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Upper respiratory tract symptoms and salivary immunoglobulin A of elite female gymnasts: a full year longitudinal field study

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ABSTRACT: The aim of this study was to determine the frequency of upper respiratory tract symptoms (URS) in elite female gymnasts during a training season. In addition, we aimed to observe the extent to which salivary immunoglobulin A (sIgA) is associated with URS in these athletes, including potential effects of the season and timing of sample collection. Over one year, 18 elite female gymnasts completed URS and fatigue questionnaires weekly and provided 1 mL of saliva after a minimum 36 h of rest (morning or afternoon) to measure relative sIgA concentration (= mean absolute sIgA value of the week divided by the mean absolute sIgA value of the weeks without URS). Mean weekly URS and mean relative sIgA values per gymnast correlated negatively ($r = -0.606$, $P = 0.022$). Most URS were noted in the most fatigued gymnasts (7.4 ± 10.1 vs. 2.5 ± 5.6 ($P < 0.001$) for 'normal' and 2.1 ± 3.7 ($P = 0.001$) for 'better than normal' rested). In spring, relative sIgA was higher compared to autumn (112 ± 55 vs. $89 \pm 41\%$, $P < 0.001$) and winter ($92 \pm 35\%$, $P = 0.001$), while during summer, relative sIgA appeared higher compared to autumn (110 ± 55 vs. $89 \pm 41\%$, $P = 0.016$). The interaction effect with timing of sample collection showed higher relative sIgA values in morning samples in spring and summer compared to afternoon samples, with the inverse observed in autumn and winter ($F = 3.565$, $P = 0.014$). During a gymnastics season, lower relative sIgA values were linked to higher susceptibility to URS in elite gymnasts. However, relative sIgA values were influenced by season and timing of sample collection and thus should be considered when interpreting sIgA data.

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INTRODUCTION

Elite sport performance requires an optimal training load (TL) balanced with effective recovery [1]. Both interruptions of training schedules due to recurrent illnesses or injuries and/or falling ill before or during competitions will impair performance outcome and should be avoided. During the 2016–2018 Summer and Winter Olympic Games [2, 3], 5–9% of athletes encountered at least one illness episode [4]; similar findings were also reported in younger competitors during the Youth Olympics (14–18 years old) [5]. Respiratory diseases, in particular upper respiratory tract infections (URTI), accounted for about 47 to 70% of reported illnesses for Summer and Winter Games respectively, for which some studies state that females might be at higher risk [2, 3], while others claim the opposite [6]. In the latter study, up to 48% of athletes reported becoming ill during or immediately after a multiple day race event (tour de ski), with 85% of symptoms related to URTI.

A possible contributor to higher infection odds in athletes is a fall in salivary immunoglobulin A (sIgA) concentration. SIgA is an antibody found in saliva that serves as an immunological barrier with the ability to neutralize pathogens responsible for causing URTI [7]. Therefore, sIgA is considered a first line defence to counter infections and has been recognized as a marker for the mucosal immune system in athletes [8–11]. However, the debate about sIgA's usefulness in this context is still ongoing, since several other factors such as nutrition, anxiety and sleep, as well as infections, influence sIgA synthesis and/or secretion, which may be the reason for not identifying an association with URTI [12].

TL is considered one major determinant for the development of respiratory infections in athletes [13] in addition to other stressors such as travel, psychological stress, insufficient calorie intake, impaired sleep quantity and quality, and behavioural and environmental

factors [14]. Both high acute and chronic TLs have been associated with increases in upper respiratory symptoms (URS) [6, 15]. Athletes appear more susceptible to infections for a short period (3–72 h) after acute high intensity and/or endurance events (suggested as the theory of 'the open window' [16]) due to transient disturbances in usual immune homeostasis relative to exercise intensity and duration [17]. In gymnasts, a decrease in sIgA immediately (< 5 minutes) after training and competition sessions has been observed, without a concomitant increase in URTI [18], while others found no unambiguous effect of gymnastic TL on sIgA [19].

In most sports, athletes need to specialize early in one discipline to reach the international senior top level, with high TLs beginning from a young age. Increased awareness of infections caused or at least facilitated by overload in young athletes (< 18 years of age) is developing [20] and susceptibility to URS may be higher because of incomplete development of their adaptive immune system [21, 22]. Furthermore, while most longitudinal studies focus on outdoor endurance sports such as sailing and tour de ski [6, 10], intermittent indoor sports such as artistic gymnastics are not given much consideration in this research domain. Studies of intermittent sports are largely performed on young male athletes, in non-elite populations and/or lasted no longer than 8 weeks [18–19, 23–25]. Yet the question remains how URS and the underlying immune system interact throughout a whole season in young elite populations, especially in female athletes with a potentially higher illness risk [2, 3]. Female elite artistic gymnasts train up to 40 h a week to reach the top international level [26] and therefore seem an ideal population to study. Hence the aim of this prospective study was to determine the frequency of URS in elite female artistic gymnasts during an entire training season and to observe to what extent sIgA can be associated with URS in these young elite athletes.

