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

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Contributions

FDL, MR, RC, MD, MSC, MMa, MS and SD performed experiments; MD, MSC and SD included patients; FDL, MD, EN, KC, MGG, SD, BR, JT and PC analyzed results and made the figures; MD, EN, KC, MGG, MMi, TY, AS, JT and PC designed the research; FDL, MR, EN, KC, MGG, JT and PC wrote the paper. All authors read and approved the final version.

Conflict of interest

No conflict of interest to declare

Clinical trial details

DrePAnon clinical trial, UMIN000042826, UMIN-CTR Clinical Trial

Data sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Abstract

Patients with sickle cell anemia (SCA) have long been discouraged from physical activity (PA). The aim of the present study was to assess the impact of increasing daily step counts on physical fitness, pain and vascular function in patients with SCA. Thirty-eight patients with SCA were recruited and equipped with a Fitbit wrist-worn accelerometer-based PA tracker for 5 weeks to objectively quantify their baseline daily step counts. Patients were then randomly assigned to one of the three groups: 1) control group: no specific information regarding PA was given for 8 weeks (N=12); 2) PA1 group: daily step counts increased by 25% of baseline for 8 weeks (N=12); 3) PA2 group: daily step counts increased by 25% for 4 weeks, then by 50% for 4 additional weeks (N = 14). Pain intensity and frequency decreased after the intervention in the PA1 and PA2 groups. In addition, patients from these two groups increased the distance walked in 6 minutes. Arterial stiffness decreased in both PA1 and PA2 groups, without any change in the autonomic nervous system activity. Several inflammatory markers slightly decreased in the PA2 group. Incubation of cultured endothelial cells with patient plasma showed a decrease in the percentage of ICAM-1 positive cells in the PA2 group. This study is the first to show that increasing daily PA by a simple way (i.e., increasing daily step count of 25-50%) for 8 weeks is sufficient to decrease pain, and improve physical condition and vascular function of patients with SCA.

Key words: Sickle cell anemia, physical activity, vascular function, inflammation

Introduction

Sickle cell anemia (SCA) is genetic disorder caused by a single mutation in the β-globin gene, resulting in the production of an abnormal hemoglobin (Hb), called HbS (1). When deoxygenated, HbS may polymerize causing a mechanical distortion of red blood cells (RBCs; sickling phenomenon). RBCs from patients with SCA are very fragile causing chronic hemolytic anemia. In addition, the abnormal rheology of RBCs from patients with SCA plays a key role in the occurrence of various clinical complications, such as painful vaso-occlusive crisis (2). Chronic hemolysis also promotes inflammation, oxidative stress and alterations in nitric oxide bioavailability, leading to chronic vasculopathy and chronic pain (3-6).

Patients with SCA have a low exercise capacity and it has long been suspected that the biological changes occurring during acute physical exercise could increase the risk of HbS polymerization, RBC sickling and acute complications. However, several studies have shown that acute exercise of mild-to-moderate intensity does not result in consistent RBC alterations or frequent clinical complications (7).

Because regular physical activity (PA) improves health in the general population and in patients with chronic cardiovascular, respiratory or metabolic diseases (8-12), recent studies have investigated the biological and physiological impact of different kind of training programs in patients with SCA (7). Most of these studies have been conducted in laboratory conditions with exercise training sessions being accurately calibrated (exercise duration, intensity, frequency, recovery period) and supervised by highly qualified medical staff (7). These research protocols also required substantial organizational efforts to ensure the participation and availability of patients with SCA. Although these studies have reported benefits on exercise tolerance, muscle function and some biological markers (7, 13-16), clinical benefits of such training programs have been poorly tested in SCA. Moreover, such training programs are not easily transposable to settings where medical resources are limited, particularly in sub-Saharan Africa, where SCA is highly prevalent and health systems often face resource constraints. Simple, easy-to-perform PA that is not burdensome for patients with SCA and does not require medical supervision could represent a promising solution, providing a compromise between low-intensity structured exercises and experimental conditions that are difficult to implement in these regions. The aim of the present study was to assess the impact of increasing regular PA by increasing daily step counts on physical fitness, pain and vascular function in patients with SCA from Dakar (Senegal).

