Journal of Science in Sport and Exercise
https://doi.org/10.1007/s42978-022-00186-w

ORIGINAL ARTICLE

Inflammatory Biomarkers in Elite Cross-Country Skiers After a Competition Season: A Case–Control Study

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Received: 8 October 2021 / Accepted: 20 July 2022

Abstract

Purpose Whether elite athletes, who have been exposed to vigorous-intensity exercise combined with other stressors, have elevated systemic low-grade inflammation, remains largely unclear. To address this question, we studied the levels of six inflammatory cytokines as potential biomarkers of a low-grade inflammatory state in elite athletes after an 11-month training and competition season.

Methods We collected sera from 27 Finnish elite cross-country skiers and 27 gender- and age-matched, moderately-exercising controls. The serum concentrations of C-reactive protein (CRP), interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), glycoprotein acetyls (GlycA), interleukin 10 (IL-10), and interferon gamma (IFN-γ) cytokines were quantified as surrogate markers of low-grade inflammation.

Results The athletes were found to have significantly lower concentrations of CRP ($P=0.0232$) and higher concentrations of IL-10, TNF-α, and IFN-γ ($P=0.0097$, $P=0.0256$, and $P=0.0185$, respectively) than the controls. No significant differences between athletes and controls were detected in the concentrations of IL-6 and GlycA. The inflammatory score (IS) did not differ significantly between athletes and controls.

Conclusion The results of this study argued against the hypothesis of a significant chronic low-grade inflammation in response to prolonged high-performance exercise among elite endurance athletes.

Keywords Athlete · Biomarker · Chronic low-grade inflammation · Exercise · GlycA · Inflammation

Introduction

Elite athletes commonly engage in hours of intense daily exercise throughout their training and during competitive seasons. Repeated strenuous bouts of exercise and very high training loads are often demonstrated to induce a transient, partial suppression of several immune functions in athletes. However, the clinical impact of these well-characterized systemic and mucosal responses in innate and acquired immune parameters is poorly documented [32, 37]. On the other hand, regular vigorous-intensity exercise combined with other stressors and lack of sufficient recovery time faced, especially by elite athletes, can cause an overwhelming and sustained pro-inflammatory response, leading to systemic low-grade inflammation [8, 12, 31]. A systemic low-grade inflammatory state and immune dysregulation have also been implicated as mediators in the development of overtraining syndrome [12]. Moreover, the inflammatory state associated with regular strenuous exercise may

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Published online: 19 September 2022

be related to detrimental modifications to the epigenome, possibly predisposing athletes to muscle and organ damage and subsequently chronic diseases [20]. Chronic low-grade systemic inflammation plays a critical role in the initiation and progression of atherosclerosis, insulin resistance, metabolic syndrome, and cancer [19, 44].

Cytokines, the glycoproteins involved in the regulation and modulation of immune responses, are produced by a broad range of cells, including immune cells, skeletal muscle, connective tissue, and adipose tissue [2]. Most studies investigating the effects of exercise on specific pro- and anti-inflammatory biomarkers in athletes have been conducted by evaluating cytokines before and after either moderate or intensive bouts of physical performance. A single strenuous exercise bout induces an initial (1–24 h after exercise) release of the pro-inflammatory cytokines interleukin 6 (IL-6), interleukin 1β (IL-1β) and tumor necrosis factor alpha (TNF-α), and these markers normally return to basal level in 24–30 h [8, 25]. This activation is followed by an anti-inflammatory response (24–72 h after exercise) with the release of the anti-inflammatory or regulatory cytokines interleukin 10 (IL-10), interleukin 4 (IL-4), interleukin 13 (IL-13), and interleukin 1 receptor antagonist (IL-1Ra) [1, 8]. The resolution of inflammation is crucial for restoring tissue homeostasis in response to exercise and improving muscle function and muscle recovery [6]. It must be acknowledged, however, that several cytokines have pleiotropic effects. With exercise, for example, IL-6 is released into circulation as a myokine from muscle, initially stimulating an anti-inflammatory systemic environment [34].