MATERIALS AND METHODS

Participants

This study included 18 elite female gymnasts (16.4 ± 3.4 years) from the High Performance School of Artistic Gymnastics in Ghent (Belgium), including European, World and Olympic championship finalists and medallists. Participants aged 18 and under (n = 14) resided in a boarding school Sunday to Thursday and returned home for Friday and Saturday nights. The adult athletes (n = 4) stayed in student homes during the week. Participants and their parents were informed about the rationale and risks of the study both orally and in written form. All athletes, together with parents if they were under 18, gave written consent to participate in this study, which was approved by the ethical review board of the University Hospital of Ghent (Belgium). The experiments were performed in accordance with the ethical standards of the Helsinki Declaration.

Intervention, measurements and data collection procedure

Data collection commenced after the summer break in July 2015 and ended after the season (July 2016). During this period gymnasts continued with their normal training regime, which consisted of 9 to 10 training sessions per week for a total of 28 to 32 hours per week. Before the first training of every week (after a minimum of 36 hours without training, which was scheduled by their coaches), every gymnast completed a health and fatigue questionnaire and provided 1 mL of unstimulated whole saliva for the measurement of salivary IgA (sIgA). This took place on Sunday afternoon (between 2:00 PM and 3:00 PM) or on the following Monday morning (between 7:00 AM and 8:30 AM). Due to logistical issues, samples were not collected during holidays, foreign training camps or competitions. Prior to saliva collection, anthropometric measures of height (Seca Stadiometer) and body weight (Seca balance) were recorded to calculate body mass index (BMI, weight (in kg) divided by length^2 (in m^2)) and gymnasts performed an incremental ramp exercise on a treadmill (H/P/Cosmos) to determine their maximal oxygen uptake (= $\dot{V}O_2$max). The test started at 5 km·h^{-1} with a 1.5% treadmill gradient. Every 30'', speed increased by 0.5 km·h^{-1} to volitional exhaustion. Pulmonary gas exchange (Jaeger Oxycon Gas Analyzer) was registered on a breath-by-breath basis. $\dot{V}O_2$max was defined as the highest 30'' average achieved during the test.

Data collection procedure: questionnaires

Gymnasts completed a health [27, 28] and fatigue questionnaire [10] about the previous week (with the opportunity for oral clarification from the researcher) which included the following questions: (1) Do you suffer from an allergy? If so, did you experience any symptoms last week? (2) Which and how many prescribed and non-prescribed medications did you take during the last week? When did you take them? Did you visit a doctor? (3) How rested do you feel today? ('Worse than normal', 'Normal' or 'Better than normal') [10]. Additionally, they rated their fatigue on a Visual Analogue Scale between 0 (totally not rested) and 10 (fully rested) [29]. The last part of the questionnaire comprised a symptom checklist [27, 28]. Symptoms included: sore throat, mucus in the throat, runny nose, coughing, repeatedly sneezing, fever, joint aches, weakness, headache and loss of sleep. Subjects self-reported the duration and severity of their symptoms, the latter by recording whether they kept to their normal training regime (scored as 1), adapted their training regime (scored as 2) or stopped training (scored as 3). For each symptom, the number of days was multiplied by the severity score and then summed to provide the weekly URS score. A score of ≥ 12 was indicative of a URS episode [27]. The aetiology of the symptoms was not further examined.

Data collection procedure: salivary IgA collection and assessment

Participants were informed orally and through information sheets about the procedure before the first saliva collection (Figure 1). They were asked (1) to refrain from caffeine and food with high sugar content or acidity in the hour before collection, (2) not to eat a main meal in the hour before collection, (3) to avoid cleansing their teeth in the 45 minutes before collection and (4) not to visit a dentist in