Methods

More details are given in the supplemental material.

Subjects and protocol

Thirty-eight men with SCA, at steady-state, from Dakar (Senegal), 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%). 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 (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. At the end of this 5-week period, a follow-up visit (V1) was scheduled for the measurement of blood biological and physiological parameters. Participant groups were randomly assigned to one of the 3 following groups: 1) a control group: 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 baseline for 4 more weeks (PA group 2; PA2; N = 14). After the 8-week intervention period (i.e., last visit; V3), the same biological and physiological parameters were measured.

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

Pain intensity and frequency were determined using the standardized questionnaire developed by Smith et al. (17). The pain interference subscale was used to quantify the impact of pain on daily functioning. SCA-specific treatments information was collected.

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), and 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 (18).

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 ((19-21), Memory Belt, Suunto, Vaanta, Finland).

Six-minutes walking test

Patients performed a 6-minute walking test (6MWT) to measure the maximum walking distance covered in 6 minutes (22-24).

Biological parameters

Hematological, biochemical and plasma inflammatory markers were analyzed.

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 to test the impact of plasma on human umbilical vein endothelial cells (HUVECs) activation. See supplemental materials for further information.

Statistics

See supplemental materials for further information.

Results

Increasing daily step count increases exercise capacity and decreases pain

Table 1 shows no difference in the clinical characteristics between patients from the three groups at the time of inclusion. The number of days for monitoring the baseline physical activity before the intervention period was not different between the three groups (44.31 ± 8.49, 45.27 ± 11.35 and 39.50 ± 5.0 days for the control, PA1 and PA2 groups, respective). During the baseline period, daily medication frequency did not differ significantly between the groups, and no episodes of VOC or ACS were reported in the study population. Mean daily step counts were compared between the three groups at baseline and after the 8-week intervention. Control

subjects did not increase significantly their daily step count (+14.3 ± 33.3%, $p = 0.22$; Figure 1A) or their 6-min walking distance (+2.8 ± 7.7%, $p = 0.09$; Figure 1B). In contrast, both the PA1 and PA2 groups significantly increased their daily step count (+25.3 ± 25.2%: $p < 0.05$ and +51.8 ± 36.5%: $p < 0.0001$, respectively; Figure 1A). As a consequence, the PA1 and PA2 groups increased their 6-min walk distance (+8.9 ± 6.8% in the PA1 group: $p < 0.05$, +16.2 ± 12.9% in the PA2 group: $p < 0.0001$; Figure 1B), with the PA2 group having the highest increase (PA1 group vs control group: $p < 0.05$; PA2 group vs control group: $p < 0.01$; PA2 group vs PA1 group: $p < 0.05$). The figure 1C shows the positive correlation between the changes in daily step count and the changes in the 6-min walking distance when the 3 groups are combined ($r = 0.30$; $p < 0.05$). The frequency of daily pain decreased in both the PA1 and PA2 group ($p < 0.05$ and $p < 0.01$, respectively) after intervention, while it did not change significantly in the control group (Figure 1D). Mean pain intensity decreased in both the PA1 and PA2 groups after the 8-week intervention ($p < 0.05$ for both) while it remained unchanged in the control group (Figure 1E). The number (Figure 1F) and dosage (data not shown) of medication per day (mainly iron supplement and acetaminophen) was not different between the three groups before or after the 8 weeks of intervention. After the 8-week intervention, daily medication frequency was 0.68 ± 0.20, 0.56 ± 0.29 and 0.58 ± 0.22 for the control, PA1 and PA2 group, respectively. Interference with daily activities decreased in the PA1 ($p < 0.05$) and PA2 ($p < 0.01$) groups after the 8 weeks of intervention (Figure 1G). No association was found between the percentage of changes in these parameters and HbF levels.