The aim of this study was to quantify concentrations of four specific blood inflammatory cytokines [IL-6, TNF-α, interferon gamma (IFN-γ) and IL-10] as surrogate markers of a systemic low-grade inflammatory state at the end of a hard and stressful training and competition year; this was done by comparing a group of elite cross-country skiers with gender- and age-matched nonathletes. In addition, concentrations of the acute-phase protein, C-reactive protein (CRP) and glycoprotein acetyls (GlycA), a novel biomarker reflecting systemic inflammation, were quantified in both the athletes and controls.

Materials and Methods

Study Design, Population, and Collection of Blood Samples

This prospective observational case-controlled study was conducted during the Finnish Nordic Ski Championships in Äänekoski, Finland; an event at the end of the skiers' competition season. The event was held between March 28th and April 1st, 2019. The study included the athletes belonging to Finland's National Nordic Ski Team competing at an international level and defined as elite level athletes [23]. As one athlete declined to participate, the number of athletes in this study was 27. The athletes' training season started at the beginning of May 2018 and the competition season at the beginning of November 2018. Thus, the athletes had experienced heavy physical stress for 11 months. For every athlete, one healthy and moderately exercising (< 6 h/week) control subject was recruited from the students and staff of Turku University Hospital and Turku University in Finland. The controls ($n = 27$) were matched with the athletes according to gender and age (± 2 years). The control subjects were studied in Turku, Finland, between April 2nd and 11th, 2019, using the same study protocol as the athletes in Äänekoski.

The study nurse interviewed the athletes and controls during the study visit to obtain clinical data and health-related information. The clinical characteristics of the study subjects are presented in Table 1. The athletes' training load information was collected from their daily training diary data from of the previous 11 months. The training information from one athlete could not be obtained.

The blood samples were collected by the study nurse a day before the start of the National Championships. Each athlete had followed an individual training protocol when preparing for the following day's race. As the time frame between the last training bout and the blood sampling varied, we did not record the exact time. The athletes were not required to fast because of the circumstances of the competition. Therefore, the controls were also not required to fast. The blood samples were centrifuged to separate serum and plasma. The separated samples were then aliquoted and frozen first at −20 °C and then at −80 °C for later analysis.

The study complied with the Declaration of Helsinki as revised in 2000 and all study-related activities were conducted according to Good Clinical Practice. The study protocol was approved by the Ethics Committee of the

Table 1 Clinical characteristics of the whole study population

Variables	Athletes ($n = 27$)	Controls ($n = 27$)
Age	27.1 (20.0 – 40.9)	27.4 (20.7 – 40.4)
Female	13 (48 %)	13 (48 %)
BMI[a]	22.05 (18.0 – 24.8)	24.0 (18.4 – 35.2)
Exercise load (h/week)	15 (11 – 17) ($n = 26$)	< 6

Values are presented as mean (range) or as number (%)

[a]*BMI* body mass index

Hospital District of Southwest Finland (ETMK Dnro: 5/1801/2019). Written informed consent was obtained from all study participants prior to the study.

Serum CRP, IL-6, TNF-α, IL-10, and IFN-γ Analysis

Serum CRP concentrations were measured in duplicates with a commercial high-sensitivity immunoassay using the IMMULITE 2000 Analyzer (IMMULITE 2000 High-Sensitivity CRP, Diagnostic Products Corp., Los Angeles, CA, USA). The detection limit was 0.1 mg/L. Serum cytokines were analyzed as duplicates with a 4-plex cytokine ELISA kit (#115433hu) according to the manufacturer's instructions using Quansys and Q-View software (Quansys Biosciences, Logan, UT, USA). The detection limits for the cytokines were: IL-6 1.04 pg/mL, TNF-α 2.43 pg/mL, IL-10 1.44 pg/mL, and IFN-γ 3.37 pg/mL. The analysis was performed in a single day using the same calibration and setup to minimize variation.

