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ORIGINAL ARTICLE

# Long-term swimming training modifies acute immune cell response to a high-intensity session

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## Abstract

**Purpose** Long-term training influence on athletes' immune cell response to acute exercise has been poorly studied, despite the complexity of both chronic and acute adaptations induced by training. The purpose of the study is to study the influence of a 4-month swimming training cycle on the immune cell response to a high-intensity training session, during 24 h of recovery, considering sex, maturity, and age group.

**Methods** Forty-three swimmers (16 females, 14.4 ± 1.1 years; 27 males, 16.2 ± 2.0) performed a standardized high-intensity session, after the main competition of the first (M1), and second (M2) macrocycles. Blood samples were collected before (Pre), immediately after (Post), 2 h after (Post2h) and 24 h after (Post24h) exercise. Haemogram and lymphocytes subsets were assessed by an automatic cell counter and by flow cytometry, respectively. Subjects were grouped according to sex, competitive age groups, or pubertal Tanner stages. Results express the percentage of relative differences from Pre to Post, Post2h and Post24h. Upper respiratory symptoms (URS) and training load were quantified.

**Results** At M2, we observed smaller increases of leukocytes (M1: 14.0 ± 36.3/M2: 2.33 ± 23.0%) and neutrophils (M1: 57.1 ± 71.6/M2: 38.9 ± 49.9%) at Post; and less efficient recoveries of total lymphocytes (M1: − 22.0 ± 20.1/M2: − 30.0 ± 18.6%) and $CD19^+$ (M1: 4.09 ± 31.1/M2: − 19.1 ± 24.4%) at Post2h. At Post2h, the increment of $CD4^+/CD8^+$ was smaller in youth (M1: 21.5 ± 16.0/M2: 9.23 ± 21.4%), and bigger in seniors (M1: 3.68 ± 9.21/M2: 23.2 ± 15.0%); and at Post24h late pubertal swimmers' $CD16^+56^+$ recovered less efficiently (M1: − 0.66 ± 34.6/M2: − 20.5 ± 34.2%).

**Conclusions** The training cycle induced an attenuated immune change immediately after exercise and a less efficient recovery of total lymphocytes, involving an accentuated $CD19^+$ decrease. The concomitant higher URS frequency suggests a potential immune depression and a longer interval of susceptibility to infection.

**Keywords** Immune system · Swimmers · Training session · Training cycle · Leukocytes · Lymphocyte subsets

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**Abbreviations**

| | |
|---|---|
| %FM | Fat mass percentage |
| ANOVA | Analysis of variance |
| ATS | ANOVA-type statistic |
| AUL | Arbitrary units of load |
| BM | Body mass |
| BMI | Body mass index |
| CD | Cluster of differentiation |
| CIPER | Interdisciplinary center for the study of human performance |
| EDTA | Ethylenediaminetetraacetic acid |
| FFM | Free fat mass |
| HR | Heart rate |
| INSA | National Health Institute Doutor Ricardo Jorge |
| M1 | First moment of evaluation |
| M2 | Second moment of evaluation |
| MATS | Modified ANOVA-type statistic |
| NK | Natural killer |
| Post | Immediately after exercise |
| Post 24h | 24 h after exercise |
| Post 2h | 2 h after exercise |
| Pre | Before exercise |
| RBC | Red blood cells |
| SD | Standard deviation |
| URS | Upper respiratory symptoms |
| WBC | White blood cells |

## Introduction

At the time of important competitions, an healthy immunological, metabolic, hormonal, circulatory and respiratory condition along with an optimized functional capacity is needed (Hellard et al. 2013). This optimal functionality allows the athlete to achieve the best performance, and is usually the result of an adequate balance between training loads and recovery throughout the different phases of the periodization of a training season (Mujika et al. 1995). However, in endurance sports, such as swimming, during the cycles of high training volume and intensity that include consecutive training sessions with little recovery time in between, athletes may experience a temporary diminished performance concomitant with an immunodepression state (Rama et al. 2013; Gleeson 2007).

Exercise immunology literature is consistent regarding post-exercise cell counts (leucocytosis, neutrophilia, monocytosis, and lymphocytosis) after a single bout of high-intensity swimming (Kargotich et al. 1997; Morgado et al. 2014, 2016) or other endurance sports (Zhang et al. 2006; Natale et al. 2003; Yamada et al. 2000; McCarthy et al. 1992, 1991). The higher circulating number of these cells suggests an acute overall increased surveillance of both innate and acquired cellular immunity, thus conferring a temporary improved host defence that has been associated with the "fight or flight" response (Walsh et al. 2011).

During initial recovery period, swimming studies report leucocytosis and neutrophilia (Ferrer et al. 2009; Kargotich et al. 1997), while monocytes recover to baseline levels and lymphocytes decline (Kargotich et al. 1997). A delayed leucocytosis and neutrophilia has been reported at 1 h after cycling (Gabriel et al. 1992; Green et al. 2003) and at 2 h after running (Gabriel et al. 1992; Yamada et al. 2000). It was also observed 3 h into recovery after different types of cycling tasks (McCarthy et al. 1991, 1992; Natale et al. 2003) and after a resistance exercise circuit (Natale et al. 2003).

