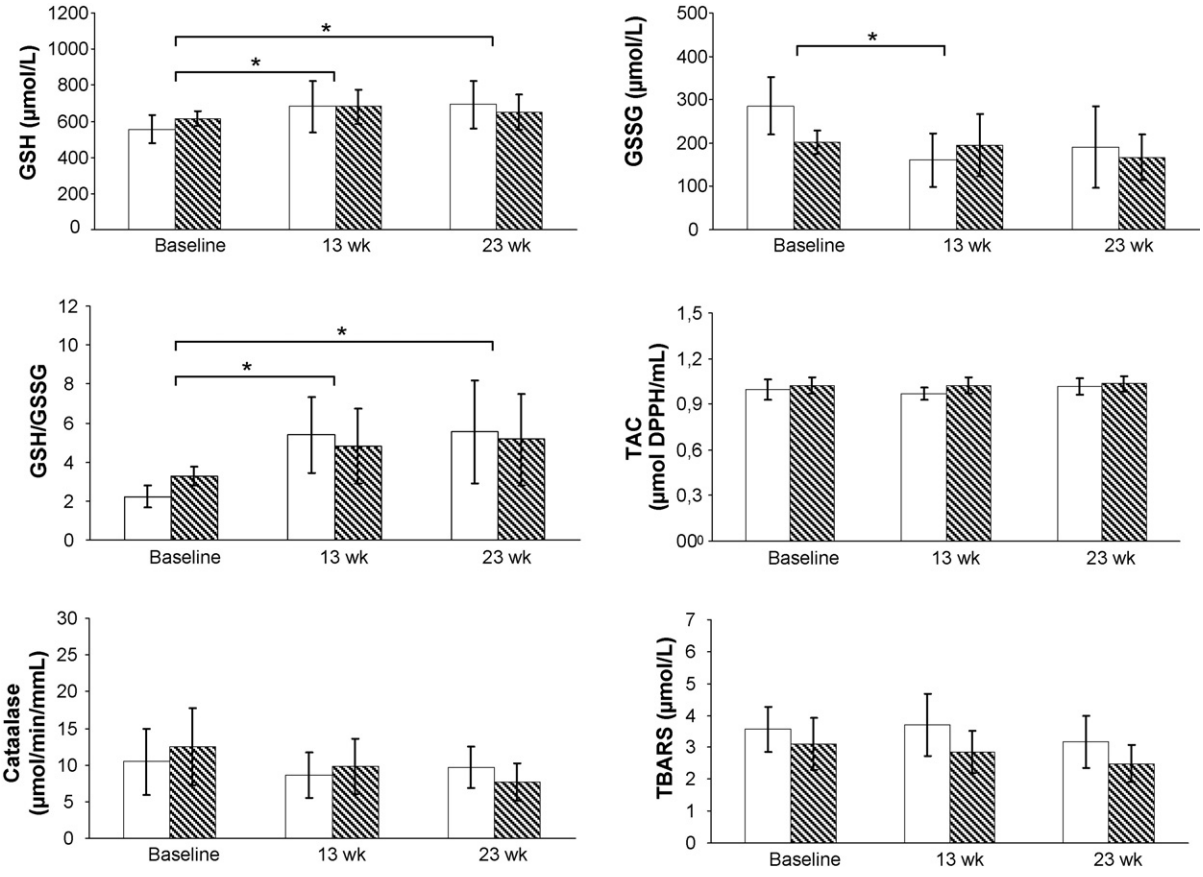

Fig. 1. Blood parameters of the redox status in male (open bars) and female swimmers (hatched bars) during training. GSH, Reduced glutathione; GSSG, oxidised glutathione; TAC, total antioxidant capacity; TBARS, thiobarbituric acid-reactive substances. Error bars denote 95% CI. $^{*}p < 0.05$: significant differences between time points for all participants.

D (which, nevertheless, is synthesised in the body upon exposure to sunlight), calcium, magnesium, and (only the girls) phosphorus. The intake of other nutrients involved in antioxidant mechanisms (vitamin C, vitamin A, and selenium) was adequate.

Body weight, height and body mass index increased significantly during the study (Supplemental file 3) with no significant difference between sexes or sex-by-time interaction. Performance improved during the study ($p < 0.001$, Supplemental file 3) with no difference between sexes or sex-by-time interaction.

