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Full Length Research Paper

Resistance to respiratory illness and antibody response in open water swimmers during training and long distance swims

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The immune response in open water swimmers during training and long-distance swims (LDS) as well as their resistance to respiratory illnesses has not been investigated. The immune, metabolic and hematological response was determined in experimental and control groups of swimmers (7 and 8 athletes, respectively) during 6 months of training, as well as in the experimental group during each one of three LDS (>6 h) carried out during this training period. At the end of six months of training, the average pre-exercise levels of serum IgG, IgA, IgM and salivary IgA, decreased significantly: 48, 34, 64 and 45%, respectively. The average serum antibodies levels did not change during the first LDS, and increased significantly between the second and fourth hours of the other two swims. Salivary IgA decreased drastically in the first two hours (80%) of all swims. LDS carried out during training in open waters significantly suppressed pre-exercise serum and salivary antibody levels, although these changes did not affect the resistance of the swimmers to respiratory illnesses. The adaptation of the immune response was expressed in a significant increase of antibodies during consequent LDS.

Key words: Antibodies, metabolic and hematological parameters, adaptation.

INTRODUCTION

Swimming and salivary IgA

Physical activity has been shown to moderately reduce pre-exercise salivary IgA levels throughout training in most of the studies done in different sports (Gleeson, 2000), the results are contradictory in swimming. The decrease of salivary IgA during training periods of three months (Tharp and Barnes, 1990; Taymazov et al., 2003) and seven months (Dhabhar et al., 1997) was observed both before and after the daily exercise of elite swimmers. Pre-exercise salivary IgA diminished 4% with each successive month of training and post-exercise levels diminished by 8.5% with each additional kilometer and 7% with each successive month of training (Gleeson et al., 1995). The studies by the same authors showed a reduction of salivary IgA after each training session, but a significant increase was observed in the levels between sessions over 2.5 months of training (Gleeson et al., 2000). In another two studies done over periods of four and six months, no notable changes in the different salivary antibodies were observed in the swimmers (McKinnon and Hooper, 1994; Pyne et al., 2001).

Since the first report of research on the close relationship between frequency of respiratory illnesses and a reduction in the concentration of mucosal immunoglobulins (Levando et al., 1988), several studies have been done in this respect for different sports and in particular for swimming. The overall conclusion of analyzing the results of recent studies with elite swimmers is that an increased risk of respiratory illnesses exists when there is a decrease in salivary IgA (Gleeson, 2000). A higher rate of respiratory illnesses was found in swimmers who showed diminished levels of salivary IgA1

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Table 1. Experimental and control group data.

Group (sex)	N	Age years	Weight (kg)	Anaerobic Threshold (m/s)	Velocity during LDS (m/s)
experimental (m)	4	32.5 ± 4.2	76.9 ± 4.9	1.13±0.1	0.84 ± 0.07
(w)	3	30.1 ± 2.0	65.3 ± 3.3	1.25 ± 0.1	1.04 ± 0.03
Control (m)	4	38.3 ± 4.5	84.2 ± 3.1	1.20 ± 0.1	
(w)	4	28.3 ± 2.1	63.2 ± 4.2	1.02 ± 0.3	

subclass during training (Gleeson et al., 1995), but this relationship was not observed in another study by the same authors (Gleeson et al., 1999a).

Swimming and serum immunoglobulins

Information about changes in systemic immune response is limited. However, a slight increase of blood IgA in swimmers at the end of seven months of training has been observed (Gleeson et al., 1995). In another study by the same authors, however, no significant changes were observed (Gleeson et al., 2000). Furthermore, the changes in immunological functions during training and prolonged swimming in open waters have not been investigated. Therefore, the aim of this work was to monitor possible respiratory illness as well as the changes in blood and salivary immunoglobulin pre-exercise levels, measured before training sessions over 6 months of training of elite swimmers and during three consequent long-distance swims carried out during this training period.