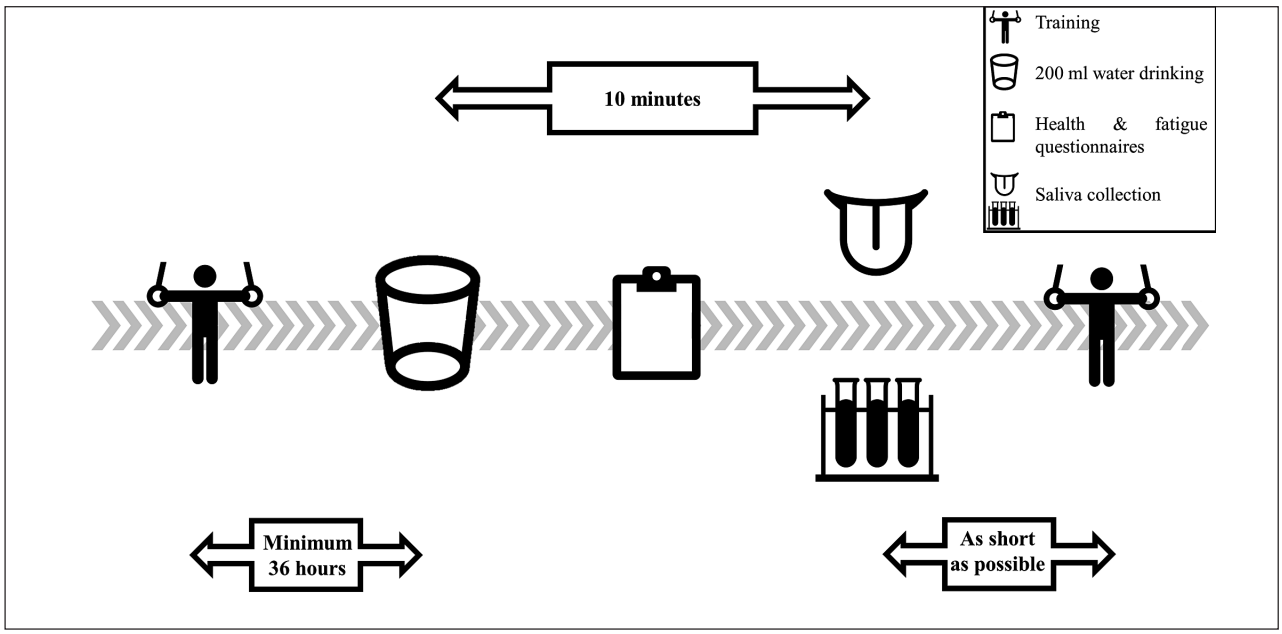

FIG. 1. Flow chart of the study.

the 24 hours before collection. The collection process started with drinking 200 mL of water 10 minutes before the sample collection, as prescribed by the salivary analysis kit (Salivary Secretory IgA Enzyme Immunoassay Kit, Salimetrics, USA).

Saliva collection began 10 minutes after drinking 200 ml of water; participants were instructed to swallow first to empty the mouth then, with their head slightly tilted forwards, saliva was collected by passive drool into a sterile vial until 1 mL was collected and collection time was measured. Subsequently saliva samples were weighed to estimate saliva volume, based on assumed mass density of 1 mg·mL^{-1} [30], and stored at -20°C until further analysis. On the day of analysis, the samples were thawed to room temperature and centrifuged for 15 minutes at 3000 rpm (= 1.4 RCF) to remove mucins. The concentration of sIgA (μg·mL^{-1}) was determined using an ELISA assay kit (Salivary Secretory IgA Enzyme Immunoassay Kit, Salimetrics, USA). Duplicate saliva samples were analysed with 25 μL of saliva per analysis. The intra-assay coefficient of variability (CV) was 5%. The lower limit of sensitivity calculation resulted in 19.78 μg·mL^{-1}. The saliva flow rate (mL·min^{-1}) was calculated by dividing the saliva volume by the collection time. Subsequently, the secretion rate (μg·min^{-1}) was determined by multiplying the absolute sIgA value with the saliva flow rate. To obtain the weekly relative sIgA concentration, the mean absolute sIgA value of the week was divided by the mean absolute sIgA value of the weeks without URS. This approach adjusts for individual variances in the data, making it possible to search for relative changes in sIgA, independent of intra- and interindividual differences in absolute sIgA values [10].

Statistical analysis

Before every analysis, the normality of the parameters was checked using the Shapiro-Wilk test. Outliers (> 1.5 interquartile range) were identified through boxplots and deleted from the analysis where appropriate. Means, standard deviations (SD) and minimum and maximum values were calculated using descriptive statistics. For every parameter, 95% confidence intervals (CI) were reported. Cohen's effect sizes (ES) were calculated for every significant difference and evaluated as trivial (0–0.19, T), small (0.20–0.49, S), medium (0.50–0.79, M) and large (≥ 0.80, L) [31].

Correlations between individual characteristics (age, $\dot{V}O_2$max and BMI) and immune parameters (saliva flow rate, absolute sIgA, sIgA secretion rate, relative sIgA, URS and URS episodes) were calculated using Pearson's correlation (between-person correlation on person-centred data). The relationships between the gymnasts' relative sIgA concentrations and their weekly mean URS and total number of URS were analysed using a linear regression with 'r' representing the correlation coefficient while 'r^2' denotes the determination coefficient (only values > 0.30 were considered clinically relevant).

Differences in saliva parameters between morning and afternoon sampling were identified through a linear mixed model (LMM), with the diagonal covariance matrix showing the best Akaike's information criterion (AIC). AIC is a criterion which assesses the relative quality of statistical models and so indicates the model most likely to be correct and so best to select based on balancing changes in goodness-of-fit vs. variance in number of parameters.