4. Discussion

In the present study we examined changes in indices of the redox and iron status, as well as nutrient intake in child swimmers during a 23-week training period, which was effective in improving swimming performance. To our knowledge, this is the first study to investigate these parameters in children athletes of both sexes for such long time.

Boys and girls did not differ in the response of the redox status to training. In accordance with this, children did not differ in the response of their blood redox status to acute swimming.[5] Cavas and Tarhan[3] also found no differences in antioxidant enzymes between boys and girls during a month of training. Additionally, Özbay and Dülger[24] and Inal et al.[25] found no differences between boys and girls nonathletes in markers of the redox status with the exception of catalase.[25] Based on these studies, we can conclude that prepubescent boys and girls do not differ in their blood redox status.

The significant increase in GSH/GSSG, resulting from an increase in GSH and a decrease in GSSG, suggests an improvement of the antioxidant status during training. Moderate exercise has been proposed as an antioxidant,[26] therefore the training regimen employed in this study may have served as an enhancer of the antioxidant capacity. The fact that TAC, catalase and TBARS did not change implies that not all indices of the blood redox status respond to training similarly. It may also indicate that the redox status of glutathione is a more sensitive marker of adaptations to training. Elokda and Nielsen[27] have also suggested that GSH/GSSG is the most sensitive marker of oxidative stress in response to training. This implies that erythrocytes may be more responsive to oxidative stress than plasma. Improvement of the redox status with training has been regularly reported in adult athletes[1] but we are unaware of studies monitoring the redox status of glutathione or TAC during

Table 1
Iron status of the swimmers during the study period [mean (95% CI); boys, $n = 11$; girls, $n = 13$]

	Baseline	13 week	23 week	Normal range[a]
Hematocrit (%)[b]				
Boys	39.7 (38.4–41.1)	40.0 (38.6–41.4)	39.0 (37.7–40.3)	34–43
Girls	39.4 (38.0–40.7)	40.2 (38.9–41.5)	39.5 (38.3–40.8)	
Hemoblobin (g/dL)				
Boys	13.2 (12.6–13.7)	13.1 (12.6–13.6)	13.0 (12.5–13.4)	12–15
Girls	13.0 (12.5–13.4)	13.1 (12.7–13.6)	13.1 (12.7–13.5)	
Erythrocyte count (M/μL)				
Boys	4.9 (4.7–5.1)	4.9 (4.7–5.2)	4.8 (4.6–5.1)	3.9–5.1
Girls	4.7 (4.5–4.9)	4.8 (4.6–5.0)	4.7 (4.5–4.9)	
Iron (μg/dL)				
Boys	75 (53–97)	74 (52–95)	72 (51–93)	50–120
Girls	70 (59–80)	49 (37–62)	75 (58–92)	
TIBC (μg/dL)				
Boys	297 (279–314)	305 (276–334)	321 (305–337)	250–425
Girls	309 (283–334)	293 (258–327)	310 (283–338)	
Transferrin saturation (%)				
Boys	26 (18–33)	24 (18–30)	22 (16–29)	20–50
Girls	23 (19–27)	17 (13–22)	24 (19–30)	15–50
Ferritin (ng/mL)				
Boys	28 (19–37)	29 (23–36)	39 (23–55)	7–140
Girls	32 (26–39)	30 (24–36)	30 (23–38)	

TIBC, Total iron-binding capacity.
[a] Data from ref. [22].
[b] $p < 0.05$: Significant main effect of time.

training in children. In another study,[6] child swimmers exhibited higher oxidative stress and lower antioxidant capacity compared to untrained counterparts at rest. This may seem to contrast with the findings of the present study but factors such as training level and study design (cross-sectional vs. longitudinal) render the two studies difficult to compare. Regarding catalase, Cavas and Tarhan[3] found an increase after training only in girls. Concerning lipid peroxidation (estimated from

Table 2
Training stress in the swimmers during the study period [mean (95% CI); boys, $n = 11$; girls, $n = 13$]