Regarding the effect of long-term swimming seasons (lasting from 3 to 7 months) at rest, decreased values of $CD56^{+}$ NK cells (Rama et al. 2013; Gleeson et al. 1995, 2000), neutrophils and monocytes (Morgado et al. 2012) were observed. Therefore, it appears that intense training over long periods affect the number and function of innate and acquired immune cells at rest, possibly contributing to upraise the risk of infection (Walsh et al. 2011).

However, the acute (Kargotich et al. 1997; Morgado et al. 2014, 2016; Tauler et al. 2008) and chronic (Gleeson et al. 1995; Morgado et al. 2012; Mujika et al. 1996b; Rama et al. 2013; Teixeira et al. 2014) immune systemic changes in response to swimming have been addressed separately. A more integrated approach is desirable, since the acute and chronic immune cell changes can influence the potential of the swimmer to respond properly to high-intensity training sessions throughout the course of the swimming season. This ability is indispensable to acquire positive adaptations and ultimately to guarantee training attendance. To our knowledge, the influence of the long-term training on the acute immune cell changes to a swimming session has not yet been studied.

Thus, this study aimed to investigate the influence of a 4-month training cycle of a swimming season over the immune cell response to a high-intensity swimming training session integrated in the normal training process, during a 24-h recovery period, whilst controlling for the effects of sex, maturity, and age group. In athletes, we expect to grasp the cumulative effects of the training process.

## Methods

### Participants

Forty-three swimmers, 16 females ($14.4 \pm 1.1$ years.), and 27 males ($16.2 \pm 2.0$ years.), undertaking 15–18 h of pool training and 4–7 h of dryland training per week, volunteered to participate in this study.

After receiving detailed information about the aim of the study and the possible risks of the investigation, either the subjects or their parents, as appropriate, provided written informed consent to participate. All procedures were approved by the Ethics Committee of the Faculty of Human Kinetics of the University of Lisbon and were conducted in accordance with the Declaration of Helsinki for human studies (World Medical Association 2008).

During the period of observation athletes were asked not to take dietary supplements, nor any kind of medication other than that prescribed for episodes of acute illness.

The swimmers had different competitive swimming backgrounds ($5.5 \pm 0.3$ mean years of competitive experience with a range of 4–11 years) and were included into different age groups according to the regulation of the National Swimming Federation and the Ligue Européene de Natation (youth: $n = 26$, 13–14 years in females and 14–15 years in males; juniors: $n = 10$, 15–16 years in females and 16–17 years in males; seniors: $n = 7$, $\geq 17$ years in females and $\geq 18$ years in males) or into different maturity groups (late pubertal: $n = 28$ years; mature: $n = 15$ years).

## Study design

This study used an observational design with a follow-up of the second macrocycle of a swimming winter training season lasting 17 weeks. Swimmers followed the training program set by the coaches.

The evaluation of the swimmers was made at two moments: M1 (after the main competition of the first macrocycle) and M2 (after the main competition of the second macrocycle).

At each moment of evaluation, swimmers performed a standardized high-intensity swimming session designed by experienced coaches and framed in the training periodization. Immunological profile was scrutinized before (Pre), immediately after (Post), 2 h after (Post2h) and 24 h after (Post24h) the training sessions at the two moments of evaluation. Data collected also included subjects' chronological age, body composition measurements, and an indicator of biological maturity (pubertal Tanner stages). Athletes were instructed not to consume anything but water after 10 p.m. of the preceding day, to have a minimum of 8 h rest before testing, and not to perform extenuating exercise in the previous 24 h. To standardize pre-exercise food intake and to avoid extending the duration of their fasted state, participants consumed a sandwich with butter and a juice after the body composition measurements and the resting blood sample collection. The experimental sessions took place between 6:30 and 10 a.m.

Throughout the follow-up season the incidence of upper respiratory symptoms (URS) was monitored weekly and training load and mean intensity of all scheduled swimming sessions were quantified. The characteristics of the training regimens and competition schedules were not modified by the present study in anyway nor any swimmer suffered from major injury or sickness preventing them from training for more than 1 day.

## Body composition measurements

Stature and body mass were measured after wakening in the fasted state wearing a bathing suit without shoes. Stature was measured to the nearest 0.1 cm (Siber-Hegner anthropometric kit). Body mass and fat mass percentage (%FM) were assessed using Bioelectrical Impedance Analysis (TANITA BC-601 body composition scale monitor) with a measuring current of 50 kHz, 100 μA. Body mass index (BMI) was calculated as body mass (BM; kg) divided by the square of the stature (m). Fat mass (FM) was calculated according to the formula: $\text{FM (kg)} = \text{BM} \times \%\text{FM}/100$. Free fat mass (FFM) was calculated according to the formula: $\text{FFM (kg)} = \text{BM} - (\text{BM} \times \%\text{FM}/100)$.