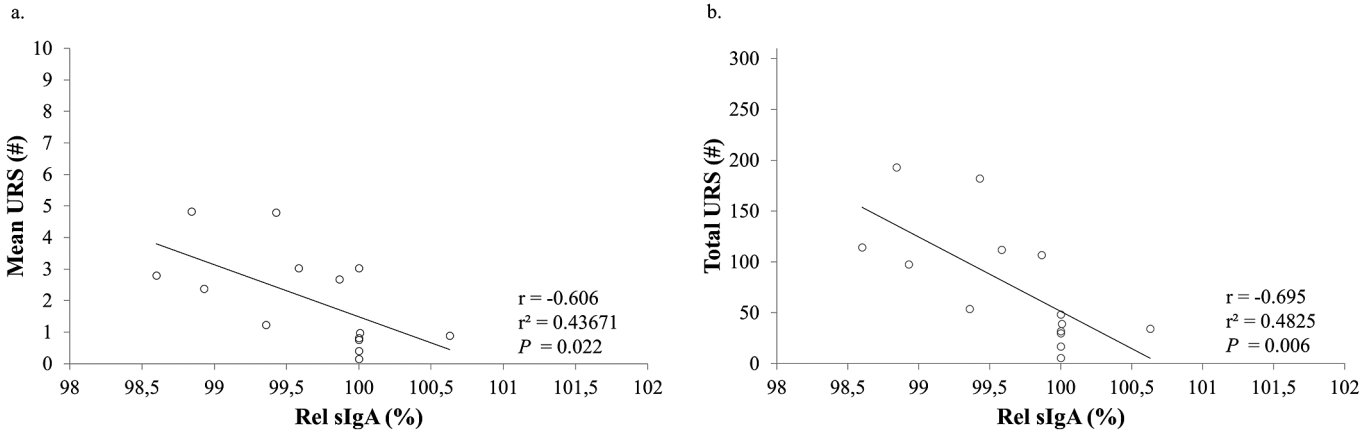

FIG. 2. (a) Linear regression between the mean upper respiratory tract symptoms (URS) per week and relative salivary Immunoglobulin A (sIgA).
(b) Linear regression between the total URS and relative sIgA.
Note: # = amount

To analyse whether a difference occurred in relative sIgA value observed in URS episodes compared to periods without an URS episode, an LMM was executed, including the evaluation of AIC. Main effects were compared using the Bonferroni method to evaluate estimated marginal means of the fitted model. The timing of saliva collection (morning or afternoon) was included in the analysis as a factor to measure possible influences of this parameter by exploring possible interaction effects through fixed effects.

LMM was also used to identify differences in relative sIgA values during different fatigue levels and during different seasons, with timing of saliva collection included as a factor to identify interaction effects through fixed effects. Main effects were compared using the Bonferroni method to evaluate estimated marginal means of the fitted model. Here the diagonal covariance matrix showed the best AIC. Differences in URS and URS episodes between seasons were also identified using this method. Lastly, differences in URS and URS episodes according to fatigue level were analysed with LMM with fatigue level as a fixed factor and the comparison of main effects using the Bonferroni method to evaluate estimated marginal means of the fitted model.

Data were analysed using IBM SPSS software (version 24). Significance was set at $P < 0.05$.

RESULTS

Over a period of 56 weeks, 661 saliva samples were gathered from 18 gymnasts (body mass 45.7 ± 7.3 kg, height 153.2 ± 6.9 cm, BMI 19.4 ± 2.0 kg·m^{-2} and $\dot{V}O_2$max 52.13 ± 4.46 mL·min^{-1}·kg^{-1}, which is 139% of predicted values for their sex, age and weight).

TABLE 1. Descriptive values as means (± standard deviation (SD)) per gymnast, minimum – maximum range and 95% Confidence Interval (CI).

Parameters	Mean ± SD	Min – Max	95% CI
Flow rate (mL·min^{-1})	1.00 ± 0.41	0.35 – 2.06	0.81 to 1.19
Absolute IgA (μg·mL^{-1})	130 ± 39	80 – 214	112 to 148
IgA secretion rate (μg·min^{-1})	119 ± 50	54 – 240	96 to 142
Relative IgA (%)	101 ± 5	96 – 120	99 to 103
URS (#)	100 ± 85	7 – 310	60 to 139
URS episodes (#)	2.28 ± 2.91	0 – 9	0.94 to 3.62
Fatigue score (-1: *Worse than normal rested*; 0: *Normal rested*; 1: *Better than normal rested*)	-0.01 ± 0.10	-0.29 – 0.18	-0.06 to 0.04
Fatigue VAS (/10)	5.54 ± 0.58	4.78 – 6.41	5.27 to 5.81

Note: IgA = Immunoglobulin A; URS = Upper Respiratory Tract Symptoms; # = Amount; VAS = Visual Analogue Scale

TABLE 2. Differences between morning (n = 382) and afternoon (n = 279) saliva parameters in means (± standard deviation (SD)) and 95% Confidence Interval (CI) of the difference, computed using the linear mixed model. ES (Effect Size), F (= variance between the sample means/variance within the samples) and *P*- values from the linear mixed model are presented.