METHODS

Swimmers and training data

The subjects of the present study were seven swimmers (experimental group) in training for ultra long distance swims (LDS) in open waters, who were born, live and train in Mexico City (altitude, 2200 m). These swimmers were training for the marathon around Manhattan Island as well as the crossing of the English Channel and the San Pedro Channel. The control group included 8 swimmers with the same training, but without the long distance swims. Written informed consent was obtained from the participants and throughout the study, daily communication was maintained with them. During training, all swimmers were under constant medical supervision by a complete capillary blood analysis and physical examination every 2- 3 weeks to detect respiratory illness. The data of the experimental and control groups is presented in Table 1.

The anaerobic threshold (4 mmol/l lactate) that reflected the performance of swimmers in open water was determined before this study by the 5 x 400 m trial, with gradually increased velocity (range 1.03-1.35 m/s). The experimental swimmers were trained for different Olympic distances during the 10 - 15 years prior to this study. The performance level of these swimmers was high, but does not reach the level that distinguished an elite swimmer (1.4 - 1.6 m/s).

The controlled training program for all swimmers consisted of up to 160 km/month, 25% of which was intense swimming (>4 mmol/l of lactate) and 75% of which was less intense. During the 6 months of training three LDS were carried out (between 7 am and 3 pm) by swimmers of experimental group in a salt water lake (18 - 21°C, 1800 m altitude): one of 6 h (month 1) and two of 8 h (months 3 and 6). Additionally, in the fourth month these athletes swam 20 - 30 min in very cold open waters (8 - 10°C at an altitude of 4000 m).

Nutrition protocol during the LDS

During all LDS every swimmer drank approximately 300 - 400 ml per hour, alternating each hour between two different beverages. One was a combined commercial beverage (50 g of proteins and carbohydrates in a proportion of 2:1 respectively) and the other a commercial electrolyte beverage diluted 1:1 with water (7 - 10 g of carbohydrates). On the average 15 ± 3 g/h of carbohydrates, 18 ± 4 g/h of proteins and 400 ml of liquids were consumed. The intake was moderately low in carbohydrates but sufficient for carrying out LDS without any problems. Body weight change during the LDS varied from swimmer to swimmer, varying between no change and a moderate increase of approximately 0.5 kg.

Sampling

Samples of capillary blood (from a finger) (Levando et al., 1988) and unstimulated whole mixed saliva (Gleeson et al., 1999a) were taken before, during (every one to two hours) and after the LDS. The research was approved by the Institutional Ethics Committee (Superior Medical School, National Polytechnic Institute). Except for total proteins, the blood chemistry parameters (glucose, triglycerides, urea, lactate and cholesterol) as well as the creatine kinase (CK) and lactate deshidrogenase (LDH) activity were determined using routine procedure with enzymatic reagents (RANDOX). The coefficient of variation (CV) for measurement was between 1 and 3%, depending on the parameter. The hematological parameters were determined with a QBC analyzer (Beckton Dickinson). IgA, IgG and IgM were measured in serum and saliva by the ELISA method with two measurements/sample and three dilutions/measurement, using a commercially prepared specific isotype antiserum (Cattly and Raykundalia, 1989). Protein concentration was measured by the Biuret method (RANDOX). Concentrations of the salivary immunoglobulin values are expressed in mg/g of total protein. The CV for all determinations was < 8% for saliva and < 5% for serum. But in LDS3, in which there were significantly decreased initial IgM levels, the CV increased 12 and 8%, respectively.

In order to evaluate the effects of the LDS nutritional regimen on changes in blood parameters and salivary IgA concentration, the control group of swimmers followed the same nutritional protocol during 8 h of rest, and samples of capillary blood and unstimulated whole mixed saliva were taken every 2 h.