	Baseline	13 week	23 week	Normal range[a]
Leukocyte count (k/μL)				
Boys	9.63 (8.58–10.69)	10.12 (9.03–11.21)	8.82 (7.97–9.66)	4.5–13.5
Girls	8.80 (7.79–9.80)	9.38 (8.20–10.55)	8.89 (7.18–10.61)	
Neutrophils (k/μL)				
Boys	4.96 (4.33–5.59)	5.30 (4.33–6.28)	4.44 (3.37–5.50)	1.8–8.0
Girls	4.17 (3.73–4.60)	4.60 (3.90–5.29)	3.98 (2.91–5.06)	
Lymphocytes (k/μL)				
Boys	3.66 (3.15–4.18)	3.69 (3.34–4.04)	3.56 (3.21–3.91)	1.5–6.5
Girls	3.75 (3.23–4.26)	3.58 (2.86–4.30)	3.66 (3.12–4.20)	
Monocytes (k/μL)				
Boys	0.76 (0.64–0.89)	0.82 (0.74–0.89)	0.81 (0.64–0.99)	0.4[b]
Girls	0.75 (0.64–0.87)	0.83 (0.69–0.97)	0.85 (0.76–0.94)	
CK (U/L, 37 °C)				
Boys	129 (98–160)	173 (139–206)	157 (112–201)	20–200
Girls	127 (101–152)	179 (110–248)	196 (103–289)	20–180
Cortisol (ng/mL)[c]				
Boys	127 (76–178)	75 (45–104)	59 (44–73)	10–380
Girls	94 (49–139)	49 (34–65)	56 (39–72)	

CK, Creatine kinase.
[a] Data from ref. [22].
[b] Mean value, no range available.
[c] $p < 0.05$: Significant main effect of time.

either TBARS or malondialdehyde), results have been contradictory, ranging from decrease[4] to no change (present study) to increase.[3] This discrepancy may stem from differences in the markers of lipid peroxidation used, training program, training status and/or age.

Training did not affect the iron status of the swimmers, and the serum iron concentration was moderate. This minimises the possibility of iron to induce formation of free radicals. The only other study that monitored prepubescent swimmers found that iron status deteriorated during training.[10] The training volume and frequency in that study were higher than in the present one, implying a dependence of iron status on training load.

Nutrient intake can affect both the redox and iron status.[11] The observed changes in glutathione during training cannot be attributed solely to nutrition, as antioxidant nutrient intake did not change with time. Notably, the children met the RDA for iron, which apparently contributed to maintaining a normal iron status. The children did not meet the RDA for other important micronutrients, calcium being probably the most critical one. Additionally, folate and magnesium intakes fell short of the corresponding RDA, indicating partly suboptimal dietary habits. Underreporting is always an issue in nutritional studies[28] and may have occurred in the present study too. Thus, actual nutrient intakes may have been higher than calculated.

Regarding macronutrient intake, a high contribution from fat and a low contribution from carbohydrate to total energy were found. Intake of saturated fatty acids exceeded the recommended 10% of total energy, and cholesterol intake exceeded the recommended 100 mg per 4.184 MJ,[12] averaging 121 and 109 for boys and girls, respectively. Protein intake averaged 2.0 g/kg for both sexes, being twice the recommended for nonathletic children.[11] This and the adequate iron intake were due to the high consumption of meat products at the cost of excessive intake of saturated fatty acids and cholesterol. Our findings suggest that nutritional monitoring of children athletes and nutritional education of children along with their parents can be useful during growth and participation in sport.

A limitation of our study was the absence of a control group, since we were unable to motivate a sizeable sample of children nonathletes to undergo regular anthropometric, nutritional and biochemical monitoring along with the athletes.

5. Conclusion

The present study contributes data to the poorly explored field of monitoring the redox status of children athletes during training. Child swimmers improved the glutathione status during a 23-week training period, although the intake of antioxidant nutrients did not change. Iron intake was adequate, and iron status was not compromised during training. Intakes of carbohydrate, total fat, saturated fatty acids, cholesterol, folate, vitamin E, calcium and magnesium were suboptimal. Finally, boys and girls did not differ in any of the redox, iron or nutritional status parameters measured.

Practical implications

- The antioxidant capacity of children may improve partially during a 23-week period of swimming training, thereby contributing to a better health status.
- Swimming training does not impair the iron status of children who consume adequate iron with their diet.
- The diet of children athletes may be inadequate with regard to some macro- and micronutrients, so nutritional monitoring and guidance are advisable.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jsams.2008.05.005.

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