## Maturity: tanner stages

After receiving detailed instructions, the participants self-assessed their degree of genital organ, breast, and pubic hair development using a questionnaire (Tanner 1962) accompanied by figures and were then grouped according to pubertal stage. Stage 1 corresponds to the pre-adolescent state; stages 2, 3, and 4 correspond to the sexual maturation stages, respectively, early, mid, and late pubertal states; and stage 5 corresponds to mature state (Tanner 1962). This method has been shown to be valid and reproducible for the evaluation of maturity in studies addressing the immune response to exercise in adolescents (Boas et al. 1996; Timmons et al. 2006).

Female subjects were asked to point out the days of menstruation.

## Swimming training season

The observed 4-month training cycle was the second macrocycle of a swimming competitive season (Fig. 1). This macrocycle started with a development period characterized by an increasing training volume, intensity, and frequency that lasted until the end of the specific preparatory subphase reaching the peak of training load of the season by week 23. Afterwards the training load was progressively reduced preparing for the National Championships.

## Quantification of the training load

Training load of each training session was assessed by quantifying the volume (total amount of metres swum) and arbitrary

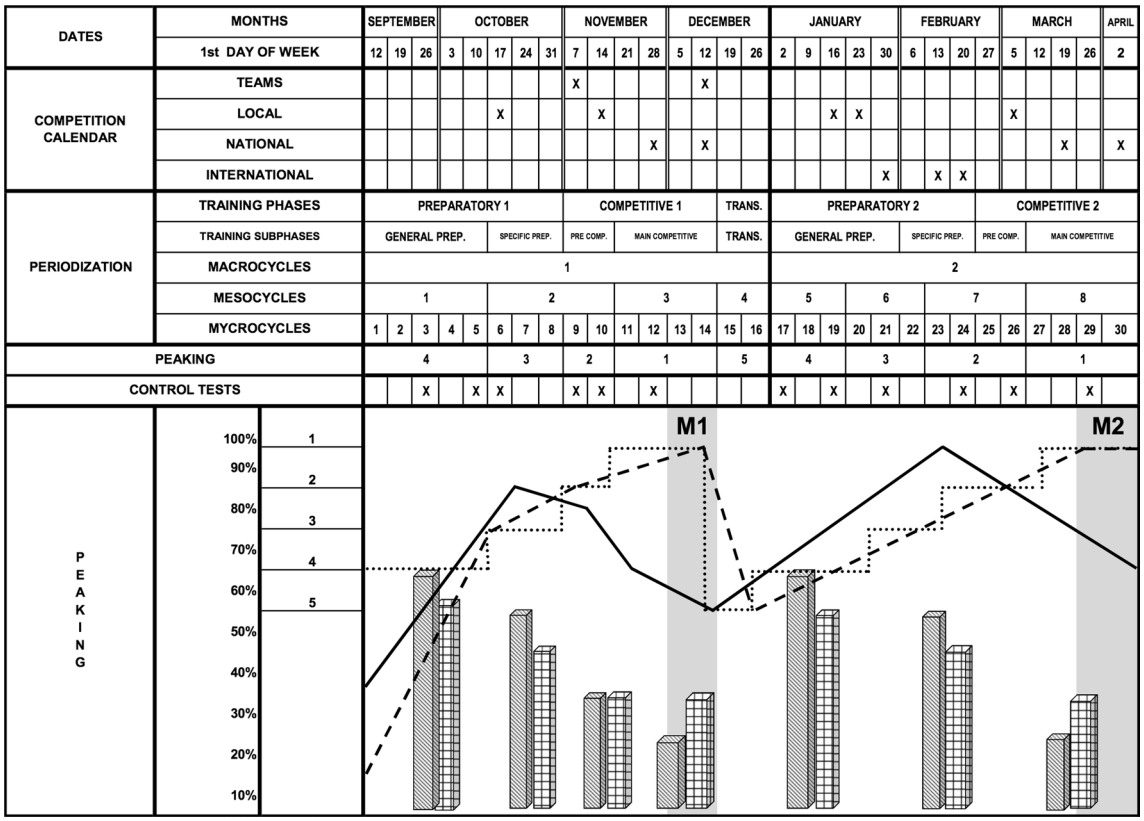


**Fig. 1** Periodization of the swimming winter training competitive season in which the 4-month training macrocycle is incorporated and schedule of the two moments of evaluation. ▨, Dryland training; ▤, technique training; continuous line, volume; dotted line, intensity; dashed line, peaking. M1, 1st moment of evaluation; M2, 2nd moment of evaluation; TRANS., transition; GENERAL PREP., general preparatory; SPECIFIC PREP., specific preparatory; PRE COMP., pre-competitive; Peaking 1–5, peaking index, where 1 is peaking at 100%, 2 at 90%, 3 at 80%, 4 at 70% and 5 at 60%; 10–100%, peaking percentage

units of load (AUL) based on the work of Mujika et al. (1995, 1996a), Rama et al. (2013) and Morgado et al. (2012).