Statistical analysis

Data are presented as the mean ± SD. Comparison of two groups was analyzed by using Student's unpaired two tailed t-test and the

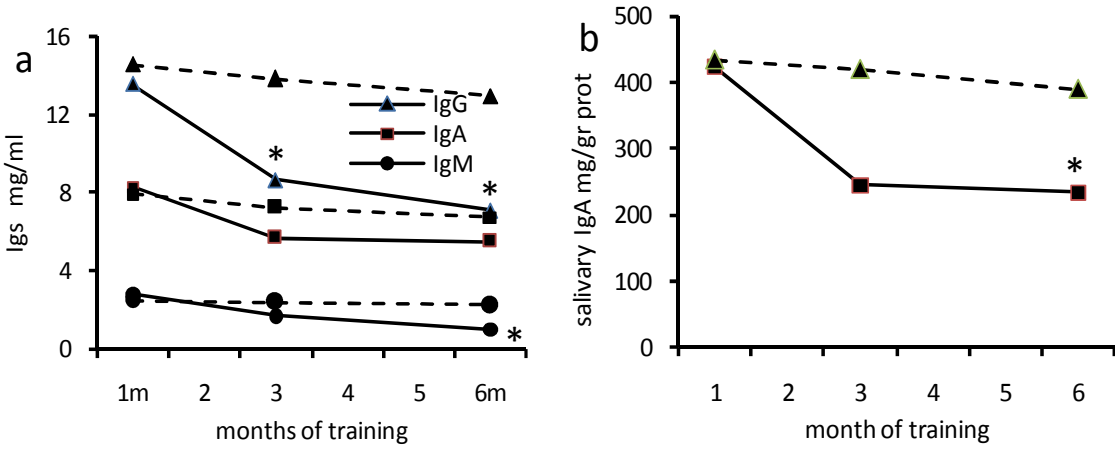

Figure 1. Average pre-exercise levels of serum (a) and salivary IgA (b) antibodie changes during six months of training. The dashed line reflects changes in the control group (* - $p < 0.05$).

Pearson correlation. All analyses were performed using the statistical program SigmaStat (SPSS Inc).

RESULTS

Pre-exercise levels of all parameters

The change in pre-exercise levels of the different antibodies measured before each one of the three LDS reflected the effect of the swimmer's training during the 6 months of study. According to one study (Dimitriou et al., 2002), the antibody levels in the morning are double those found in the afternoon. In the current study, there was an exponential decrease over the 6-month training period in average pre-exercise levels of serum IgG (48%, $p = 0.015$), serum IgA (34 %, $p = 0.25$), serum IgM (64 %, $p = 0.047$) and salivary IgA (45%, $p=0.048$) (Figure 1a and b). In the control group no significant changes were observed in the levels of different antibodies during training, although a tendency to a decrease was found: 8, 9, 11 and 10% for serum IgG, IgA, IgM and salivary IgA, respectively ($p>0.1$).

Pre-exercise levels of granulocytes decreased 27% ($p = 0.099$) and those of agranulocytes (limpho+monocytes) increased 15% ($p = 0.059$). Pre-exercise levels of metabolic and hematological parameters did not present significant changes during training, with the exception of urea, which increased 17% ($p < 0.05$, data not presented).

Changes in antibody levels during the LDS

Slight changes in the concentration of the total proteins (less than 5%) as well as of hematocrit and hemoglobin (less than 2%) during the LDS confirm that the changes in the plasmatic volume were less than 5% and therefore did not significantly influence the measurements of the serum antibody. Only in saliva were significant changes observed in the concentration of the total proteins. Therefore, we supposed that it was necessary to normalize the concentration of salivary IgA to that of the total proteins.

During an 8 h rest period of the control group (Figure 2), when the same nutritional regimen was applied as that given to swimmers during the LDS, a significant increase was observed in serum IgA ($p < 0.01$) and a significant decrease in serum IgM ($p < 0.05$), but only between the 6th and 8th h. These changes were not related to variations in plasma volume. During the same period the salivary IgA in the control group did not change significantly. When the percentage of changes in antibody levels in the experimental group was corrected to those found in the control group, the variations in all antibody levels in the first LDS were insignificant (Figures 3a, b and c). On the other hand, in the following two LDS there was a significant increase of IgG between hour 2 and 4 of swimming (Figure 3a), with a positive correlation ($r = 0.9$, $p=0.018$) between LDS2 and LDS3. The changes in average serum IgA during LDS2 and LDS3 reflected a very similar pattern (Figure 3b), with a positive correlation between LDS2 and LDS3 ($r = 0.98$, $p=0.003$). The levels of IgM increased moderately and similarly in LDS2 and LDS3 ($r = 0.93$, $p = 0.018$) (Figure 3c).