Increasing daily step count decreases arterial stiffness and blood pressure

Both CF-PWV (Figure 2A) and CR-PWV (Figure 2B) decreased in the PA1 and PA2 groups after the 8 weeks of increasing daily step count (p ranging from < 0.05 to < 0.0001) while they remained unchanged in the control group. After the 8 weeks, the PA1 and PA2 groups had lower CF-PWV ($p < 0.05$ and $p < 0.01$, respectively) and CR-PWV ($p < 0.01$ for the two groups) than the control group. We observed a trend for a negative correlation between the increase in step count and the decrease of CF-PWV when all individuals were combined (Figure 2C; $r = -0.25$; $p = 0.06$). In the same way, a significant negative correlation was observed between the changes in the 6-min walking distance and the decrease of CF-PWV (Figure 2D; $r = -0.34$; $p < 0.01$). Systolic (Figure 2E) and diastolic (Figure 2F) blood pressures were significantly lower after the 8 weeks of increasing daily step count compared to before in the PA2 group ($p < 0.01$ and $p < 0.05$ for SBP and DBP, respectively). No significant change was observed in the two

other groups (i.e., control and PA1 groups) but both the PA1 and PA2 groups had lower systolic and diastolic blood pressures after the 8 weeks of increasing daily step count compared to the control group ($p < 0.001$ for SBP and $p < 0.01$ for DPB). The percentages of change in SBP and DBP correlated with the percentages of change in CF-PWV ($r = 0.36$; $p < 0.05$ and $r = 0.41$; $p < 0.01$, respectively; Figures 2G and 2H). No association was found between the percentage of changes in these parameters and HbF levels.

It has been previously demonstrated that having SBP ≥ 120 mmHg or DBP ≥ 70 mmHg (i.e., relative systemic hypertension) may increase the risk for pulmonary hypertension and renal dysfunction (25). The percentage of patients having a value for SBP ≥ 120 mmHg or DBP ≥ 70 mmHg was 75% in the control group, 58% in the PA1 group and 57% in the PA2 group ($\chi^2 = 0.93$; $p = 0.63$). After the 8-week intervention, the percentage of patients with relative systemic hypertension was almost the same in the control group (83%) while it decreased in both the PA1 and PA2 groups (25% and 29%, respectively; $\chi^2 = 12.96$; $p < 0.01$).

Resting heart rate (Figure 3A) and markers of the ANS activity (Figures 3B-3E) did not change with the 8-week intervention in any group and did not differ between the three groups.

Increasing daily step count has no impact on hemolysis

No difference between the three groups and no change in leucocytes and platelets count were observed (data not shown). In addition, no difference between the groups and no change between the first and second visit was observed for Hct (Figure 4A) or hemolytic markers (i.e., free Hb, LDH and total bilirubin levels; Figures 4B-4D)

Increasing daily step counts reduces inflammation

CRP did not change over time and was not different between the three groups (Figure 5A). IL-6 concentration did not change over time in the three groups but we observed a lower level after the 8-week intervention in the PA1 group compared to the control group, and a trend for the PA2 group for having lower value too (Figure 5B). TNF-α did not significantly change in the three groups but the values were significantly lower in the PA2 group compared to control group after the 8 weeks of increasing daily step count (Figure 5C). IFNγ decreased significantly after the 8 weeks of intervention in the PA1 and PA2 groups, while increasing in the control group (Figure 5D). Incubation of HUVECs with the plasma of patients showed that the

percentage of HUVECs positive for ICAM-1 and ICAM-1 expression decreased in the PA2 group after 8 weeks of increasing daily step count (Figures 5E and 5F).

Discussion

The present study demonstrated that increasing daily step count of 25-50% for 8 weeks is sufficient to increase physical capacity, decrease pain frequency and intensity, improve vascular function and decrease inflammation in patients with SCA from Dakar (Senegal).

