Letter to the Editor

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The impact of exercise on the variation of serum free light chains

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To the Editor,

Measurement of serum free light chains (sFLC) has become an established method for screening, prognostic evaluation, and monitoring of multiple myeloma and related monoclonal gammopathies [1]. In recent years it was shown that non-clonal elevations of sFLC, measured as the κ- and λFLC sum (ΣFLC), can act as a global marker of immune stimulation, and may be linked to severity of immune disease [2]. In a cohort of 15,859 individuals all aged above 50 years, Dispenzieri et al. found that ΣFLC is a significant predictor of worse overall survival in persons without plasma cell disorders [3]. sFLC as such is regarded a biomarker of frailty in the elderly.

Due to the well-defined clinical role of sFLC, the long-term biological variation of monoclonal FLC in patients with monoclonal gammopathies [4] and polyclonal FLC in healthy controls were recently assessed [5]. Immune status is not solely influenced by pathogen exposure or inherent immune disorders, other factors such as exercise also play a role. Acute exercise, e.g., induces immune stimulation, which is reflected by transiently increased concentrations of sensitive inflammation markers [6]. Whether or not exercise can affect FLC concentrations has to our knowledge not been assessed before.

In the present study, we hypothesized that exercise-induced immune stimulation may affect sFLC concentrations. To test this hypothesis we monitored sFLC concentrations in 37 elderly volunteers who participated in the Nijmegen Four Days Marches and walked 30 km at 4 consecutive days at a self-selected pace.

Thirty-seven elderly men and women (age 76–86 years) of the 2013 Nijmegen Four Days Marches (an annual walking event in The Netherlands) volunteered to participate in our study. The experimental set-up to determine the demographic characteristics of the volunteers, their health status and exercise characteristics are described previously [7]. Baseline blood measurements were performed 12–36 h preceding the start. Immediately after finishing on the fourth day, all baseline measurements were repeated. FLC measurements were measured in batches of frozen and anonymized sera. Sera aliquots were stored at –20 °C directly after collection and thawed directly before analysis. Serum FLC analysis was performed on the SPA_{plus} instrument using Freelite reagents (The Binding Site Ltd, Birmingham, UK). ΣFLC was calculated as the sum of the individual measurements of both κFLC and λFLC. Values are presented as means±standard deviation.

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Three out of 37 included volunteers dropped out the walking march due to joint and exercise-related problems. These volunteers were excluded from further analysis. The exercise was performed under temperate ambient conditions. Participant characteristics, health status and exercise characteristics of the remaining 34 subjects are presented in Table 1. With dehydration defined as a body mass loss of 2% or more, eight out of the 34 volunteers were dehydrated at least 1 day during the walking exercise. We observed an exercise-induced plasma volume expansion of 3.0±2.5% over the 4 days, which was due to a small, but significant decrease of hematocrit from 0.40 L/L at baseline to 0.38 L/L directly after 4 days of exercise ($p<0.0001$).

At baseline the mean κFLC concentration was 19.2 mg/L (range 8.4–50.3), the mean λFLC concentration was 18.0 mg/L (range 8.3–33.0) and the mean κ/λ FLC ratio was 1.1 (range 0.6–2.4). One participant had a κ/λ FLC ratio of 2.4 and exceeded the Freelite reference range for healthy controls (0.26–1.65) [8]. Further monoclonal protein analyses demonstrated that this participant had an IgA-kappa M-protein of 3.6 g/L.

After exercise, no significant change in FLC values was observed (Figure 1). The mean κFLC concentration increased up to 20.7 mg/L ($p=0.16$). The mean λFLC concentration increased up to 19.4 mg/L ($p=0.04$), and the mean κ/λ FLC ratio remained unaffected at 1.1 ($p=0.93$). We observed a non-significant increase in average ΣFLC from 37.2 to 39.8 mg/L ($p=0.09$). After compensation for the exercise-induced plasma volume expansion, the FLC changes in the blood further minimized (Supplemental Data, Figure 1A, which accompanies the article at http://www.degruyter.com/view/j/cclm.2014.52.issue-11/issue-files/cclm.2014.52.issue-11.xml). Interestingly, this small and non-significant increase in sFLC after the 4-day walk could solely be ascribed to five participants who exhibited an increase in FLC levels of >20% (Supplemental Data, Figure 1B). In the total cohort of 34 volunteers a significant increase in serum CRP levels was measured ($p<0.001$). All five participants with increased FLC levels of >20% had CRP values that at least doubled (Supplemental Data, Figure 1C).

Despite the importance and well-defined role of FLC measurements in clinical practice, no data is available about the impact of exercise on sFLC concentrations. We now conclude in our cohort of 34 elderly volunteers that serum ΣFLC did not significantly increase after walking 120 km in 4 days. We did observe a significant increase of CRP concentration. The significant increase of CRP without a significant increase of FLC in our cohort is in line with the recent observation that polyclonal FLC and CRP provide independent information as to inflammatory status [9]. Serum CRP is an established marker of innate immunity, and serum polyclonal FLC represents B cell

Table 1 Demographics, health status and exercise characteristics of volunteers.