The main salivary IgA diminished drastically (80%) in the first three hours and maintained this level for the rest of the time of all LDS (Figure 4). The average levels (corrected to control group changes) of WBC, CK, LDH and urea were similar in all LDS (Figures 5 a, b and c). The average levels of granulocytes during LDS1-LDS3 increased drastically after the second hour, reaching their maximum compared to the basal level (275, 324 and 396%, respectively) between 6 and 8 h of swimming ($p < 0.01$). Agranulocytes also increased to 140, 142 and 135%, respectively, of the basal level in the 6th h

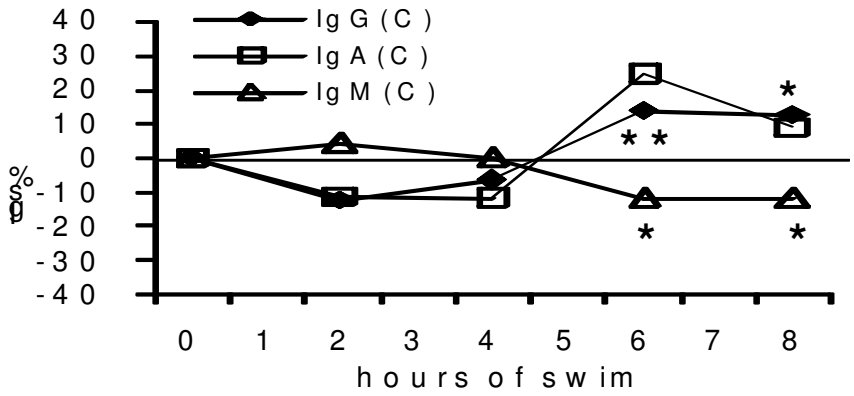

Figure 2. Average percentage level changes of serum antibodies in the control group during 8 h of rest with the same nutritional regimen as the experimental group (* - $p < 0.05$, ** - $P < 0.01$).