	Morning (Mean ± SD)	Afternoon (Mean ± SD)	95% CI of the difference	ES	F	*P*-value
Flow rate (mL·min^{-1})	1.04 ± 0.63	0.94 ± 0.59	0.03 to 0.19	0.232 (S)	4.109	0.043*
Absolute IgA (μg·mL^{-1})	132 ± 83	123 ± 64	-2 to 21	0.186 (T)	2.567	0.110
IgA secretion rate (μg·min^{-1})	123 ± 96	106 ± 74	4 to 31	0.288 (S)	6.199	0.013*
Relative IgA (%)	103 ± 52	97 ± 42	-2 to 13	0.167 (T)	2.084	0.149

IgA = Immunoglobulin A; ES = effect size: S = small, T = trivial. *$P < 0.05$.

Because of foreign competitions, training camps and holidays, not every gymnast was able to donate a saliva sample every week; on average, 37 (± 9) samples per gymnast were gathered. During this period, 33 URS episodes were registered. Twelve out of the 18 gymnasts experienced at least 1 URS episode, with 7 having a minimum of 2 episodes. One gymnast described having hay fever, which was attributed to one of her URS episodes. Descriptive values expressed as means per gymnast for saliva parameters, URS and fatigue ratings are presented in Table 1. Significant correlations were found for age and BMI (r = 0.598, $P = 0.009$) and age and number of URS episodes (r = 0.582, $P = 0.011$).

A linear regression showed negative correlations between mean URS per week and mean relative sIgA values per gymnast (Figure 2a) and between total URS and mean relative sIgA values per gymnast (Figure 2b).

Because of variable time points of sample collection, the variability between morning and afternoon measurements in saliva parameters was explored and presented in Table 2. Flow and sIgA secretion rates were lower in the afternoon compared to the morning ($P < 0.05$), but there was no effect on relative sIgA values.

No differences in relative sIgA values were found between weeks with (106 ± 55%) and without (100 ± 48%) an URS episode (F = 0.058, $P = 0.809$), and no interaction effect with timing of sampling was observed (F = 1.868, $P = 0.172$).

No differences were found in relative sIgA values (F = 1.167, $P = 0.312$) between the fatigue categories (worse than normal rested (95% CI: 72 to 104), normal rested (95% CI: 86 to 104), better than normal rested (95% CI: 87 to 120)) as shown in Figure 3. Also, no significant interaction effect was identified between fatigue level and timing of sample collection (F = 0.142, $P = 0.867$). However, both URS (F = 11.398, $P < 0.001$) and URS episodes (F = 22.318, $P < 0.001$) differed between the fatigue levels. More URS were noted in the 'worse than normal' (7.4 ± 10.1) category compared to 'normal' (2.5 ± 5.6, ES = 0.600 (M), $P < 0.001$) and 'better than normal' (2.1 ± 3.7, ES = 0.697 (M), $P = 0.001$) rested categories. Correspondingly, more URS episodes were detected in the 'worse than normal' (0.32 ± 0.47) category compared to 'normal' (0.05 ± 0.22, ES = 0.736 (M), $P < 0.001$) and 'better than normal' (0.03 ± 0.17, ES = 0.821 (L), $P < 0.001$) rested categories. As for URS and URS episodes, no difference was noted between the 'normal' and 'better than normal' rested categories (ES = 0.084, $P = 0.686$; ES = 0.102, $P = 0.590$).

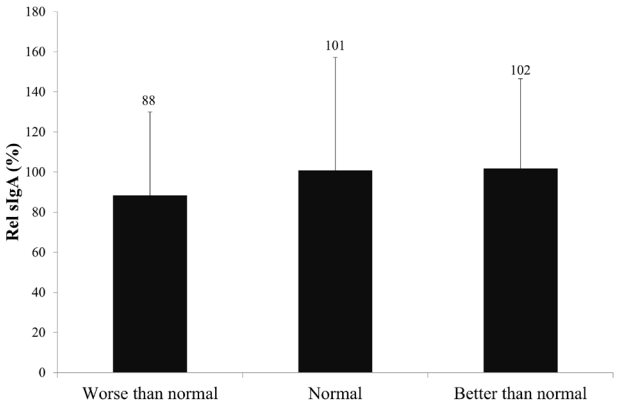

FIG. 3. Relative salivary Immunoglobulin A (sIgA) value for 'Worse than normal rested', 'Normal rested' and 'Better than normal rested'. F = 1.167; $P = 0.312$ (Linear Mixed Model).

