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ORIGINAL RESEARCH

Acute Changes in Interleukin-6 Level During Four Days of Long-Distance Walking

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Background: Interleukin 6 (IL-6) has an inflammatory effect, and its concentration in serum increases during exercise. However, no studies have assessed acute changes in IL-6 concentration after consecutive days of extreme and long-term exercise.

Objective: This study aimed to assess acute changes in serum IL-6 concentration during four days of long-distance walking.

Methods: This prospective observational study assessed 25 athletes (aged 44.8 ± 9.1 years), who covered a total of 251 km in four days. Blood samples were collected daily to assess serum IL-6 concentrations. Repeated-measures analysis of variance (with Bonferroni's post hoc test) and the Kruskal–Wallis *H*-test (with Dunn's post hoc test) were used to investigate the differences between the measures.

Results: The serum IL-6 concentrations were higher on the four days of walking (1st day: 26.8 ± 14.8; 2nd day: 14 ± 7.4; 3rd day: 9.4 ± 10.8; 4th day: 4.5 ± 0.2 pg/mL) when compared to pre-walk values (pre-walk: 2.2 ± 2.1 pg/mL; $p < 0.001$). On the first day, there was a tenfold increase compared to the pre-walk value.

Conclusion: The inflammatory response increased the serum concentration of IL-6 after four days of exercise. With the passing of days, there were reductions but not to baseline values.

Keywords: exercise, long-distance walk, inflammation, biomarker, interleukin-6, athletes

Introduction

Interleukin 6 (IL-6) is a cytokine that plays a role in the specific antigen immune response and acute inflammatory response.[1] It is produced in several types of cells and can act in a large number of tissues.[2] IL-6 plays a crucial role in the defense response and has a pleiotropic characteristic that can determine more than one phenotypic characteristic (angiogenesis, glucose metabolism, and osteoclastogenesis).[2,3]

When moderate to extreme intensity exercise (>85–90% of maximal heart rate) is performed, the IL-6 level in the blood circulation increases. Skeletal muscle contraction is the stimulus for its release; thus, it is considered a myokine as it is produced, expressed, and released by muscle and has paracrine and endocrine effects.[4,5] A reduction in the availability of carbohydrates for exercise stimulates the release of IL-6 as it can assist in the maintenance of serum glucose levels during exercise.[5]

IL-6 is an important marker since an increase in its concentration is associated with an increase in the levels of acute-phase inflammatory proteins, such as C-reactive protein,[6] the risk of cardiovascular events,[7] and the process of rupture

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or erosion of atherogenic plaques.[8] Among men, IL-6 is associated with the risk of myocardial ischemia.[9]

The majority of studies reported in the literature on this theme evaluated session (bout) exercise or a long-distance test (marathon, half-marathon, or 164km of cycling) and showed an increase in the levels of IL-6 released into blood vessels.[6–8] However, chronic training could reduce the release of IL-6 by skeletal muscle because exercise improves the energy performance of the myocytes.[10] Maintaining IL-6 concentrations promotes homeostasis in the inflammatory response and better use of the energy framework without damaging the myocytes.[4,11,12]

After the removal of physical stress, there is a tendency toward IL-6 homeostasis over time (until 48 hours post-exercise).[13,14] However, the IL-6 concentrations during exercise and the duration of this exercise remain unclear in the literature. With the stress of physical exercise, in response to acute inflammation, the serum concentration of IL-6 increases and has been shown to potentiate the effects of other cytokines,[5,15] culminating in the secretion of other biomarkers (such as PCR).[16] Specifically, this four-day walking event covered five cities, on roads with differences in level (uphill, downhill, and level), temperature, time, and distance on each day.

In the present study, IL-6 levels were measured to ascertain if the concentration increased during four days of long-term exercise. The present study aimed to provide evidence of adaptations in relation to IL-6, which would enable us to observe the inflammatory response. We hypothesized that IL-6 levels would increase on all walking days.

Methods

The present observational study performed assessments during the resistance path event, referred to as the ecological walk, which takes place in mid-west Brazil. The event is financed by the state government and is considered a tradition since it has been occurring for 27 years. The athletes are recruited two months before the event by the team of the State Secretariat of Education of the State of Goiás and participants are required to present a medical release prior to participation. Participant selection took place on two consecutive on which the interested individuals were required to walk at least 28.2 km in 3 hours and 10 minutes. The study protocol was approved by the Research Ethics Committee of the Federal University of Goiás (no. 781/2013). The procedures involving the participants were conducted according to the guidelines of the Declaration of Helsinki. All participants were informed and signed a consent form before participating in the study.