It is now widely accepted that regular physical activity or exercise training may have beneficial effects on the cardiovascular, muscle, metabolic, respiratory, blood rheological and inflammatory function/profile of various chronic diseases (26). Previous studies showed that 8 weeks of regular training in sickle cell mice decreased inflammation, blood viscosity and oxidative stress (27-29). In patients with SCA, recent studies showed that training programs (2 to 3 training sessions of 20 to 45 min per week for 6 to 8 weeks) improved muscle function, increased ventilatory efficiency and aerobic physical fitness (14, 16, 30, 31). The exercise intensity chosen for these training sessions was based on an initial submaximal/symptom-limited cardio-pulmonary exercise test performed by the patients under laboratory conditions (7, 14, 30, 31). Individualizing and calibrating training programs requires the use of advanced technical tools, such as cardiopulmonary exercise testing with gas exchanges analyzers and the determination of metabolic thresholds during exercise. Consequently, this type of approach is hardly implementable in large populations, particularly in sub-Saharan African countries where medical resources are limited and the prevalence of SCA is particularly high. In contrast, the use of daily step count as a guide for PA is easily adaptable across diverse contexts from low-income countries with limited healthcare infrastructure to high-income countries such as those in Europe and the United States. It represents a simple, scalable, and cost-effective tool that can complement existing healthcare strategies, with particular added value in low- and middle-income settings where access to care is often severely constrained. Walking is simple, free and one of the easiest ways to get more active. A recent meta-analysis conducted in the general population (227,000 participants) showed a significant inverse association between daily step count and all-cause mortality and cardiovascular mortality (32), which confirms another meta-analysis (33).

The present study shows that guiding PA in patients with SCA using a wearable activity tracker is both feasible and safe within the African context. Increasing daily step count improves

physical fitness, as evidenced by longer 6-min walking distances, and reduces pain frequency and intensity. The mechanisms at the origin of the decrease of pain are beyond the scope of this study but could involve a decrease in central nervous system sensitization due to regular PA (34). Moreover, the lower inflammatory profile found after the 8 weeks of increasing daily step count could have contributed to lower pain frequency and intensity (35). Consequently, the quality of life of the patients improved, leading to a lower interference with daily activities.

Increasing daily step count also resulted in a decrease of arterial stiffness, both in the PA1 and PA2 groups, indicating an improvement of the vascular function. These findings are in agreement with studies showing an improvement of the vascular function in patients with prehypertension after a 8 weeks program of mixed aerobic-resistance training (3 sessions of 1 hour per week (36)) or in young individuals with cardiovascular risk factors after 6 weeks of increasing daily step counts (37). A systematic review and meta-analysis showed that aerobic exercise performed more than three times per week, for sessions under 60 min and over an intervention period of up to 8 weeks, is associated with significant improvements in vascular function in patients with heart failure (38). Improvement of vascular function may have contributed to the improvement of physical fitness, systolic and diastolic blood pressures and pain in our study. Vascular function has been reported to be impaired in SCA and to participate in the occurrence of several acute and chronic complications (39-41). The development of chronic vasculopathy in SCA is multifactorial and may involve alterations in the ANS activity, inflammation and hemolysis (40, 41). We did not observe any difference in the ANS activity between the three groups and increasing daily step count had no effect. Moreover, no effect of the 8-week intervention was found on hemolytic markers (i.e., LDH, bilirubin and free hemoglobin), and no difference was observed between the three groups. In contrast, we observed some differences in cytokines levels (IL-6 and TNFα) between the 3 groups at the end of the follow-up with both PA1 and PA2 groups having less inflammation than the control group. In addition, IFNγ level was lower in the PA2 group after the 8 weeks of increasing daily step count compared to before. Regular exercise is known to decrease inflammation in the general population and in patients with various chronic disorders (26, 42) but this is the first time that a such result is observed in the context of SCA. Both IL-6 and TNFα have been shown to increase ICAM-1 expression on endothelial cells through the modulation of the STAT3 and NFκB pathway, respectively, and of the Rac1 pathway (43, 44). IFNγ has been reported to increase the expression of ICAM-1 on endothelial cells through the activation of protein kinase