Serum GlycA Analysis

A high-throughput proton NMR metabolomics platform (Nightingale Health Ltd, Helsinki, Finland) was used to analyze the serum metabolic profile as described earlier [39]. The analysis platform assesses 228 variables, including biomarkers of lipid and glucose metabolism, amino acids, ketone bodies and GlycA. GlycA consists of a complex heterogeneous nuclear magnetic resonance signal originating from the N-acetyl sugar groups presented on multiple acute phase glycoproteins in circulation: α1-acid glycoprotein, haptoglobin, α1-antitrypsin, α1-antichymotrypsin, and transferrin [27].

Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS v26.0, Champaign, IL, USA) and Prism v9.1.0 (GraphPad Software Inc., San Diego, CA, USA). Before the analysis, one control a with BMI > 30.0 was excluded from primary analysis since obesity is often associated with the appearance of a pro-inflammatory condition [4]. The distribution of the data was evaluated using the Shapiro–Wilk test and only GlycA was shown to be normally distributed. The differences between the groups in the GlycA data were assessed using independent sample t-tests and the data are reported as a mean ± standard deviation (SD). All other variables were not normally distributed and thus the differences between the groups were analyzed using the Mann Whitney U test. These variables are reported as median values with an interquartile range (IQR), where IQR equals the difference between the 25th and 75th quartiles. Ratios between IL-10: IL-6 and IL-10: TNF-α were calculated and compared between the groups.

Values for inflammatory cytokines were log-transformed and used as an input for principal component analysis (PCA) [30]. The first component corresponded with pro-inflammatory cytokines and was extracted for further analysis (hereby referred as pro-component). Loadings for each component are shown in Table 2. In addition, the inflammatory score (IS) for the samples was calculated as described previously [3]. Briefly, the values of serum cytokines were divided into quartiles, and assigned with a score ranging from 1 (lowest quartile) to 4 (highest quartile). The IS was considered to be the sum of TNF-α, IL-6, and IFN-γ scores, from which the IL-10 scores were then subtracted.

Pearson's correlation coefficient for normally distributed data and Spearman's correlation coefficient for not normally distributed data were used to analyze the association between the variables. Significance was set at $P < 0.05$.

Results

The clinical characteristics of the whole study population are presented in Table 1. The mean yearly training load of the 26 elite cross-country skiers was 766 h (range 580–902 h), i.e., on average, 15 h/week. The training typically consisted of 90% endurance (low, moderate, or high intensity), 8% strength, and 2% speed. The modes of training included running, cycling, and skiing/roller skiing. During the 5-month competition season, the athletes participated in 30–60 (median 35) events. In the matched control group, the exercise load was less than 6 h/week. At inclusion, none of the study subjects reported symptoms of an acute infection.

Cytokine levels are presented in Fig. 1. The athletes were found to have a statistically significant lower concentration of CRP ($P = 0.0232$) than the controls. The concentrations of IL-10, TNF-α, and IFN-γ were statistically significantly higher in athletes compared to controls ($P = 0.0097$, $P = 0.0256$, and $P = 0.0185$, respectively). No significant differences in the concentrations of IL-6, and GlycA were

Table 2 Loadings for each principal component (PC) with explained variance

PC	IL-6	TNF- α	IFN- γ	IL-10
0 (50%)	0.466	0.517	0.656	0.293
1 (25%)	−0.685	−0.056	0.662	−0.295
2 (15%)	0.523	−0.701	0.334	−0.336
3 (9%)	−0.193	−0.480	0.137	0.844

IL-6 interleukin 6, *TNF-α* tumor necrosis factor alpha, *IFN-γ* interferon gamma, *IL-10* interleukin 10