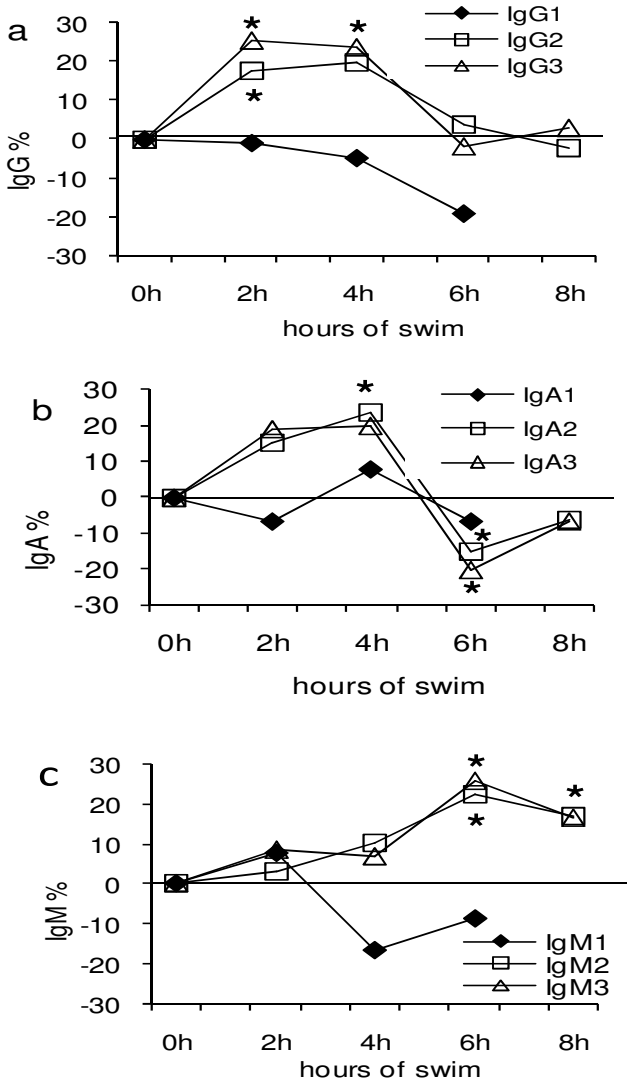

Figure 3. Average percentage level changes of IgG (a), IgA (b) and IgM (c) during three consecutive LDS (corrected to control group changes; * - $p<0.05$).

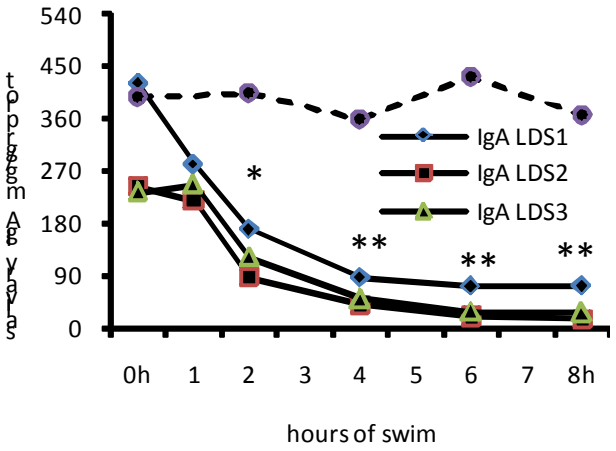

Figure 4. Average percentage level changes of salivary IgA (b) during three consecutive LDS.