The initial evaluation included seventy-one participants, 20 of whom were excluded on the first day (Figure 1). On day 2 of selection, the final 25 male participants were selected for inclusion. Athletes who covered the distance in a longer time than expected were excluded automatically. After selection, the participants were assessed on a pre-scheduled date and time for the performance of clinical trials to formalize their health conditions to participate in the walk. The mean age of the athletes was 44.8 (9.1) years, body mass 70.2 (10.8) kg, height 1.70 (0.07) m, and body mass index 23.7 (2.7) kg/m2.

Participants were required to get up at 4:30 am and start walking at 5:30 am. The sleep time was 6.5 hours (between 10:00pm and 4:30 am). Meals consumed during the day were distributed at breakfast (bread, coffee, juice, and fruit), snack I (fruit, bread, and chicken pâté), lunch (rice, beans, meat, and vegetables), snack II (fruit, bread, and chicken pâté), and dinner (rice, beans, meat, and vegetables). Food and juice were provided ad libitum. Snacks were served in the morning and afternoon while the participants were walking and moving. A serving of isotonic drink (300 mL with 18 mg of carbohydrate) was offered during each period (morning and afternoon) and all participants were guided to consume it. The calorie consumption of the participants was not monitored. The participants were accompanied throughout the four-day journey by a multidisciplinary team including doctors, physiotherapists, physical education professionals, nurses, and nutritionists.

The ecological walk took place in July and covered five cities. The roads on the route included differences in level (uphill, downhill, and level), temperature, time, and distance on all days (Table 1). The soil temperature was measured with an appropriate thermometer (AcuRite model 00606TX, IC: 6608A-606TX, FCC ID: RNE606 TX; Chaney Instrument Co., Wisconsin, USA) coupled to a digital reader (AcuRite model 00782W3; Chaney Instrument Co., Wisconsin, USA). The distance and time taken to travel each stretch was measured using a vehicle tachograph. On the first day, the participants covered the longest stretch within 10 hours. The highest temperatures were recorded on the fourth day of walking and the greatest amplitude between the minimum and maximum temperatures of the five days occurred on the fourth day at 18° and 42°C, respectively.

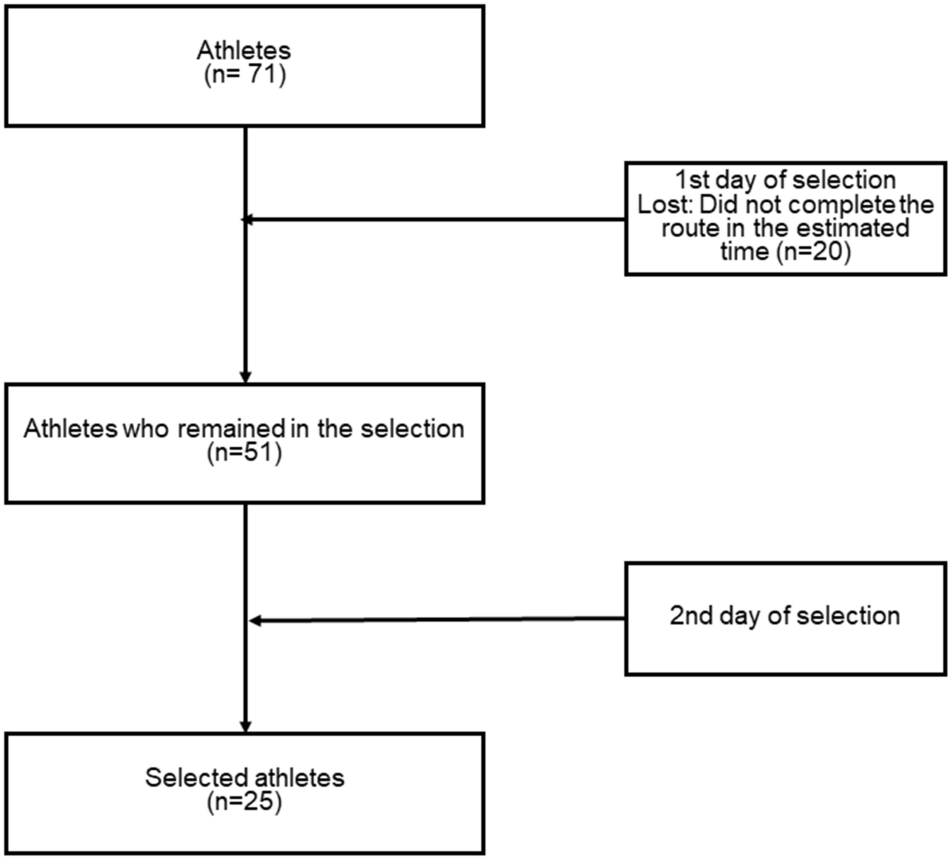

Figure 1 Design of study.