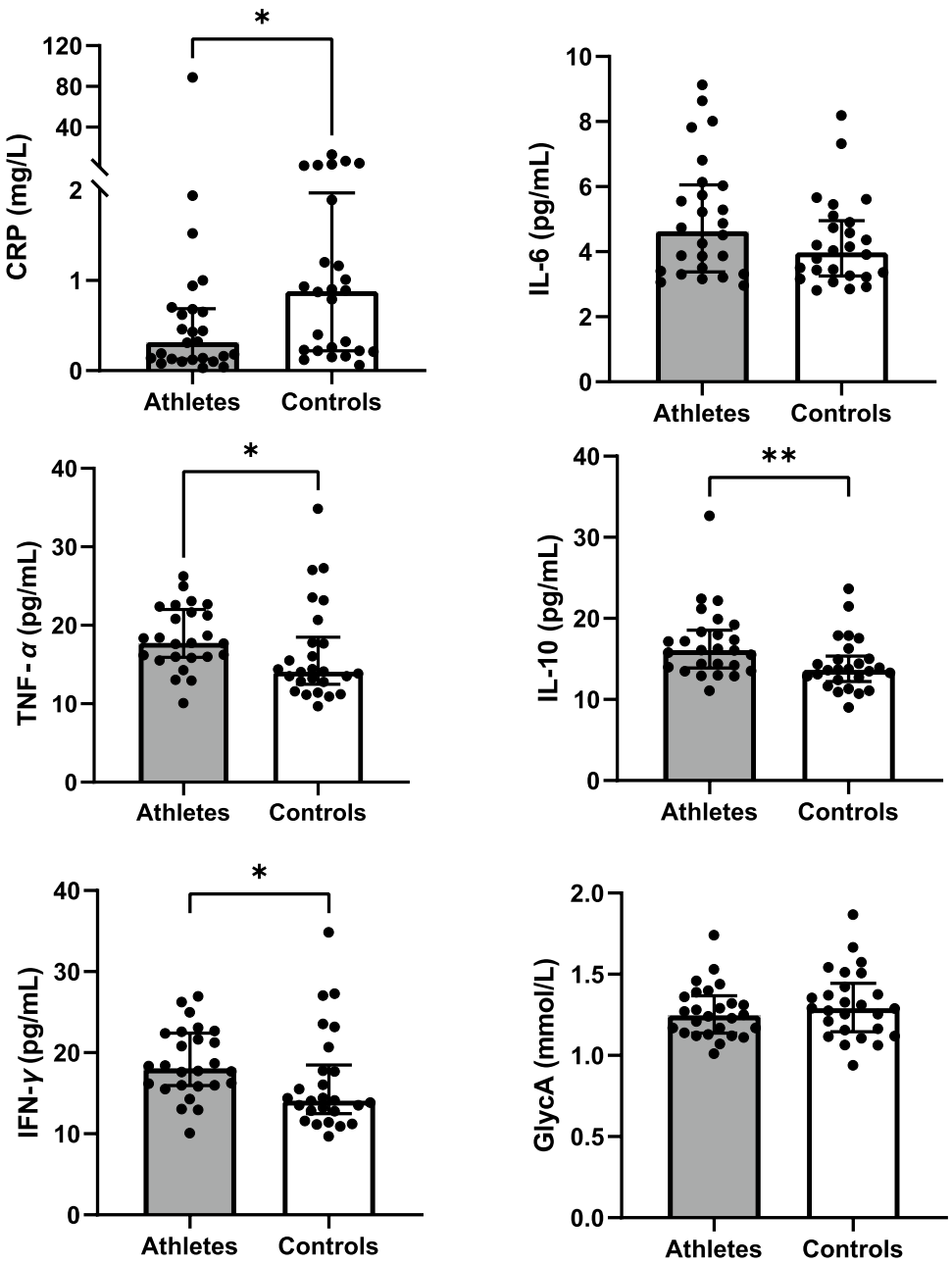

Fig. 1 Concentrations of C-reactive protein (CRP), interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), interleukin 10 (IL-10), interferon gamma (IFN-γ), and glycoprotein acetyls (GlycA) in sera of athletes and controls. The data are presented as median (IQR, interquartile range) for CRP, IL-6, TNF-α, IL-10, and IFN-γ and as mean ± SD for GlycA. *indicates a significant difference between groups. *$P<0.05$; **$P<0.01$

detected between the two groups. The pro-component and IS are presented in Fig. 2. The pro-component was found to be significantly higher in athletes ($P=0.0049$), whereas IS did not differ significantly between athletes and controls. In the ratios between IL-10: IL-6 and IL-10: TNF-α, no significant differences between the groups were detected.