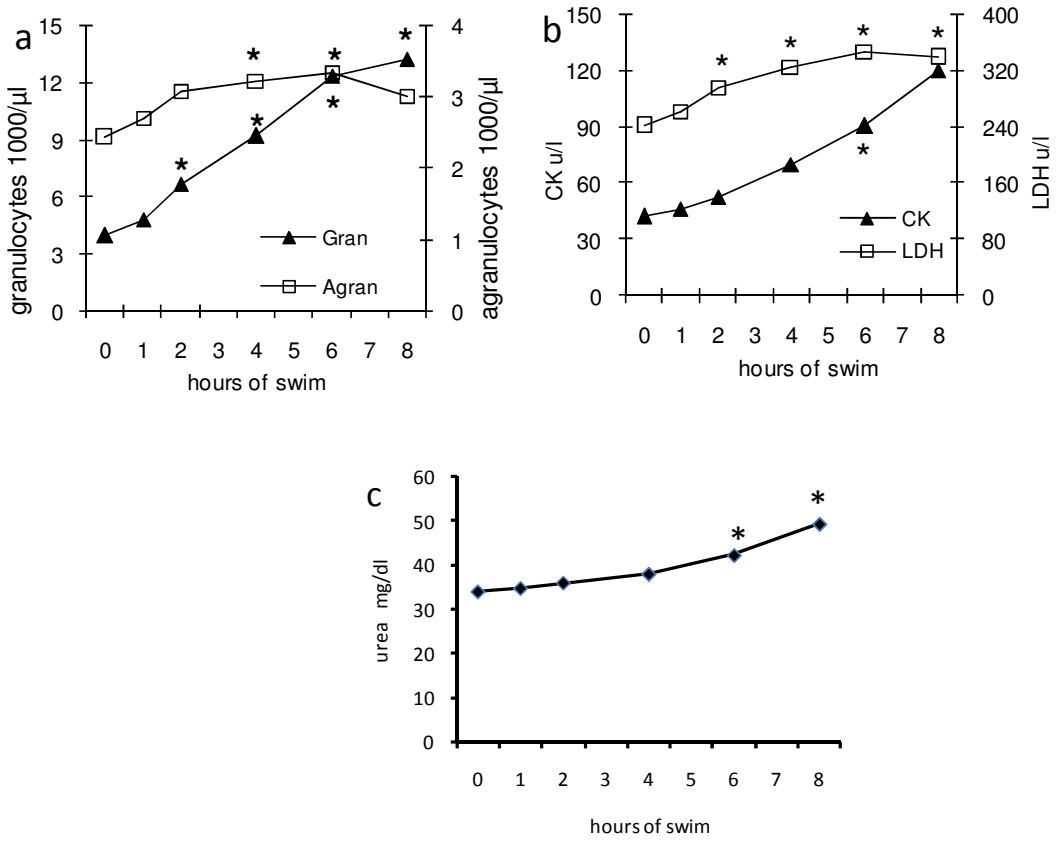

Figure 5. Average WBC (a), CK, LDH (b) and urea (c) level changes during three consecutive LDS.

($p < 0.05$).

The average CK activity during the LDS presented an exponential increase after the second hour ($p < 0.05$), and by the 8th h had reached 120 u/l, representing a 185% increase (Figure 5b). LDH activity increased significantly after the second hour of the swims, reaching a maximum level of 340 u/l, representing a 45% increase, between 6 and 8 h of swimming (Figure 5b). The average urea concentration increased significantly after the sixth hour of the swims (Figure 6c). The blood parameters such as glucose, lactate and triglycerides (corrected to variations in control group values) changed differently in