GPower software (version 3.0) was used to calculate the sample power considering a medium effect size of 0.25, a significance level of 5%, one group, five measures of the outcome variable, and the number of athletes followed during the study, giving a power of 86%.

Body mass was measured before breakfast (in a fasting state) and later in the day (before dinner) for four days using a portable digital scale, to the nearest 0.1 kg (2096PP model; Toledo, St. Paulo, Brazil). During weighing, the participants stood in the center of the scale, barefoot, shirtless, and wearing only a light pair of shorts. Height was measured using a portable stadiometer with a precision of 1 mm (Sanny, São Paulo, Brazil). The body mass index (BMI) was calculated by dividing the body mass by the square of the height (kg/m^2).

On the day of the pre-walk examinations, blood samples were collected to assess baseline serum IL-6 concentrations at a specialized laboratory. Serum samples were collected in the afternoon because of the IL-6 circadian cycle,[17] without the need for fasting. During the walk, blood samples were always collected at the end of the day, between 5:00 pm and 6:30 pm, approximately one hour after the end of the day's walk. As the athletes walked all day and the objective was to verify the changes at the end of each day, the aforementioned schedule represented the best option to minimize the effects of food[18] on the serum concentration of IL-6.

The sample was asked to remain standing for 10 minutes before collection of five milliliters of blood in a tube with ethylenediaminetetraacetic acid (First Lab, São José dos Pinhais, Brazil) and the samples were centrifuged at 1500 rotations for 10 minutes at 4°C in automatic equipment (BL 1200; Sarstedt AG & Co. KG, Nümbrecht, Germany) according to the manufacturer's guidelines. After centrifugation, the samples were transported in a container at 2°C and stored at –20°C. Serum IL-6 levels were measured 12 hours after sample collection and centrifugation using an immunometric enzyme-chemiluminescence assay (Alinity ci-series, Abbott Laboratories, Illinois, USA). The analyses were performed in duplicate and the coefficient of variation was less than 5%. The values were predicted to be between 1 and 5 pg/mL.[1]

Table 1 Anthropometric Measurements and Environmental Parameters During the Four Days of Long-Distance Walking (n = 25)

	1st Day	2nd Day	3rd Day	4th Day	η2	p
Body mass, kg						
Morning	70.3 (10.8)	69.2 (10.8) *	69.3 (10.5) *	69.4 (10.9) *	0.54	<0.001
Afternoon	68.0 (10.8)	68.6 (10.9)	68.9 (10.6) *	69.5 (10.7) *,‡	0.78	<0.001
p	<0.001	0.23	0.008	0.66		
Distance, km	70.0	59.0	67.0	55.0	–	–
Time, h	10.0	9.0	8.5	8.5	–	–
T (°C) (min–max)	21–37	19–31	22–38	18–42	–	–

Notes: *Different from pre-walk; ‡Different 3rd–4th day; η^2 – eta (effect size). At the end of the study, the body mass increased when comparing the 1st and 3rd (Δ = 0.9 kg, p < 0.001), 1st and 4th (Δ = 1.5 kg, p < 0.001), and 3rd and 4th (Δ = 0.6 kg, p <0.001).

The results are described using means, standard deviations, medians, ranges, 95% confidence intervals (95% CIs), and graphs. Data with normal distributions were compared using analysis of variance for repeated measures with post hoc Bonferroni correction (BMI and body mass). Skewed data were log-transformed and compared using the Kruskal–Wallis *H*-test with Dunn's post hoc test. Effect size (Cohen) was computed for analysis. The variation (Δ) between the days (pre and 1st day, pre and 2nd day, pre and 3rd day, and pre and 4th day) was calculated to verify the amplitude of IL-6 during the walking days. Statistical analyses were performed using R version 4.0.2 (R Development Core Team, Vienna, Austria) in the RStudio environment, version 1.2.5033 (RStudio, PBC, Boston, MA, USA). Statistical significance was set at p<0.05.

Results

The distributions of distance, time, and temperature on all days are shown in Table 1. Initially, six athletes were pre-obese when their BMI was analyzed. On the second day, one of the athletes left the classification of pre-obesity and was considered eutrophic. In total, the athletes covered 251 km with a daily average of 63 ±6.9 km in 9 ±0.5 h/day (Table 1). It should be mentioned that all athletes were required to reach the end of the established daily route at the same time.