Since one athlete had a CRP as high as 88.85 mg/L suggestive of an acute infection, although no symptoms were reported, we excluded this athlete from further statistical analyses. Excluding this athlete from the further analyses did not change the result except that the difference in IFN-γ was no longer significant ($P=0.0634$). The concentration of CRP was statistically significantly lower in athletes ($P=0.0101$) and concentrations of IL-10 and TNF-α were statistically significantly higher in athletes ($P=0.0167$ and $P=0.0262$, respectively) compared to the controls. No significant differences in the concentrations of IL-6 and GlycA between the groups were detected. The difference in the pro-component and IS also remained alike, while the pro-component was found to be significantly higher in athletes ($P=0.0083$), the IS did not differ significantly between the athletes and controls.

No significant correlation between age, training load and any of the measured cytokines was detected among the athletes or controls. Since a significant correlation between BMI and GlycA concentrations was detected in the control

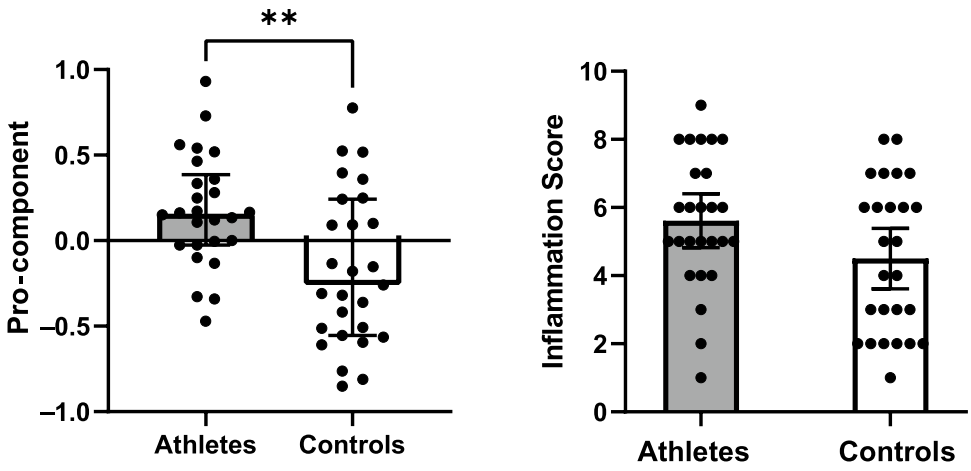

Fig. 2 Pro-component and inflammatory score (IS) in athletes and controls. IS of each subject was the sum of each pro-inflammatory cytokine interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), and interferon gamma (IFN-γ) score from which IL-10 score was subtracted. *indicates a significant difference between the groups. **$P<0.01$

group ($P=0.032$), the controls with BMI > 25 ($n=5$) were excluded from further analyses of GlycA. Among the normal-weighted controls ($n=21$), no relationship was found between BMI and GlycA concentrations, and no significant differences between controls and athletes were detected. No significant correlation between BMI and other cytokines (CRP, IL-6, TNF-α, IL-10 and IFN-γ) was detected.

When comparing the cytokine levels between genders, neither significant differences were found in the whole study group nor among the controls. Among athletes, a statistically significant difference between the genders was detected only in GlycA concentrations: male skiers had higher GlycA levels than female skiers ($P=0.002$).

Discussion

The main findings of our study were that compared to normally exercising, gender- and age-matched controls, elite cross-country skiers had a significantly lower concentration of CRP, and significantly higher concentrations of IL-10, TNF-α, and IFN-γ. These results were found in a study conducted just before their last competition at the end of an 11-month training and competition season. No significant differences in the concentrations of IL-6, GlycA, or IS were found between the groups.

