Increasing daily step counts improves physical fitness, and reduces pain and arterial stiffness in sickle cell patients

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Supplemental material

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Methods

Subjects and protocol

Thirty-eight men with SCA from Dakar (Senegal), regularly followed by the "Centre National de la Transfusion Sanguine" (CNTS) participated in this longitudinal study (*drePAnon* clinical trial, UMIN000042826, UMIN-CTR Clinical Trial; age: 31.8 ± 8.5 yrs; weight: 57.0 ± 7.2 kg; height: 177 ± 6 cm; HbS: 87.2 ± 3.0%; HbF: 10.0 ± 3.1%). All patients were at steady-state at the time of inclusion and none of them had hospitalized vaso-occlusion or acute chest syndrome (ACS) in the preceding 3 months, or transfusion in the preceding 2 months. Patients experiencing any condition impairing the walking gait, such as leg ulcers or osteonecrosis of the femoral head, were not included in the study. The protocol was approved by the Ethics Committee of Cheikh Anta Diop University (0388/2019/CER/UCAD) and was conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent. Patients with leg ulcers, osteonecrosis or who experienced stroke were not included, because it may negatively impact on walking abilities.

At the first visit to the Laboratory of Physiology and Functional Explorations (Cheikh Anta Diop University, Dakar, Senegal), patients were equipped with a Fitbit wrist-worn accelerometer-based PA tracker (Alta, Alta HR, or Inspire 2, San Francisco, CA, USA) for at least 5 weeks of follow-up under real-life conditions, in order to objectively quantify their baseline daily step counts. Instructions were given about how to wear the device, use the mobile software (Fitabase application, San Diego, CA, USA) and synchronize data. Patients were asked to wear the device continuously and to remove it only during bathing or water activities. They were asked to maintain their usual lifestyle throughout this period and encouraged to charge the device during these times or during sleeping hours. At the end of this 5-week period, a follow-up visit (V1) was scheduled to collect blood samples, perform biological assessments, and conduct various physiological tests and participant groups were then formed. Patients were randomly assigned to one of the 3 following groups: 1) a control group for which no specific information regarding PA was given for 8 weeks (control group; N = 12); 2) a group where patients had to increase their daily step counts of 25% above the previous 5-weeks daily step counts, for 8 weeks (PA group 1; PA1; N = 12); 3) a group where patients had to increase their daily step counts of 25% above the previous 5-weeks daily step counts, for 4 weeks, and then of 50% of their initial daily step counts for 4 more weeks (PA group 2; PA2; N = 14). Regular phone calls were made with patients from groups PA1 and PA2 to remind them their targeted daily step counts. After the 8-week intervention period, the last visit (V3) was programmed and

the same biological and physiological parameters than the ones assessed before the intervention were measured in all three groups. Between visits V1 and V3, an intermediate visit (V2) was appointed to reinforce the protocol instructions, particularly for participants in the 25–50% group. During this visit, they were instructed to increase their daily step count by 50% compared to their baseline value. Daily questionnaires regarding daily pain and medication intake were also competed by the patients throughout the eight-week intervention period.

Daily pain diary, interference with daily living activities and daily medication

Pain intensity (score ranging from 0 to 10; 10 being the highest pain) and frequency were determined using the standardized questionnaire developed by Smith et al. (1). The pain interference subscale was used to quantify the impact of pain on daily functioning. This instrument evaluates the degree to which pain impairs seven fundamental domains of daily life: general activity, mood, walking ability, occupational performance, interpersonal relationships, sleep quality, and overall enjoyment of life. Each domain is assessed using a 0–10 numerical rating scale, with higher scores indicating maximal interference. The daily use of SCA-specific treatments, including analgesics and iron supplementation, was assessed during the baseline period and the subsequent 8-week follow-up. Daily medication frequency was calculated as the proportion of days on which patients used these treatments relative to the total number of days in each period.