In Figure 4a, the differences in relative sIgA values for different seasons are presented (F = 9.174, $P < 0.001$), with 95% CI for relative sIgA values during spring 105 to 118, summer 97 to 115, autumn 83 to 96 and winter 85 to 100. In spring, relative sIgA was higher compared to autumn (ES = 0.505 (M), $P < 0.001$) and winter (ES = 0.421 (M), $P = 0.001$) values, while during summer, higher values of relative sIgA were measured compared to autumn (ES = 0.420 (M), $P = 0.016$).

A significant interaction effect was noted between season and timing of sample collection (F = 3.565, $P = 0.014$), as shown in

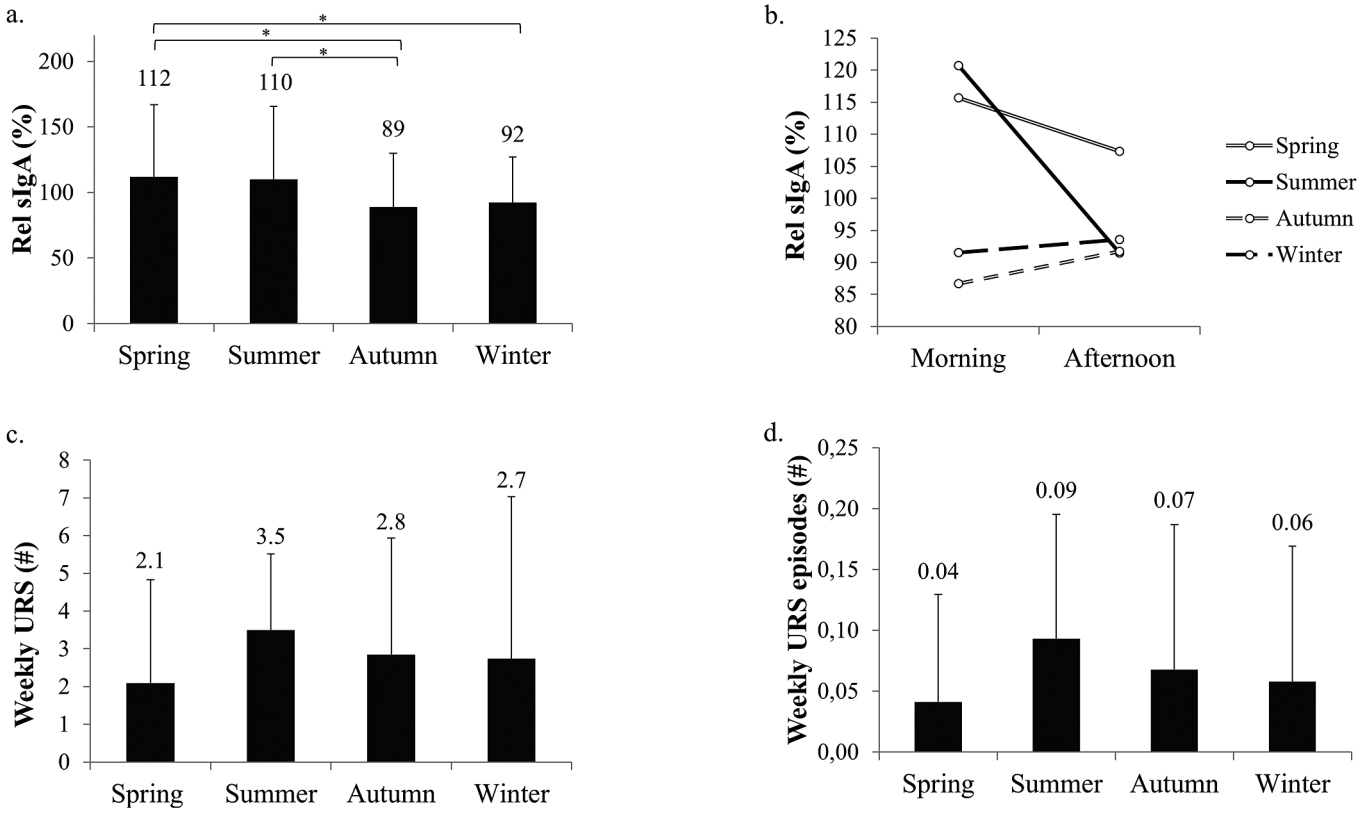

FIG. 4. (a) Relative salivary Immunoglobulin A (sIgA) values for the different seasons. Spring vs. autumn: $P < 0.001$; spring vs. winter: $P = 0.001$; summer vs. autumn: $P = 0.016$.
(b) Interaction between season and timing of sample collection (morning vs. afternoon) in relative sIgA values.
(c) Mean weekly upper respiratory tract symptoms (URS) for the different seasons.
(d) Mean weekly URS episodes for the different seasons.
Note: Values are expressed as means ± standard deviations (SD).