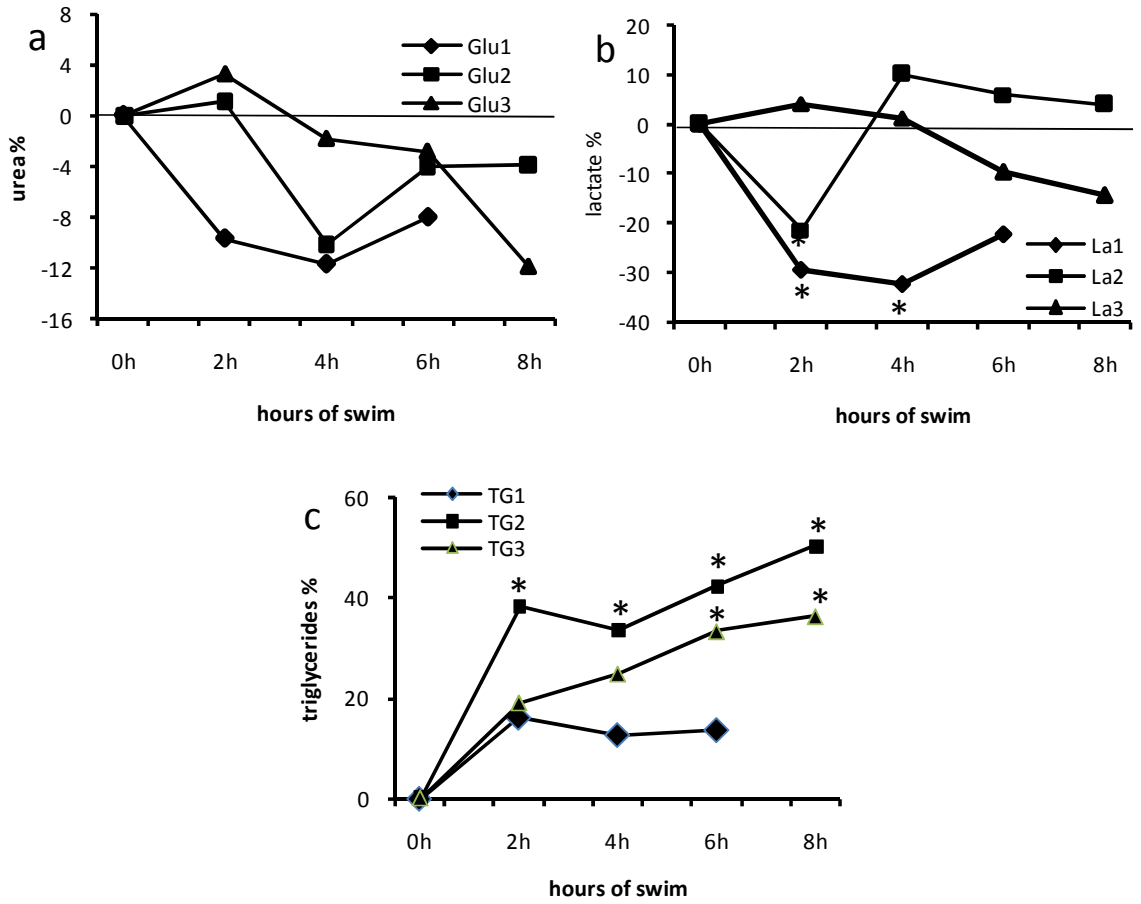

Figure 6. Average glucose (a), lactate (b) and triglycerides (c) level changes during three consecutive LDS.

the three LDS, indicating a metabolic adaptation process.

Glucose and lactate showed the same pattern of changes during all LDS, with more stability in LDS2 and LDS3. The average levels of triglycerides (Figure 6c) increased significantly in LDS2 and LDS3 from the 2nd h of the swims, evidencing their mobilization.

Despite a significant reduction in serum and salivary antibodies during the training period, the swimmers did not present any respiratory illnesses during this period or in the 3 months following the study.

DISCUSSION

Antibodies and training

In the present study, pre-exercise levels of metabolic parameters did not change significantly during training, except for a significant increase in the urea and triglyceride concentration, which confirmed an increment in the participation of proteins and lipids in energy production.

The response of different antibodies in the control group was not significant during training, but all pre-exercise serum antibodies and salivary IgA levels decreased to a great extent during the training sessions. Whereas the significant decrease in pre-exercise salivary IgA coincided with the data of other investigators (Tharp and Barnes, 1990; Gleeson et al., 1995; Taymazov et al., 2003), the significant decrease in serum antibodies was contradictory to other studies (Gleeson et al., 1995; 2000). This contradiction can probably be explained by two special conditions in the present study: a) the three consecutive LDS that were carried out during training, and b) the fact that these LDS were carried out in relatively low water temperatures that affected the hormonal response (Leppäluoto et al., 2008). The relative importance of these two conditions is evidenced by what occurred to the three swimmers that continued training after this study, but without doing any LDS. Their pre-exercise serum and salivary antibody concentrations, determined between 3 and 5 months after the end of the current study, returned to the pre-study levels (data not presented).

The mechanisms by which pre-exercise serum immunoglobulin levels diminished during the training period are unknown. Most likely this decrease resulted from a combination of psychological and physiological

responses to excessive training (McKinnon, 1999) by means of a decrease in the biosynthesis of antibodies or an increase in their catabolism. It is known that prolonged exercise does not reduce the number of B lymphocytes or antibody-producing cells (Nilssen et al., 1998), the inhibition of the activation of B cells found in the present study may be caused by stress hormones or by prostaglandins derived from monocytes (McKinnon, 1999; Tvede et al., 1989). These same factors are probably responsible for increasing the hepatic catabolism of IgA (Evans et al., 2002).

In the current study, pre-exercise levels of lymphocytes increased, whereas the levels of antibodies (especially IgG) decreased, the latter coinciding with reports of an increase in B-lymphocyte proliferation with diminished IgG levels (Taymazov et al., 2003). The metabolism of serum IgG differs from that of the other immunoglobulin classes in that IgG has the longest half live in circulation and the lowest catabolic rate (Waldmann and Strober, 1969). The metabolism of IgA has not been studied intensely even though it is synthesized in quantities of 66 mg/kg of body weight per day, which far exceeds the combined daily synthesis of all the other isotypes. Since hepatocytes are the main catabolic site of IgA, exercise can increase the hepatic catabolism of this antibody, probably through the action of stress hormones (Burgess and Stanley, 1994). The high velocity of IgA synthesis is the probable reason for the moderate decrease in its average pre-exercise serum level observed during this study.