To quantify the AULs a stress index scale was established in reference to the theoretical values of blood lactate accumulation usually associated with the different training zones: I—warm-up and recovery, II—endurance 1, III—endurance 2, IV—endurance 3, V—lactate tolerance, VI—lactate production and VII—sprint. The volume accomplished in each training zone was quantified (mI, mII, mIII, mIV, mV, mVI and mVII). The magnitude of the load (AUL) was obtained from the ratio between the weighed volume, calculated by adding the volumes swum in each training zone multiplied by the respective index (1, 2, 3, 4, 6, 8, 10) as suggested by Rama et al. (2013), and the total volume was effectively completed, according to the formula:

$$\text{AUL} = \frac{1\,\text{mI} + 2\,\text{mII} + 3\,\text{mIII} + 4\,\text{mIV} + 6\,\text{mV} + 8\,\text{mVI} + 10\,\text{mVII}}{\text{swimming training session volume}}$$

This was performed for all season sessions of the competitive season.

The weekly load was characterized by the sum of the load of all the training sessions of the week and the comparison between the two moments of evaluation was performed based on the mean of the weekly training load of the whole macrocycle and of the 4 weeks prior to each moment.

## Standardized swimming training session

The swimming session that was performed to evaluate the acute effect of exercise on the immune system started with a 1500 m standardized warm-up lasting 30–35 min. This was followed by a high-intensity main task that lasted 50 min and

a 500 m recovery task (8 min of duration). The main task was designed to induce maximal lactate accumulation and had a total distance of 1000–1200 m, depending on the age group considered. For the youth group the main task consisted of two sets of four repetitions of 75 m front crawl on a 5-min cycle, with 10 min of active recovery between sets. This reduction of distance aimed to elicit an effort situated at the same maximal lactate accumulation zone of intensity, thereby levelling the physiologic impact between groups. The swimmers were instructed to perform each repetition at 90–95% of 100 m freestyle personal best race time. The session designed for juniors and seniors was identical to the latter, except with repetitions of 100 m.

To determine the mean effort intensity percentage (%) in relation to the personal best at the 100 m freestyle race the times of each repetition were registered.

A heart rate (HR) monitor (Polar RS800CX™, Kempele, Finland) was used during each training session to calculate percentage of estimated maximal heart rate.

### Immune system parameters

Peripheral venous blood samples were collected via standard procedures before (Pre, between 6:00–6:30 a.m. in the fasted state), immediately after (Post), 2 h after (Post 2 h) and 24 h after (Post 24 h) each swimming training sessions. Venous blood was collected into tubes containing EDTA for assessment of hemogram and leukogram and for counting of total lymphocytes and subsets ($CD3^+$, total T lymphocytes cells; $CD4^+$, T helper cells; $CD8^+$, T cytotoxic cells, $CD16^+56^+$, natural killer (NK) cells; and $CD19^+$, B cells). Hemogram and leukogram were performed in an automated haematology analyzer (Coulter LH 750, Beckman) which produced information about the following parameters: haemoglobin concentration (g $dL^{-1}$), hematocrit (%); and counts of white blood cells, namely leukocytes, neutrophils, monocytes, and eosinophils. Total lymphocytes and subsets were counted by flow cytometry (FACS Calibur, BD Biosciences), using the commercial kits from BD Biosciences (BD multitest IMK kit). Results were expressed as number of cells·$10^9$ $L^{-1}$ for leukogram parameters and as number of cells·$\mu L^{-1}$ for total lymphocytes and subsets. Plasma variation was calculated and Post, Post 2 h and Post 24 h values were corrected for plasma volume variation (Dill and Costill 1974).

### Upper respiratory symptoms

Athletes self-reported upper respiratory symptoms (URS) using daily log books as described in Rama and colleagues (Rama et al. 2013). The list of symptoms includes headache, fever, ear pain, chills, runny or blocked nose, pharyngitis/tonsillitis, bronquitis, asthma, phlegm, cough, conjunctivitis, itchy, watery eyes, nausea/vomiting, and diarrhoea. According to Bishop (2006), one episode was defined as the repetition of more than two symptoms on at least two consecutive days, and a new episode was considered after a minimum interval of 10 days following the previous one. Additionally, all swimmers were asked to indicate the medication they were on.

### Statistical analysis

The statistical analyses were performed using the software IBM SPSS Statistics (version 21; IBM Corp., Armonk, N.Y., USA) and the R software (version 2.15.1; R Core Team), both for Windows, with a significance level of 5%.

Descriptive statistics, including means and standard deviation (mean ± SD) were performed for all outcome measurements.

To have values that reflect the acute change of the immune parameters at each moments of evaluation, the percentage of relative differences from Pre to Post, Pre to Post 2 h and Pre to Post 24 h were calculated according to the formula:

$$\mathrm{RV} = \frac{X - Y}{Y} \times 100$$

where RV is the percentage of relative difference, $X$ is the final value and $Y$ is the starting value.