C. ICAM-1 plays a crucial role in the pathophysiology of SCA by promoting the adhesion of sickle cells to the endothelium, contributing to vaso-occlusion and tissue damage (45-47). In non-sickle cell patients with chronic pain, a positive correlation has been reported between pain intensity and the plasma concentration of the soluble form of ICAM-1 (48) and any change in pain intensity over time was reflected by changes in ICAM-1 levels. Although we did not measure directly the soluble form of adhesion molecules in the plasma of the patients, incubation of HUVECs with plasma showed a decrease of the percentage of HUVECs positive for ICAM-1 and a decrease in ICAM-1 MFI in the PA2 group after the 8 weeks interventions. The changes in ICAM-1 expression observed in the PA1 group were of lower magnitude than for the PA2 group and did not reach statistical significance. Our findings suggest that the slightly lower inflammation after 8 weeks of increasing daily step count could result in lower endothelial cells activation, which could have participated to the improvement of the vascular function. Other mechanisms, such as a decrease in oxidative stress or an increase in nitric oxide (NO) production, could also be at the origin of the improvement in vascular function found in the present study. A decrease in oxidative stress has been previously reported in sickle cell mice following a multi-week training protocol (49), while an increase in NO production was suggested by the results of Grau et al (16) observed in a small group of children with sickle cell anemia (SCA) after a 6-week training protocol conducted in laboratory conditions.

An important limitation of the present study is the non-inclusion of women. Women were not included because hormonal changes that may occur during the menstrual cycle could affect some key parameters measured in this study, such as arterial stiffness. For instance, it was demonstrated that flow-mediated dilation of the brachial artery and arterial compliance increased from the follicular to the late follicular phase, decreased during the early luteal and then re-increased during the late luteal phase (50). Moreover, potential confusions may exist between painful symptoms related to SCA and other pain (specifically, painful menstruation, which may be common in young women). Finally, the level of menstrual flow may affect the severity of anemia, periodically impacting lifestyles and the level of physical activity. Future studies should include women but specific analyses to characterize the luteal and follicular phases of the menstrual cycle will be needed to take into account the impact of both regular physical activity and hormonal changes on clinical, biological and physiological parameters. A second limitation is the fact that patients included in the present study were rather active with a mean daily step count ranging from 3000 to more than 9500-10000. The limited number of car ownership and the underdeveloped public transportation network in Dakar are probably at

the origin of the active lifestyle of the inhabitant of this city. While this active lifestyle could be probably present in other African regions, it is not the case in other world regions, such as in Europe or USA. Further studies conducted in regions where sedentary behavior is more common are needed to test whether a 25-50% increase in daily step count is sufficient to improve pain, vascular function and physical capacities of SCA individuals. Finally, the limited sample size of the present study does not allow to identify an optimal step count threshold that could provide the greatest clinical and biological benefits to the patients.

In conclusion, this study is the first one to show that increasing daily PA by a simple way (i.e., increasing daily step count of 25-50%) for 8 weeks is sufficient to improve the physical condition and vascular function of patients with SCA, as well as to decrease pain frequency and intensity. Although increasing daily step count of 25% or 50% resulted in very similar effects, it seems that the 50% condition had slightly greater effects, notably on inflammation. Most importantly, this change in PA behavior had no negative impact nor adverse event in the participants. It is recommended that physicians encourage patients with SCA to engage in regular daily PA, with objective monitoring of daily step count using validated trackers or connected devices (e.g., wearables or smartphones), and to progressively increase their daily step count by at least 25% above their baseline level. Long-term clinical effects of increasing daily PA need to be investigated in the future.

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Table 1: Clinical characteristics of the patients at the time of inclusion

	Control group (n = 12)	**PA1 group (n = 12)**	**PA2 group (n = 14)**
VOC rate (number/year)	0.46 ± 0.46	0.37 ± 0.44	0.25 ± 0.33
ACS rate (number/year)	0.36 ± 0.74	0.47 ± 0.92	0.36 ± 0.84
Priapism (number and %)	2 / 16.7	2 / 16.7	3 / 21.4
Stroke (number and %)	0	0	0
Moderately elevated albuminuria (number and %)	7 / 58.3	10 / 83.3	11 / 78.6
Proteinuria (number and %)	1 / 8.3	2 / 16.7	0
Hydroxyurea treatment (number and %)	1 / 8.3	0	0

VOC: vaso-occlusive crises; ACS: acute chest syndrome.