Figure 4b. Relative sIgA (%) values in spring and summer collected in the morning were higher than those collected in the afternoon, while values in autumn and winter were higher when collected in the afternoon compared to the morning.

No significant differences were seen in URS ($F = 1.421$, $P = 0.235$) and URS episodes ($F = 1.183$, $P = 0.315$) between the different seasons (Figure 4c and 4d). The 95% CI for URS during spring, summer, autumn and winter were respectively 1.26 to 2.93, 2.43 to 4.58, 2.01 to 3.69 and 1.80 to 3.68. The respective 95% CI values of the URS episodes during spring, summer, autumn and winter were 0.01 to 0.08, 0.05 to 0.14, 0.03 to 0.10 and 0.02 to 0.10.

DISCUSSION

This prospective study reported a negative association between number of URS episodes and the mean relative sIgA values in elite female gymnasts over an entire gymnastics season. Number of URS episodes and age were positively related. Secondly, the interpretation of relative sIgA concentrations in elite gymnasts depended partly on the interaction between the 'timing of the sample collection (morning vs. afternoon)' and across the 'seasons'. Relative sIgA concentration was chosen as an appropriate parameter since the influence of timing of sample collection alone was not significant for this, and it is an accepted method to employ to avoid the large variation in interindividual differences seen in absolute sIgA values [10]. Furthermore, immune function and URS frequency have never been monitored together in elite gymnasts throughout a season, with the inclusion of potential seasonal effects.

As sIgA values might be influenced by several parameters including oral health, nutrition, sleep, and diurnal and seasonal variations [12], this study tried to minimize their possible interference by controlling for the influence of oral health and nutrition in the study design, by monitoring fatigue as a possible derivative of sleep quality and by integrating the influence of diurnal and seasonal variations into the analyses.

The sIgA values of the gymnasts were similar to those of elite sailors [10], as was the frequency rate of URS episodes (2.5/gymnast/56 weeks vs. 2.7/sailor/50 weeks), which was also comparable with a swimming population (4.8/swimmer/75 weeks) [10, 15]. Hence no differences in URS rate could be detected despite the dif-

ference in athletic population, discrepancies in methodology for determining an URS episode (by questionnaire vs. physician-based vs. a combination of both) and the different training circumstances and environmental factors with an impact on risk for respiratory infection (e.g. air pollution because of the magnesia alba in the gymnasia inducing respiratory problems [32]; cold, dry air leading to bronchoconstriction or coughing [33]; limited airway damage in hot and humid conditions [34]). The frequency of URS episodes in athletes appears similar to that of the general adult population [35]. However, the frequency of URS and its timing might be different in athletes depending on peak training or competition periods in the season, suggesting a possible different aetiology compared to the general population. Therefore, these results indicate that indoor intermittent sports in adolescent athletes do not result in an altered risk for URS episodes as compared to adult endurance (outdoor) athletes. In fact, considering that there is a positive correlation between gymnasts' age and URS episodes, younger athletes might be more protected against URS episodes, possibly because of lower TL and stress [36].

The significant negative correlation between relative sIgA values and total URS episodes supports previously published data in elite Yachtsmen [10]. Lower values are associated with higher URS risks, which may also be concluded from studies using the absolute sIgA concentration and sIgA secretion rate [8, 9, 27, 37]. However, sIgA values (relative, absolute and secretion rate) probably are not the single or even major determinant for this outcome, since URS may develop through different mechanisms, including both viral and bacterial pathogens, allergy and air pollution [38] and other stressors including diet and psychological parameters [37].

One factor that may be associated with lower sIgA values and subsequently more URS episodes is the fatigue status of the individual athlete. Although no significant relationship was apparent here between the relative sIgA concentration and the fatigue categories (Figure 3), a similar pattern was seen in another study [10], with lower relative sIgA concentrations for a 'worse than normal' rested state compared to the 'normal' and 'better than normal' rested state. Furthermore, in the present study, those gymnasts reporting feeling fatigue levels that were 'worse than normal' when rested experienced more URS compared to the other fatigue categories; thus gymnasts with more URS felt more fatigued. Monitoring fatigue may therefore be a useful surrogate indicator to detect (particularly) lower sIgA values and URS, since fatigue may reduce the ability to counter infections [8]. This needs to be further examined as it is not clear whether avoiding excessive fatigue and consequent negative performance outcomes might be a sufficient preventative measure for developing URS without having to resort to salivary IgA determinations as a predictor of this risk profile.