Normality of the outcome variables was analysed using the Shapiro–Wilk test. To verify if participants were within the "clinically normal" values associated with each variable, the one-sample $t$ test was used to compare group means with the upper or lower limits of the reference interval (INSA 2011; Lewis et al. 2006).

It was evaluated if sex, maturity, and swimming age group, influenced the effect of training on the immune response to the swimming session using nonparametric mixed-design ANOVAs. The within-subject factor was the moment of evaluation (two levels: M1 and M2), which is referred as the effect of training, and the subjects' factors were the aforementioned influential variables. The nonparametric mixed-design ANOVA has an ANOVA-type statistic (ATS) for each effect, and also a modified ANOVA-type statistic (MATS) for the subject's factor. The option for the nonparametric approach was due to the violation of the assumptions of parametric mixed ANOVA, namely the normality of the dependent variables in each factor's level, homogeneity of variances or sphericity. This nonparametric analysis was performed with the *nparLD* package (Noguchi et al. 2012) from the R software. Subsequent analyses were performed according to procedures adopted previously (Morgado et al. 2016, 2017).

To analyse the influence of training over the acute immune response to exercise, independently of any of the factors tested, nonparametric Wilcoxon test was used.

Heart rate comparison between groups at each moment of evaluation was assessed using parametric one-way Anova test and the correspondent non-parametric test of Kruskal–Wallis when normality was not assumed.

## Results

The participant's characteristics, including demographics and body composition related variables, are presented in Table 1.

No subject reported stages 1, 2 or 3 of the Tanner classification. Swimmers' physical characteristics slightly changed over the training macrocycle, reflecting little increases in stature, body mass, and FFM.

The main sets of the swimming training sessions were accomplished at the requested high intensity in relation to their personal best time at the 100 m freestyle race: 92.3 ± 4.7% at M1, and 93.4 ± 7.2% at M2.

Neither the absolute maximal heart rate achieved nor the percentage of the expected maximal heart rate were significantly different between swimming age groups or between maturation groups at any of the two moments (Table 2).

The baseline immune profile of the participant was within the reference interval associated for with each variable at both moments of evaluation (Lewis et al. 2006). Plasma volume variation according to Dill and Costill (1974) from M1 to M2 was 5.80 ± 8.37% ($t = -4.541$, $p = 0.000$).

### Seasonal training workload

Training load characterization of the whole macrocycle and of the 4 weeks before each moment of evaluation is presented in Table 3.

The load score of the 4 weeks prior to M2 was lower, and the volume and load score of the whole macrocycle

**Table 1** Mean ± SD values of the demographics and body composition of female ($n = 16$) and male ($n = 27$) swimmers at the first (M1) and second (M2) moments of evaluation

| Swimmer characteristics | Females | | Males | |
|---|---|---|---|---|
| | M1 | M2 | M1 | M2 |
| Age (year) | 14.4 ± 1.05 | 14.7 ± 1.06 | 16.2 ± 2.01 | 16.5 ± 2.05 |
| Stature (cm) | 162.2 ± 6.13 | 163.5 ± 6.23* | 173.6 ± 6.42 | 174.3 ± 6.18* |
| Body mass (kg) | 54.3 ± 8.87 | 55.4 ± 6.60* | 64.7 ± 7.85 | 66.0 ± 7.31* |
| BMI (kg $m^{-2}$) | 20.6 ± 1.96 | 20.5 ± 1.82 | 21.5 ± 3.0 | 21.8 ± 1.82 * |
| FM (%) | 25.0 ± 3.33 | 24.8 ± 2.91 | 16.6 ± 3.05 | 16.6 ± 2.90 |
| FM (kg) | 13.6 ± 0.71 | 13.8 ± 0.68 | 10.7 ± 0.48 | 11.0 ± 0.48 |
| FFM (kg) | 40.7 ± 5.02 | 41.6 ± 4.69 * | 54.0 ± 7.05 | 55.0 ± 6.29 * |

*BMI* body mass index, *FM* fat mass percentage, *FFM* fat-free mass

*Different from M1 ($p < 0.05$)

**Table 2** Mean ± SD values of maximal heart rate and of percentage of the expected maximal heart rate, and respective ranges representing minimum and maximal values, achieved in the standardized high-intensity swimming training session performed at the first (M1) and second (M2) moments of evaluation

| | M1 | | M2 | |
|---|---|---|---|---|
| | Maximal HR achieved | % HR max | Maximal HR achieved | % HR max |
| Swimming age groups | | | | |
| Youth | 175 ± 8 [166–196] | 88.2 ± 3.8 [84–99] | 170 ± 5 [161–180] | 83.0 ± 2.5 [81–91] |
| Juniors | 181 ± 9 [165–194] | 91.7 ± 4.9 [84–99] | 175 ± 11 [160–191] | 87.5 ± 5.6 [81–97] |
| Seniors | 177 ± 11 [164–188] | 91.0 ± 5.6 [84–97] | 169 ± 10 [162–186] | 87.8 ± 6.2 [84–95] |
| Difference between age groups | $F = 1.609$; $p = 0.219$ | $F = 2.044$; $p = 0.149$ | $F = 1.118$; $p = 0.342$ | $F = 1.203$; $p = 0.316$ |
| Maturity groups | | | | |
| Late pubertal | 176 ± 9 [165–196] | 89.3 ± 4.7 [84–99] | 171 ± 8 [160–191] | 86.7 ± 4.0 [81–97] |
| Mature | 178 ± 9 [164–192] | 89.9 ± 4.3 [84–97] | 173 ± 8 [163–185] | 87.8 ± 4.0 [83–94] |
| Difference between maturity groups | $F = 0.154$; $p = 0.697$ | $F = 0.117$; $p = 0.735$ | $F = 0.413$; $p = 0.526$ | $F = 0.432$; $p = 0.517$ |