Regular exercise appears to modify and dampen the inflammatory responses initiated by acute physical exercise, although controlled studies on elite endurance athletes are scarce [15, 28]. The recovery of 49 well-trained male triathletes was examined by measuring inflammatory biomarkers altogether five times from 2 days before until 19 days after an Ironman triathlon [26]. The main finding was that concentrations of CRP and IL-6 increased after the race and were still significantly elevated 5 days (CRP and IL-6) and 19 days (CRP) after the long-distance triathlon. This finding was interpreted to indicate prolonged low-grade systemic inflammation, possibly reflecting incomplete muscle recovery [26]. In a 6-week follow-up study with eight endurance-trained cyclists performing 2 weeks of normal training, 2 weeks of intensified training and 2 weeks of recovery training, no significant changes in weekly measured levels of IL-6 or TNF-α were detected [17]. Roth et al. [31] examined six collegiate wrestlers each month throughout a 5-month wrestling season and found that TNF-α and IL-8 levels modestly increased late in the season compared with the preseason. The authors interpreted the low-grade inflammatory state to be associated with the long duration of the season and chronic exposure to high physical and mental stress [31]. Similarly, plasma concentrations of TNF-α were reported to be higher among ten elite Greco-Roman wrestlers during a heavy training period compared to preseason levels and nonathlete controls [43]. In line with these studies, the results of our study showed a higher concentration of TNF-α in elite skiers compared to nonathlete controls, putatively reflecting the sustained burden of the physical and mental stress over the 11-month season.

Interestingly, no significant differences in the IL-6 concentrations between skiers and normally exercising controls were found in this study. IL-6 is generally classified as a pro-inflammatory cytokine. However, when contracting skeletal muscle synthesizes and releases IL-6 into systemic circulation (myokine function), it can have anti-inflammatory and immunosuppressive effects [34]. IL-6 can stimulate the synthesis of the anti-inflammatory cytokine IL-10 and the IL-1 receptor antagonist and inhibit the production of the pro-inflammatory cytokine TNF-α [28, 34]. The magnitude of the exercise-induced IL-6 response is dependent on the intensity and duration of exercise and an individual's endurance capacity and level of adaptation to training [14, 28]. Dysregulated, continual synthesis of IL-6 has a pathological effect on chronic inflammation and

autoimmunity [12]. Epidemiological studies in the general population have shown reduced IL-6 and TNF-α concentrations in adults, at rest, with higher levels of physical activity and fitness, even after adjustment for potential confounders such as BMI [14, 35]. One explanation for our results might be the adaptation of the athletes to repeated physical stress and hence the lack of interference of IL-6 on TNF-α production inhibition.

IFN-γ, a pleiotropic cytokine, induces antiviral, antiproliferative, and immunomodulatory effects on numerous target cells. At low concentrations, IFN-γ is considered to be an anti-inflammatory cytokine [21]. Single bouts of moderate and strenuous exercise decrease the capacity of peripheral blood T cells to produce IFN-γ, which has been hypothesized to increase the risk of infection [36]. The evidence is scarce and the issue regarding the effect of sustained and intensified exercise on IFN-γ production remains controversial. Regular moderate exercise is shown to increase the concentration of IFN-γ in individuals unaccustomed to exercise [41, 42]. In a study of 13 elite kayakers, the concentration of IFN-γ in the kayakers before the training season was found to be significantly lower compared to seven nonathletes [7]. We found unexpectedly higher concentration of IFN-γ in our elite skiers compared to normally exercising controls. This result could also reflect the adaptation of the athletes' immune system to repeated physical stress at the end of the training and competition season. However, more studies are needed to support this interpretation.

In our study population, a significantly higher concentration of anti-inflammatory cytokine IL-10 was detected in athletes compared to controls. Normally, IL-10 appears later in exercise or during recovery as a compensatory mechanism to counteract rising pro-inflammatory cytokines [28, 36]. The higher concentration of IL-10 in our athletes compared to the controls is in line with other previous findings [16, 18] as well as with a recently published study by Sellami et al. [33]. In this study, participation in a high-intensity sport was associated with higher IL-10 concentration as well as longer telomere length, suggesting slower aging and a potentially healthier phenotype [33]. Similar to these results, master athletes practicing lifelong regular exercise have been reported to have increased plasma concentration of anti-inflammatory cytokines, with IL-10 levels similar to young adults, mirroring postponement of immunosenescence and age-related inflammation [24].