The body mass measured in the morning decreased from the second day when compared to the first day of walking (2nd day: Δ = 1.1 kg, p<0.001), 3rd day (Δ = 1 kg, p=0.001) and 4th day (Δ = 0.9kg, p=0.01). At the end of the day, the body mass increased when comparing the 1st and 3rd (Δ = 0.9 kg, p < 0.001), 1st and 4th (Δ = 1.5 kg, p < 0.001), and 3rd and 4th (Δ = 0.6 kg, p <0.001).

The serum concentration of IL-6 was higher on the four days of walking than in the pre-walking period (p<0.001) (Table 2). The following increases in IL-6 concentration were observed: 1st day, 24.6 pg/mL (p<0.001); 2nd day, 11.8 pg/mL (p<0.001); 3rd day, 7.2 pg/mL (p<0.001); and last day, 2.3 pg/mL (p<0.001). Compared

Table 2 Serum Concentrations of IL-6 (pg/mL) Observed on the Four-Day Walk (n=25)

	Mean (SD)	95% CI	Median	Min–Max
Pre-walk	2.2 (2.1)	1.3–3.0	1.62	0–11.9
1st day	26.8 (14.8) *	20.7–32.9	22.9	9.2–78.3
2nd day	14.0 (7.4) *	10.9–17.0	11.6	4.5–45.5
3rd day	9.4 (10.8) *	4.9–13.9	6.5	1.5–44.0
4th day	4.5 (4.2) *	2.8–6.2	3.1	1.5–20.7
p	<0.001			
η^2	0.84			

Note: *Different from pre-walk. η^2 – eta (effect size).

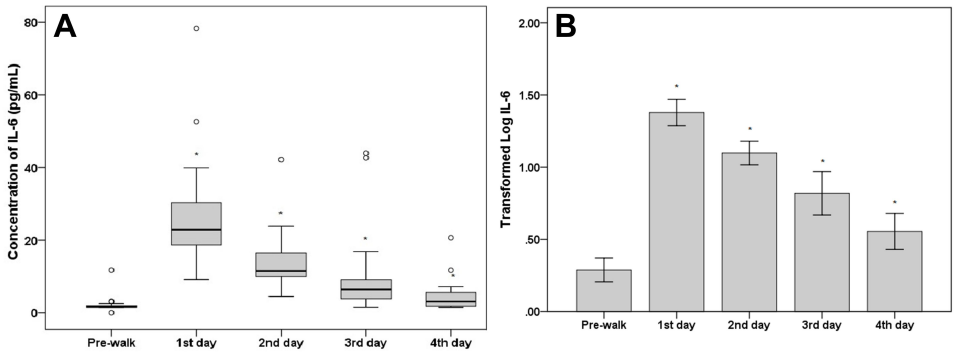

Figure 2 Box (**A**) and Log transformed bar (**B**) plots of IL-6 concentrations recorded during the four-day walk. The medians and 95% confidence intervals are shown after Log transformation (**B**). *Difference of pre-walk.

to the IL-6 concentration on the first day of walking, the following reductions were observed: second day (Δ=12.8 pg/mL, p<0.001), third day (Δ=17.4 pg/mL, p<0.001), and fourth day (Δ=22.3 pg/mL, p<0.001). Between the second and third days (Δ=4.6 pg/mL, p=0.001) and the second and fourth days (Δ=9.5 pg/mL, p<0.001), there were also reductions. Between the third and fourth days, there was a reduction of 4.9 pg/mL (p=0.001). The analysis showed a large effect size (η^2=0.84).

The distribution of serum IL-6 concentrations over the four-day walk is shown in Figure 2A. The concentrations of IL-6 were subjected to logarithmic transformation as the values were not normally distributed (Figure 2B). After the transformation, the difference in values over the four days in relation to the baseline values (p < 0.001) and between the days (p < 0.001) remained significant.

Discussion

The results showed a significant increase in the serum concentration of IL-6 on the days of the walk when compared to the pre-walk values. On the second day, the concentrations of IL-6 decreased, but did not reach the baseline values at the end of the 251km covered in four days.