HR, heart rate; [min–max], range representing minimum and maximum absolute heart rate values; % HR max, percentage of the expected maximal heart rate (calculated as 208–0.7 × age)

**Table 3** Mean ± SD weekly values of the training volume (m), and load score (AUL) of the whole macrocycle and of the 4 weeks before the first (M1) and second (M2) moments of evaluation

| Training load parameters | M1 | M2 |
|---|---|---|
| Volume (m) | | |
| 4 weeks | 30,696 ± 3991 | 29,157 ± 7397 |
| Whole macrocycle | 30,050 ± 5120 | 37,302 ± 10,331* |
| Load score (AUL) | | |
| 4 weeks | 12.0 ± 0.8 | 11.4 ± 0.8* |
| Whole macrocycle | 11.9 ± 0.6 | 12.3 ± 0.8* |

*AUL* arbitrary units of load

*Different from M1 ($p < 0.05$)

that preceded each moment of evaluation were higher at M2, than at M1.

### Influence of training on the acute immune cell changes in response to exercise

Considering the effects of the 4-month training cycle on the response to the training session, at M2, the increase of leukocytes and neutrophils from Pre to Post was smaller (Fig. 2a, b) and the decrease of total lymphocytes (Fig. 2c) and the magnitude of variation of $CD19^+$ (Fig. 2d) from Pre to Post 2 h were bigger. The effects on $CD16^+56^+$ from Pre to Post 24 h were dependent on maturity [$F(1, \infty) = 4.470$, $p = 0.035$] with a bigger negative variation at M2 in late pubertal swimmers (Fig. 2e) and the changes of $CD4^+/CD8^+$ ratio from Pre to Post 2 h were dependent on swimming age group [$F(1.881, \infty) = 10.847$, $p = 0.000$] with a more accentuated increase of $CD4^+/CD8^+$ ratio in seniors and less accentuated increase in youth at M2 (Fig. 2f).

### Upper respiratory symptoms

The number of episodes of URS was monitored weekly (Fig. 3).

When comparing the two moments of evaluation, during the 4 weeks before M2 there was a higher frequency of URS episodes than along the 4 weeks prior to M1.

## Discussion

In the present investigation, to understand the influence of training over the acute immune changes in response to intense prolonged exercise, a representative high-intensity swimming training session was performed after the main competition of the first macrocycle and after the main competition of the second macrocycle, of a 4-month swimming training cycle.

To replicate investigations and to compare results, it has been suggested that if significant plasma volume changes are expected to influence the results, either the corrected or both the measured and corrected data should be presented (Kargotich et al. 1998). Additionally, resting and/or pre-exercise blood samples taken over the course of a training season should adopt consistent sampling procedures by enforcing a standard sampling posture and resting interval for each sample.

Bearing in mind all the physiological mechanisms that can affect cell quantification (McMurray 1983; Nielsen et al. 1984; Kargotich et al. 1998) and function of the immune system, in our study the comparisons between the magnitude of response of the immune parameters to the training sessions at the two moments was made by calculating relative differences in percentage, based on the post-exercise values corrected for plasma volume variation. Thus, as we are not comparing absolute values we may argue that our results were not affected by plasmatic volume changes.

At the end of the training cycle, the lower increase of leukocytes and neutrophils from Pre to Post suggests an attenuated acute response, maybe resulting from a smaller recruitment of cells from the reservoirs or marginated pool of cells (Kruger and Mooren 2014). Furthermore, the higher magnitude of the decrease from Pre to Post2h of total lymphocytes and $CD19^+$ subset suggests a less efficient recovery of the acquired immunity (in particular $CD19^+$ lymphocytes) in the first 2 h after the intense training session, and a longer interval of immune susceptibility to infection than at the beginning of the macrocycle.