Figure legends

Figure 1: Physical activity and pain parameters in the three groups. Comparisons of the PA data (1A-B), pain parameters (1D-E) and main medication (1E-F) between the three groups, before and at the end of the 8-week intervention. Correlation between the changes in step count and the 6-min walking distance (1C). V1: before the 8-week intervention; V3: after the 8-week intervention. *p < 0.05; **p < 0.01; ****p < 0.0001.

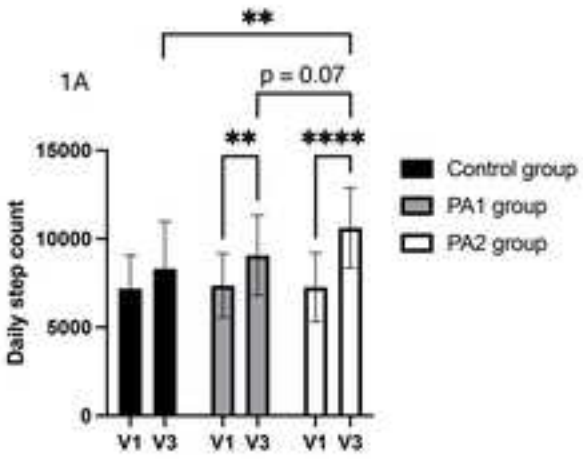
Figure 2: Pulse wave velocities and blood pressures in the three groups. Comparisons of the vascular function parameters (2A, 2B, 2E, 2F) between the three groups, before and at the end of the 8-week intervention. Correlation between the changes in vascular function parameters and the changes in step count or in the 6-min walking distance (2C, 2D, 2G, 2H). V1: before the 8-week intervention; V3: after the 8-week intervention; ΔCF-PWV: changes in the carotid-femoral pulse wave velocity; ΔSBP: changes in systolic blood pressure. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.

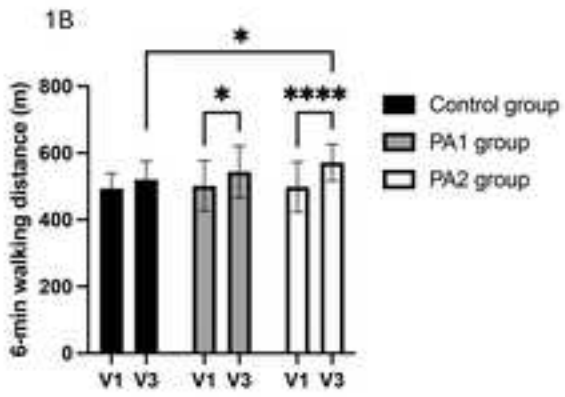
Figure 3: Comparisons of the autonomic nervous system activity parameters between the three groups, before and at the end of the 8-week intervention. V1: before the 8-week intervention; V3: after the 8-week intervention. HR: heart rate; SDNN: standard deviation of all normal RR intervals (SDNN); (LF: low frequencies (0.04–0.15 Hz); HF: high frequencies (0.15–0.40 Hz).

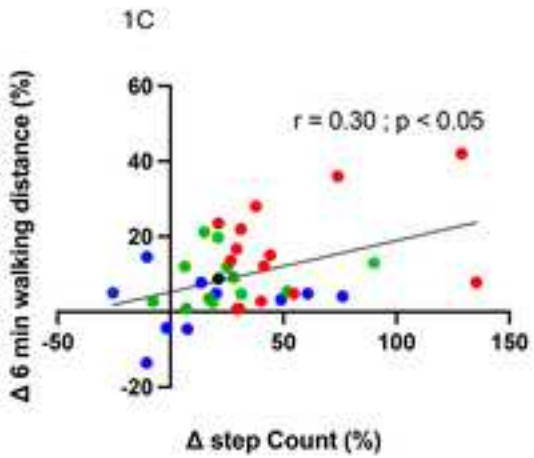
Figure 4: Hematological and biochemical parameters in the three groups. Comparisons of hematocrit (Hct, 4A), free hemoglobin concentration (FreeHb, 4B), lactate dehydrogenase levels (LDH, 4C) and total bilirubin (4D) between the three groups, before and at the end of the 8-week intervention. V1: before the 8-week intervention; V3: after the 8-week intervention. *p < 0.05.