Studies that evaluated IL-6 found an increase in sessions of acute exercise lasting up to an hour or between one and four hours, as in the case of marathons (full and half).[13,19,20] The duration varied between 6 and 48 hours, some with 10 hours of daily exercise.[14,21,22] The present study is different in that we evaluated the concentration of IL-6 at the end of each of four days of long-term exercise. It is worth mentioning that the increase in IL-6 concentration depends on the intensity and duration of the exercise, so only 50% of the variation in plasma IL-6 concentration is due to the duration of the exercise.[23]

The increase in the concentration of IL-6 can be up to ten times greater[6] than the values measured before the exercise was performed, similar to that found at the end of the first day of walking in the present study, which was twelve times higher than the baseline values. A study conducted by Sahl et al[22] evaluated cyclists who traveled for almost 200 km a day for 14 days for an average of ten hours per day. However, IL-6 concentrations were measured only after fourteen days and were found to have increased significantly. Thus, collecting blood samples every day and in the late afternoon is an important factor due to the diurnal variation in IL-6 levels.[17] Blood samples were collected following the end of the daily effort in the present study, but were collected five minutes later event, three hours, and 12 hours and showed significant increases after a session of resistance exercise, or cycling, marathon, or ultramarathon.[13,14,19,24]

The intensity and duration of the exercise influences the maintenance of elevation of IL-6 concentration after exercise, mainly after high-intensity exercise.[25] In the present study, the duration may have contributed to the increase in IL-6 concentrations since the kilometers walked on the first and third days were the highest, but the route was flat. The routes on the second and fourth days were mostly uphill. Analysis of IL-6 concentrations followed by four hours of soccer practice and cycling and the maintenance of continuous elevation suggested that the number of muscle groups required during exercise may increase the IL-6 concentration, since American football players use muscle groups in both the upper and lower limbs, while cyclists only use muscle groups in the lower limbs.[26] Walking athletes may have recruited more muscle fibers (type I)[27] and other muscle groups such as those involved in posture and with greater oxidative capacity (higher oxygen consumption) during uphill moments.[25]

Muscle contraction is the stimulus for the production, expression, and release of IL-6 during exercise with a longer duration,[28] as performed by the athletes who walked during the four days. It seems that the main role of increasing IL-6 production during exercise is to minimize the inflammatory response[29] and promote maintenance of the serum level of glucose, perhaps being released at times when there is a lack of glycogen in the muscle cells.[5,11] These findings suggest that the release of IL-6 is regulated by the availability of energy substrate[5] which may explain the increase in IL-6 concentrations observed among the athletes in the current study. As the athletes received 300 mL of isotonic solution (with 18 mg of carbohydrates) twice a day (morning and afternoon) with water consumption ad libitum, it can be speculated that the replacement was not sufficient for the athletes because the duration and intensity of exercise can influence the isotonic replacement requirements.

The present study showed an increase in IL-6 concentrations 12 times higher than baseline values on the second day, and there was a reduction that did not return to baseline values on the last day of walking. These results are associated with a pro-inflammatory response and demonstrate that athletes who are subjected to extreme exercise are at risk of skeletal[30] and cardiac[19] muscle damage with long-term exercise. The daily concentration of IL-6 can be used to investigate the inflammatory response during long-term exercise and assist in the development of strategies to decrease its release and minimize its effects. In practice, this can be performed by further assessing food consumption and water replacement among athletes, since a rise in IL-6 concentration increases the release of cellular glucose.[31] This study is only the second to assess the behavior of IL-6 in extreme exercise. The only other study conducted to date in this regard evaluated 14 days of cycling and the assays were performed only at the beginning and after the end of the 14-day course.[22]

In addition, IL-6 was dosed in isolation as its elevation due to tissue damage stimulates an acute inflammatory response, which, alone or potentiating the effects of other cytokines,[5,15] induces the secretion of other markers (PCR, serum amyloid A, fibrinogen, and α1-antichymotrypsin) related to the inflammatory response.[16] IL-6 is a cytokine secreted by skeletal muscle during exercise,[11,27,32] and has been correlated with several biomarkers.[12,13] IL-6 also presents an important increase in concentration during exercise of moderate to high intensity and long duration[7,14,22,33] in addition to being associated with the risk of a cardiac event and survival.[7]

Limitations

The current study had some limitations such as the inability to evaluate the levels of cardiac markers to investigate cardiac function during extreme performance and the correlation with IL-6 concentration. Also, others inflammatory markers (such as protein C-reactive and tumoral necrosis factor-α). Tracking food consumption, measures of exercise intensity (heart rate or subjective perception of effort), and glucose during the course may add to understanding of the relationship with the inflammatory response. We were unable to measure IL-6 concentrations during follow-up to determine how long it took the body to return to homeostasis.

Conclusion

The inflammatory response increased the serum concentration of IL-6 after each of the four days of exercise. With the passing of days, there were reductions in IL-6 concentrations, but not to baseline levels. However, further research needs to consider the consumption of carbohydrates, glucose concentration, others inflammatory markers and isotonic replacement during long-term exercises.

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Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that there are no conflicts of interest.

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