The idea of a potential immune depression and a longer interval of immune susceptibility to infection were considered in this investigation. Although the training load intensity of the 4 weeks prior to each evaluation decreased marginally from M1 to M2, the training load of the whole macrocycle preceding M2 was higher than M1. Concomitantly, the number of URS tended to be higher at M2 than at M1. This evidence suggests a potential immune depression, and we can speculate that this may result from the cumulative effects of the swimming training loads. Throughout adolescence, especially during puberty, the physiological levels of some hormones (e.g. catecholamines, cortisol, growth hormone, oestrogen, and testosterone) in association with a differential effect of these hormones and cytokines on lymphocyte subsets (Nemet and Eliakim 2010; Steensberg et al. 2001) may also influence the exercise-induced immune changes and, therefore, should be considered. Timmons et al. (2006) have reported that when exposed to equal exercise conditions, alterations in some immune cellular and humoral components were smaller in pre- and early-pubertal boys (10 years) versus adult men (22 years) and recovery from strenuous exercise was faster in children than in the adults (Timmons et al. 2004). Although these authors have not

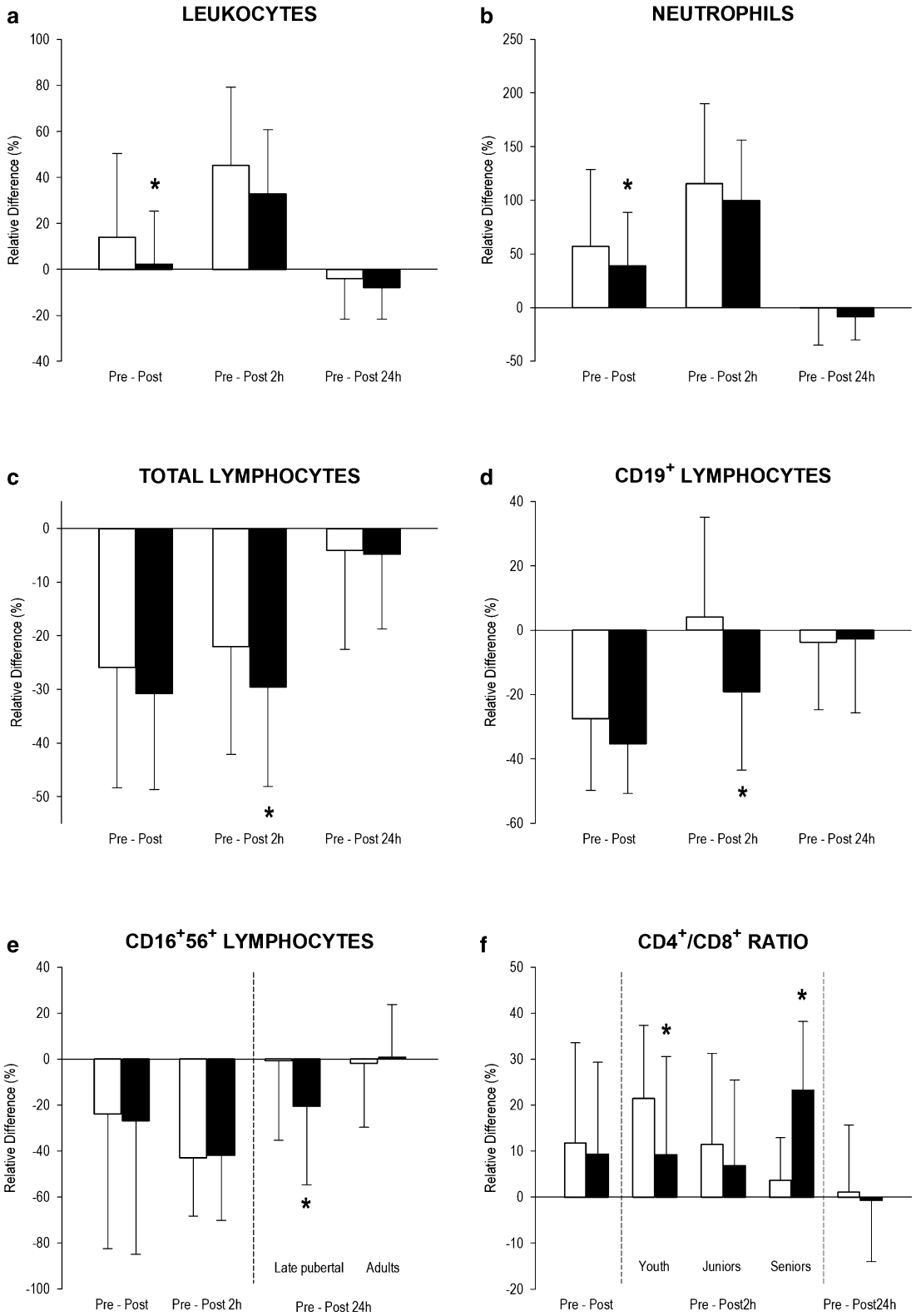


**Fig. 2** Mean ± SD of the relative difference (%) values of the leukocytes (**a**), neutrophils (**b**), total lymphocytes (**c**) and subsets $CD19^+$ (**d**), $CD16^+56^+$ (**e**), and $CD4^+/CD8^+$ ratio (**f**) cell changes in response to a standardized high-intensity swimming training session performed at the beginning (M1) and at the end (M2) of a 4-month swimming training cycle. Open square, M1; filled square M2; asterisk, different from M1