	Men (n=21)	Women (n=13)
Demographic characteristics		
Age, years	81±2	81±2
Height, cm	173±6	161±6
Weight, kg	73.6±8.5	57.7±7.0
Body mass index, kg/m^2	24.5±2.3	22.4±2.3
Lean body mass, kg	54.8±5.7	38.9±4.9
Health status		
Physical activity, h/week	4.5±4.4	4.9±4.1
≥5 times/week ≥30 min exercise,%	59	73
Blood pressure		
Systolic, mm Hg	144±19	144±16
Diastolic, mm Hg	83±11	81±10
Resting heart rate, bpm	63±16	67±13
Use of prescribed medicine		
Anti-hypertensive drugs	6 (29%)	6 (46%)
Statins	6 (29%)	1 (8%)
Analgesics	3 (14%)	1 (8%)
Anti-diabetics	2 (10%)	0 (0%)
Other[a]	3 (14%)	1 (8%)
Pathology		
Hypertension	6 (29%)	6 (46%)
Cardiovascular disease	3 (14%)	0 (0%)
Hypercholestorolemia[b]	6 (29%)	1 (8%)
Diabetes	2 (10%)	0 (0%)
Cancer (not further differentiated)	4 (19%)	1 (8%)
Other[a]	3 (14%)	2 (15%)
Exercise characteristics		
Exercise duration per day, hh:mm	7:10±1:02	7:56±0:57
Speed, km/h	4.4±0.1	3.8±0.1
Average heart rate day 1, bpm	99.1±13.7	111.5±14.4
Peak heart rate day 1, bpm	113.8±14.1	123.8±14.0
Exercise intensity day 1,%	66.5±9.0	73.9±9.8
Fluid balance		
Fluid intake, L[c]	1.88±0.8	1.90±0.8
Change in body mass, absolute kg[c]	−0.70±0.7	−0.26±0.6
Change in body mass, relative%[c]	−0.90±0.9	−0.46±0.9

[a]Volunteers who were diagnosed and treated for cancer, rheumatoid arthritis, allergy and glaucoma; [b]Hypercholesterolemia is defined as total cholesterol levels of >6.5 mmol, as previously diagnosed by a physician; [c]Daily fluid intake and changes in body mass during walking. Data are presented as mean±standard deviation.

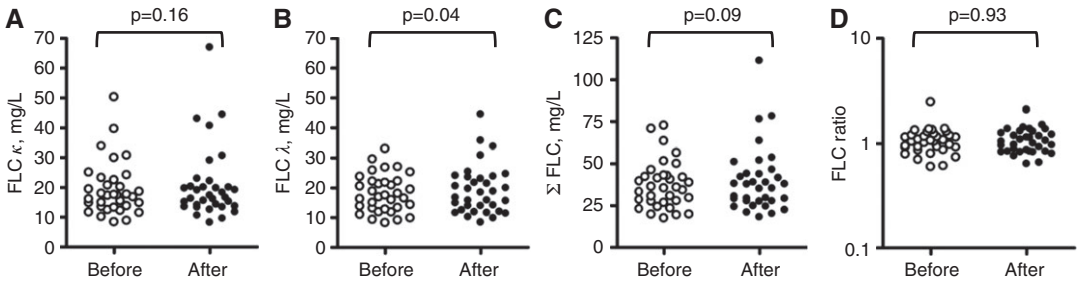

Figure 1 FLC concentrations before and after walking exercise.
(A) The average κFLC concentration increased from 19.2 to 20.7 mg/L (p=0.16). (B) The average λFLC concentration increased from 18.0 to 19.4 mg/L (p=0.04). (C) The average ΣFLC increased from 37.2 to 39.8 mg/L (p=0.09). (D) The FLC ratio remained stable at 1.08 (p=0.93). The red dotted lines indicate the reference ranges for the Freelite κ/λ FLC ratio (0.26–1.65).

activation and thus the activity of the adaptive immune system. In five participants an increased FLC concentration of more than 20% was measured. This coincided with a strong increase in CRP values in all five participants. Most likely, the CRP increase in these volunteers might be caused by an upcoming infection. Such upcoming infections trigger both innate and adaptive immune activation and this might explain the significant FLC increase in these participants [2, 9].

Limitations of this study. To minimize the analytical variation in our FLC measurements, we performed all FLC analyses as one batch on the same SPA_{plus} analyzer with the same batch of Freelite reagents. The within-run analytical variation under these conditions is minimal with CV values <4%. Our cohort was obviously biased for physical fitness, as the volunteers needed to be able to complete 4 consecutive days of walking exercise. The volunteers in our cohort were, however, not selected as healthy controls. In fact, 23 out of the 34 volunteers had at least one disease diagnosed by a physician for which they were treated. Subgroup analysis for the 'healthy volunteers' and the 'diseased volunteers', demonstrated that exercise had no significant effect on sFLC concentrations in both healthy and frail elderly individuals (data not shown). We chose to enroll only elderly volunteers in this study because FLC measurements are clinically most relevant in this age category. Another reason to study elderly is motivated by the observation that elderly have in general less physiological reserves and therefore more difficulties to balance homeostatic conditions [10]. We speculated that potential exercise-induced FLC variations would therefore be most apparent in elderly volunteers. We hypothesize that also in younger age cohorts exercise will give rise to no or only minimal FLC variation, but this needs further validation.

In conclusion, intensive 4-day walking exercise does not significantly influence sFLC concentrations. This means that large FLC association studies or FLC measurements in individual patients do not need to be compensated for recent strenuous exercise. This finding emphasizes the stability of polyclonal FLC as a biomarker.

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Conflict of interest statement

The authors state there are no conflicts of interest regarding the publication of this article. The serum free light chain analyses were performed free of charge by The Binding Site, research funding and free light chain analyses played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.
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