The production and secretion of IgA in saliva involves the interaction of multiple and complex factors, meaning that the mechanisms responsible for changes in the output of secretion of IgA after intense exercise and during periods of intensive training are speculative (McKinnon, 1999). The effects of exercise on IgA secretion may reflect alterations in epithelial IgA transport or alterations in the concentration of IgA at the base-lateral epithelial surface (Gleeson and Pyne, 1996; McKinnon, 1999). By the end of the present study salivary IgA, which is very sensitive to training, decreased 45% in relation to pre-exercise levels.

Neither during this study nor in the three following months did the swimmers present any respiratory illnesses, despite a significant reduction of serum and salivary antibodies and swim in very cold water carried out in 4th month of study. Such a reduction in these parameters coincides with the results of one study (Gleeson et al., 1999b), but is contradictory to those of other studies by the same authors (Gleeson 2000; Gleeson et al., 1995). We attribute this high resistance to respiratory illnesses in the current study to the effects on the immune response of the LDS in relatively cold waters.

Antibodies during LDS

Analysis of metabolic response during all LDS showed the similar and significant increase in protein catabolism and consequently the greater participation of proteins in energy production after 2 h of swimming. The triglyceride increase was ever greater with each LDS, indicating that an increase in lipid mobilization was also participating in energy production. The levels of glucose and lactate were stable in LDS2 and LDS3, coinciding with the elevation of urea and triglyceride levels. In the control group the antibody levels during the 8 h of following the same nutritional regimen as the experimental group only reached a significant change between six and eight hours when compared with the initial level. Possible effects of hemoconcentration can be ruled out, as the changes in the concentrations of total protein, hematocrite and hemoglobin were not significant (less than 5%).

During LDS2 and LDS3, there was an increase in antibodies after 4 h, followed by a significant decrease in serum IgG to the initial levels, a significant decrease in serum IgA below the initial levels, and no decrease in IgM. The high positive correlation between the significant increases in serum antibodies during LDS2 and LDS3 suggests that the same mechanism was at play. These data also showed that the measurements before and after long duration exercise did not show any definite trends. The mechanism of the increase in serum antibodies is not clear, although it is difficult to explain without assuming a mobilization of antibodies from some reserve, such as lymphocytes. The kinetics of the increase in antibodies does not completely correspond to the agranulocyte response (Figure 5a) during the LDS, but circulating lymphocytes represent only 1% of total lymphocytes deposited in different organs.

The decrease in antibody levels after the 4th h of LDS was very fast, which may be due to: (a) an influx of antibodies from vascular to extravascular pools, (b) an increase in antibody catabolism in the liver, or (c) a possible unknown mechanism of their capture and release (Shibuya et al., 2000).

During LDS there are clinical symptoms of muscle inflammation and muscular damage, detected by an increase in WBC, CK and LDH serum activity. Cytokines can change the activity of hepatocytes and probably immunoglobulin metabolism (Evans et al., 2002). It can be assumed that since the participants of experimental group in this study had a similar nutritional regime during the LDS and swam in water of the same temperature, their immunoglobulin response from the first to the last LDS reflects the training adaptation process of the immune system. Habituation can be related to the lower production of the stress hormones (Dhabhar et al., 1997).

The description of receptors for IgA/M made by Shibuya et al. (2000) leads us to conclude that they may be involved in another possible mechanism of antibody capture and release. The dynamic exchanges caused by exosomes should also be considered (Thery et al., 2002). The FcRn receptor, found in newborns, has also been described in adults, and a role has been assigned to it in the regulation of IgG catabolism (Israel et al., 1996).

Conclusion

A significant reduction in serum and salivary antibodies was observed over 6 months of training of high altitude swimmers in open water without affecting their resistance to respiratory illnesses. This reduction was probably due in great part to the LDS included in the training program. Salivary IgA diminished drastically after two hours of the LDS. The increase in serum antibody response during LDS2 and LDS3 probable reflects the response of immune system to the inflammation process in skeletal muscles provoked during the LDS.

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