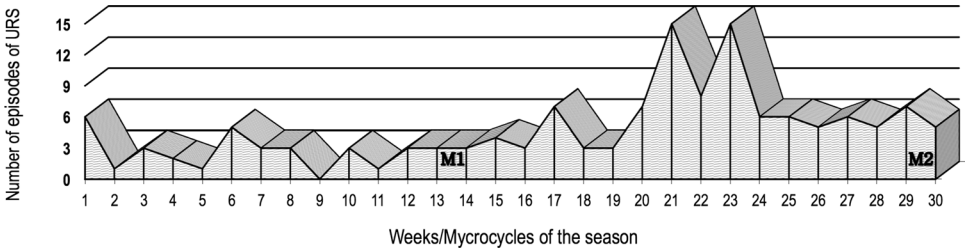


**Fig. 3** Weekly number of episodes of upper respiratory symptoms (URS) over the course of the training season in which the 4-month swimming training cycle was included (between M1 and M2; corresponding to the second macrocycle of the season)

investigated the influence of long-term training on the acute response to exercise, these results highlight the importance of controlling for age-related variables, especially during adolescence. We still have to consider seasonal variations of infectious agents (Fig. 3). In addition, the difficulty of $CD16^{+}56^{+}$ to recover in 24 h in late pubertal swimmers, and the lower magnitude of change of the $CD4^{+}/CD8^{+}$ ratio during the early recovery in the youth group, suggests that the younger athletes' immune changes were more sensitive to the influence of long-term training.

Considering the studies that control URS frequency, the information provided by participants lacked confirmation by a physician and/or laboratory analyses (Cox et al. 2008; Spence et al. 2007). Consequently, it is possible that the outcomes could have misled the conclusions, probably tending to overestimate the frequency of infections in athletes (Gleeson et al. 2004; Hellard et al. 2015); hence, it is difficult to infer between inflammation and infection (Cox et al. 2008; Spence et al. 2007). To our knowledge only another investigation conducted in Portuguese swimmers (Rama et al. 2013) studied the URS occurrence with laboratory cells count confirmation. The authors (Rama et al. 2013) reported a higher URS frequency during the hardest training phases, which is in accordance with our data (Fig. 3), despite the lack of statistical inference. Therefore, one can state that the results obtained in our investigation reinforce those obtained by Rama et al. (2013).

The reduction in the cell trafficking and cell proliferation and/or increased cell death responses (Kruger and Mooren 2014) could have relied upon the long-term adaptations of the adherence of cells to the endothelium and their redistribution amongst organs or compartments, and of the physiological responses to acute exercise, namely cardiac output, shear stress, and blood flow to working muscle, and improved ability to counteract pH and temperature changes (Adams et al. 2011). Moreover, training might have influenced catecholamines, and cortisol concentrations and their regulation of lymphocyte subset redistribution (McCarthy et al. 1991; Mignini et al. 2008). During the post-exercise recovery period cortisol acts as conditioner of the entry of lymphocytes into the circulation contributing to their return to lymphoid compartments (Nieman 1994), and regulates both lymphopenia and neutrophilia (McCarthy et al. 1991; Mignini et al. 2008). Although the overall long-term training effects of cortisol over resting lymphocytes remains unclear, and herein was not evaluated, we may speculate that cortisol's levels may partially explain the less efficient recovery change of total lymphocytes and $CD19^{+}$ subset to the swimming session at the end of the cycle.

It is commonly accepted that training load increments in well-trained athletes undertaking periods of elevated training volume and intensity can lead to the stimulation of adaptive mechanisms related to metabolic and hormonal circulatory and respiratory responses that can compromise performance and, therefore, induce an impaired immune status, including falls in the number and activity of T and B cells (Lancaster et al. 2004; Walsh et al. 2011). This conjuncture can contribute to elevate the risk of infection; nevertheless, the literature suggest that this situation may be reversible by a tapering or recovering period (Gleeson and Bishop 2005; Walsh et al. 2011). Hence, coaches and athletes ought to implement intervention and behavioural strategies during taper periods to contribute to maintain health conditions, preventing the onset of fatigue and associated diminished performance, thus helping to avoid illness and reaching the peak performance at competitions. Also, athletes should take special precautions during the first hours after intense training sessions.

## Conclusions

In the present investigation, at the end of the training macrocycle, the lower magnitude of the immediate leukocytosis and neutrophilia followed by the more prolonged recovery of total lymphocytes and B cells changes in response to the swimming session appears to dictate a general attenuated acute immune change. This change seemed even more attenuated in the younger athletes, reflected by the difficulty of $CD16^{+}56^{+}$ to recover in 24 h in late pubertal swimmers, and by the lower magnitude of the $CD4^{+}/CD8^{+}$ ratio change during the early recovery in the youth group. Concurrently, there was a higher URS frequency, which reinforces the idea of a potential immune depression and a longer interval of immune susceptibility to infection. It appears that the cumulative effects of the swimming training loads induced an overall reduction of the ability of the immune system

to respond to intense training sessions, especially in the younger athletes, which makes effective resting time more valuable.

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## Compliance with ethical standards

**Conflict of interest** The authors have no conflicts of interest to declare.

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