Blood pressure and pulse wave velocity

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured in the left arm using a manual sphygmomanometer (Omron M3; Intellisense, Kyoto, Japan), while the subject remained in a seated position. The measurements were taken three times after a 30-min period of rest. The carotid-femoral pulse wave velocity (PWV; CF-PWV) and carotid-radial PWV (CR-PWV) were measured using an automated system (Pulse Pen; DiaTecne, Milan, Italy). PWV reflects arterial stiffness (2). The CFPWV and CRPWV were measured simultaneously using two pressure-sensitive transducers. The transit time of the pulse wave was calculated by the system software. The distance between the carotid and femoral or radial measurement sites was measured over the body surface using a tape measure. The PWVs were calculated as the distance between two measurement sites divided by the transit time. To cover the entire respiratory cycle, at least 12 readings were performed successively on each subject. Three consecutive measurements were performed for CF-PWV and CR-PWV, and the mean was calculated. The same trained individual performed all PWV measurements.

Autonomic nervous system activity

The activity of the autonomic nervous system (ANS) was assessed using heart rate variability (HRV) for at least 10 min with a heart rate (HR) monitor (Memory Belt, Suunto, Vaanta, Finland) before and after the 8-weeks intervention, in supine position conditions. The relevance of using HRV to quantify ANS activity is still debated (3) but it remains the easiest and most efficient tool to ensure ANS activity measurement. A temporal analysis of HRV was performed to calculate the standard deviation of all normal RR intervals (SDNN), which reflects the global ANS activity. Spectral indices were corrected for HR (4) and determined as previously published (5). The low frequencies (LF, 0.04–0.15 Hz) are known to reflect both sympathetic and parasympathetic activities, the high frequencies (HF, 0.15–0.40 Hz) reflect parasympathetic activity and the LF/HF ratio was used as a broad index of "sympathovagal balance" (5).

Six-minutes walking test

Before (V1) and after (V3) the 8-week intervention, patients performed a 6-minute walking test (6MWT) to measure the maximum walking distance covered in 6 minutes by each patient. This test has already been used in SCA and reflects exercise capacity/physical fitness (6-8).

Biological parameters

Hemoglobin concentration (Hb), hematocrit (Hct), leucocytes and platelets counts, plasma bilirubin (BIL), C-reactive protein (CRP) and lactate dehydrogenase (LDH) levels were determined using standard laboratory tests (9). Plasma free Hb concentration was determined by the measurement of hemoglobin absorbance at 576 nm using the Cripps method (10). Plasma IL-6, TNF-α and IFN-γ levels were measured by Bio-Plex Multiplex immunoassay (Biorad, Hercules, CA, USA), using a Bio-Plex Pro™ Human Cytokine Assay kit and the BioPlex 3D platform (Biorad), according to the manufacturer's instructions.

Endothelial cells incubation with plasma

A subset of 24 plasma samples (8 per group; before and after the 8 weeks intervention) was randomly selected from the whole population for use in cell culture experiments. Human umbilical vein endothelial cells (HUVECs) from PromoCell were grown in Endothelial Cell Medium MV2 (PromoCell) and treated with plasma (5%), for 4 hrs. HUVECs were detached using accutase (Thermo Fisher Scientific), labeled with FITC-conjugated antibodies against

CD54 (Inter-Cellular Adhesion Molecule-1 [ICAM-1], clone STA; Thermo Fisher Scientific), and then analyzed using an Accuri C6 Plus Flow Cytometer (Becton Dickinson).

Statistics

Comparisons between the three groups (control, PA1 and PA2 groups) before and after the 8-week intervention were performed by a 2-way ANOVA with repeated measurements, followed by Tukey post-hoc when appropriate. A Pearson test was used to test for the presence of correlations. Qualitative data were compared with a χ^2 test. Graphpad Prism 10 (La Jolla, CA, USA) was used for statistical analyses. A p-value < 0.05 was considered as significant.

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