Timing of sampling varied depending on the scheduling of the gymnasts' training, so the possible influence of circadian rhythms on the salivary parameters could not be neglected. The timing and season of collection impacted relative sIgA concentration, with a lower relative sIgA concentration during the afternoon compared to the morning in summer – a pattern that was seen to a lesser degree during spring, but not during the other seasons. One suggestion is that elite gymnasts, especially during summer, may be more prone to dehydration at noon compared to the morning, as a consequence of travel time from home to the training hall (> 1 h drive for most of the gymnasts). This may have a negative influence on IgA secretion [39, 40], represented in the trough in relative sIgA values in the afternoon compared to the morning. Severity of dehydration was positively associated with alterations in serum Ig composition after judo practice [41], whereas a submaximal cycling test followed by overnight fluid restriction led to a decrease in saliva flow rate and in the secretion rates of salivary antimicrobial proteins, α-amylase, and lysozyme but not in sIgA [42]. However, to the best of the authors' knowledge, no studies have been conducted in which the hydration status and sIgA concentration were monitored without the interfering effects of training or fluid intake. A second suggestion involves the influence of the solar radiation-dependent vitamin D on sIgA [43]. Vitamin D metabolites fluctuate during the day. In winter there is an increase during the day, following an overnight trough, of 1.25(OH2)D, the biologically active vitamin D molecule [44]. Since vitamin D indirectly affects sIgA [43], winter (and possibly autumn) morning sIgA values may be compromised by lower vitamin D availability compared to the afternoon.

Overall, relative sIgA concentrations were higher during spring and summer than in autumn, while during spring values were also higher compared to the winter months. This could not be confirmed by another study conducted in gymnasts, where similar values were found over 3 seasons (1 measurement in autumn, 1 in winter and 1 in spring), possibly explained by the limited amount and period of sample gathering [18]. Despite the fluctuations in sIgA over the seasons, we did not observe subsequent corresponding variation in the frequency of URS or URS episodes throughout the different seasons, although higher illness rates would be expected during autumn or winter from a study with swimmers [15]. One possible explanation is that during a swimming season, TL is differently distributed, with a peak during the winter months [15], while during a gymnastics season TL remains high throughout the entire season without distinctive peaks and troughs [45]. As higher TLs are correlated with higher respiratory illness rates [13], this might explain the levelling off in the number of URS and URS episodes in the gymnasts' population throughout the season compared to the swimmers. The higher relative sIgA values during spring and summer might be ascribed to two factors. Firstly, during spring and especially during summer, gymnasts might be less fatigued because of school holidays (2 weeks in April and the whole of July and August), which offer more recovery time than during weeks of school combined with training. Secondly, vitamin D has an immune-modulatory function [46], as seen in endurance athletes who had lower vitamin D levels which were linked with lower immunoglobulin secretion rates and increased susceptibility to URTI [47]. The combination of less sunlight in winter and autumn and the indoor character of gymnastics [47] might place gymnasts

at risk of attaining insufficient vitamin D levels, possibly degrading sIgA values [46].

Practical implications

Care should be taken over whether relative sIgA measures should be used for clinical purposes, as factors such as season of the year and timing of sample collection affect its interpretation. During autumn and winter values are lower than during the rest of the year, irrespective of URS frequency. In addition, variation between morning and afternoon saliva parameters should be considered, since flow and secretion rate are lower in the afternoon. Monitoring and optimizing fluid intake/hydration status may help to alleviate this to some extent. Because of the association with relative sIgA values and URS, fatigue status may help identify periods with higher risk of reduced immunocompetence, and therefore higher risk of infection, and should be considered by coaches or team physicians of elite gymnasts. Since vitamin D may influence sIgA parameters, gymnasts may draw benefits from vitamin D supplementation, especially during winter months when sun exposure is minimal.

Limitations

The small study sample without a control group is one of the limitations of this study, while the strength of the study is the long-term follow-up of elite athletes, which is rare in the exercise immunology literature. As high intra-variability of absolute sIgA could have disturbed our results, relative sIgA values were predominantly analysed to mitigate the effect of intra-individual variances.

A possible weakness is the different time points of saliva collection. Although these analyses revealed that this factor did not influence mean absolute and relative sIgA values, a significant interaction effect with seasons was found. Secondly, measuring TL would have broadened the information about the season. Although this was the intention at the beginning of the study, due to compliance issues these data could not be included in the results. Also, the occurrence of URS or URS episodes was defined by weekly completion of a validated questionnaire about self-reported symptoms [27, 28], while daily reporting and the analysis of the pathology of the symptoms would have provided more information about the exact timing, duration and aetiology of the URS episode. Furthermore, the phase of the menstrual cycle and potential use of oral contraceptives should be taken into account as reproductive hormones may influence immunological parameters. Consequentially, our results cannot be extrapolated in males. Lastly, age could be a confounding factor that could not be integrated in our analyses due to the limited sample size.

CONCLUSIONS

During a gymnastics season, relative sIgA values are linked to the susceptibility to an URS episode. However, relative sIgA values should be interpreted with care, since they can be influenced by season and the timing of sample collection. In addition, fluctuations in URS are associated with gymnasts' feelings of fatigue, with more URS evident in the most fatigued.

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Disclosure of interest

No potential conflict of interest was reported by the authors.

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