Overall, the higher TNF-α and IFN-γ levels detected in skiers in this study comprised significantly higher pro-component level compared to nonathlete controls. It must be acknowledged that the higher IL-10 levels may have counterbalanced the inflammatory milieu in athletes in a way that the IS did not differ significantly between athletes and controls. This probably reflected not only successful compensatory mechanisms but also an adaptation to long-term training and other stressors related to professional endurance sports.

In this study, athletes had significantly lower baseline levels of CRP than normally exercising controls. The median CRP concentration in controls was 0.88 mg/L, the same as the average CRP concentration in healthy members of the general population [40]. This finding supports previous research showing that higher levels of physical activity and cardiorespiratory fitness are consistently associated with 6%–35% lower CRP concentration [29]. In the general population, an increased baseline CRP concentration may associate with an exacerbated risk of death from cardiovascular complications and all the causes, the risk being probably independent of other traditional risk factors [22].

GlycA, a novel biomarker reflecting the glycosylation states of several acute-phase proteins [23], has recently been shown to be a valuable marker of systemic inflammation and cardiovascular disease risk assessment [9–11]. A recent meta-analysis showed that exercise interventions decreased plasma GlycA concentration in sedentary adults [5]. The effect of strenuous exercise on GlycA is largely unknown. In our study population, no differences in the concentration of GlycA were found between athletes and controls. One explanation for this might be that our controls were young, healthy, and non-sedentary individuals without known risk factors for cardiovascular diseases. The mechanism associated with the higher concentration of GlycA in male skiers compared to female skiers is unclear and remains to be established. The protective effect of estrogen upon skeletal muscle inflammation and repair in women has, however, been well documented in animal models [12].

Some limitations of our study must be addressed. First, there is high inter-individual variance in biomarker concentrations and measurements of these markers vary by context. Thus, isolated testing of specific biomarkers can only provide limited information. Absolute values of the cytokines chosen for this study in a one-time blood sample could, however, be interpreted as meaningful since gender- and age-matched, normally exercising controls were used as a comparison. Second, the inflammatory responses elicited by cytokine profiling vary in different athletic disciplines as well as by duration and type of physical training [38]. We did choose one of the most strenuous endurance sport disciplines representing an exceptionally high intensity and duration of training. The third limitation is the lack of an uncontrolled environment regarding exercise and eating preceding the sample collection, which was also true for the controls. Due to the competition situation, the athletes would not have welcomed food intake restrictions and we had to use non-fasting samples. Our approach was limited by our goal to

perform an observational study without interfering with the pre-competition training process.

Conclusions

While regular strenuous exercise improves athletes' physiology and performance, there might be detrimental consequences to their health and immune responses. The results of this case–control study with elite cross-country skiers do not support the hypothesis that strenuous exercise performed in a highly demanding endurance sport induces chronic low-grade inflammation and thus predisposes athletes to chronic diseases. Our study findings are crucial when considering the performance of elite athletes, since chronic diseases characterized by persistent inflammation and immune dysregulation profoundly impair skeletal muscle strength, endurance capacity, and regeneration potential.

Acknowledgements We would like to thank Minna Hyppönen, Wilma Grönroos and Sirpa Vänttinen for technical assistance with study subjects and Nightingale Health Ltd for the GlycA analyses.

Author Contributions All the authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by RL, JKI, SP, JH and MV. The first draft of the manuscript was written by RL and all the authors commented on previous versions of the manuscript. All the authors read and approved the final manuscript.

Funding Open Access funding provided by University of Turku (UTU) including Turku University Central Hospital. Jenny and Antti Wihuri Foundation.

Data Availability Not applicable.

Code Availability Not applicable.

Declarations

Conflict of Interest NK has been an employee of Nightingale Health Ltd., at the time of the GlycA analyses but not during the data analysis or manuscript preparation. The remaining authors declare no competing interests.

Ethical Approval The study protocol was approved by the Ethics Committee of the Hospital District of Southwest Finland (ETMK Dnro: 5/1801/2019).

Consent to Participate Written informed consent was obtained from all the study participants.

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