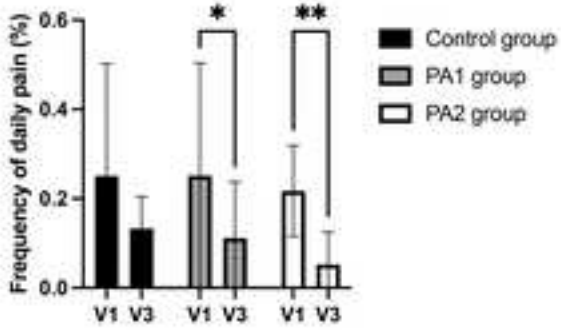
Figure 5: Inflammation and endothelial cell activation. Comparisons of plasma inflammatory molecules (5A-D) and markers of endothelial activation (5E-F) between the three groups, before and at the end of the 8-week intervention. V1: before the 8-week intervention; V3: after the 8-week intervention; CRP: C-reactive protein; IL-6: Interleukin 6; TNFα: tumor necrosis

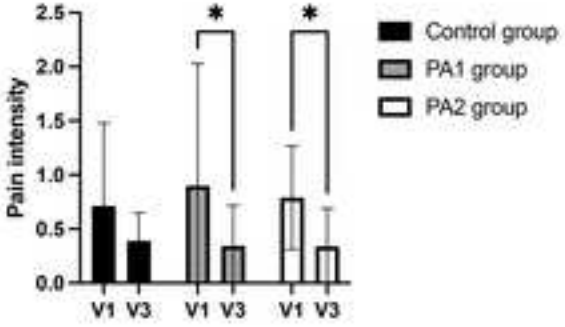
factor a; IFNγ: interferon g; ICAM-1: intercellular adhesion molecule; MFI: median fluorescence intensity. *$p < 0.05$; **$p < 0.01$.

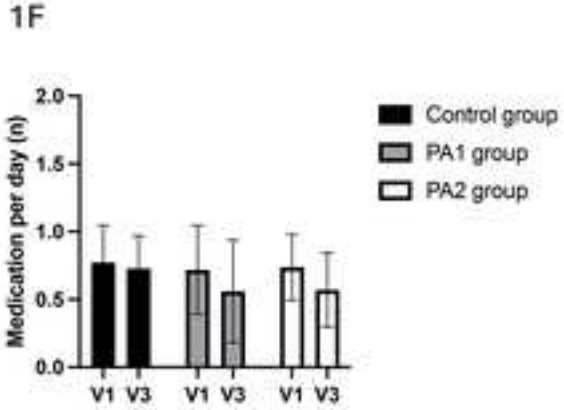

1A
**
p = 0.07
**
****
Daily step count
15000
10000
5000
0
V1 V3 V1 V3 V1 V3
Control group
PA1 group
PA2 group

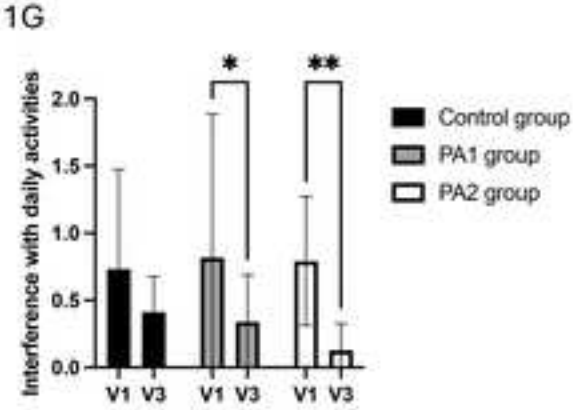

1B
*
*
****
6-min walking distance (m)
800
600
400
200
0
V1 V3 V1 V3 V1 V3
Control group
PA1 group
PA2 group

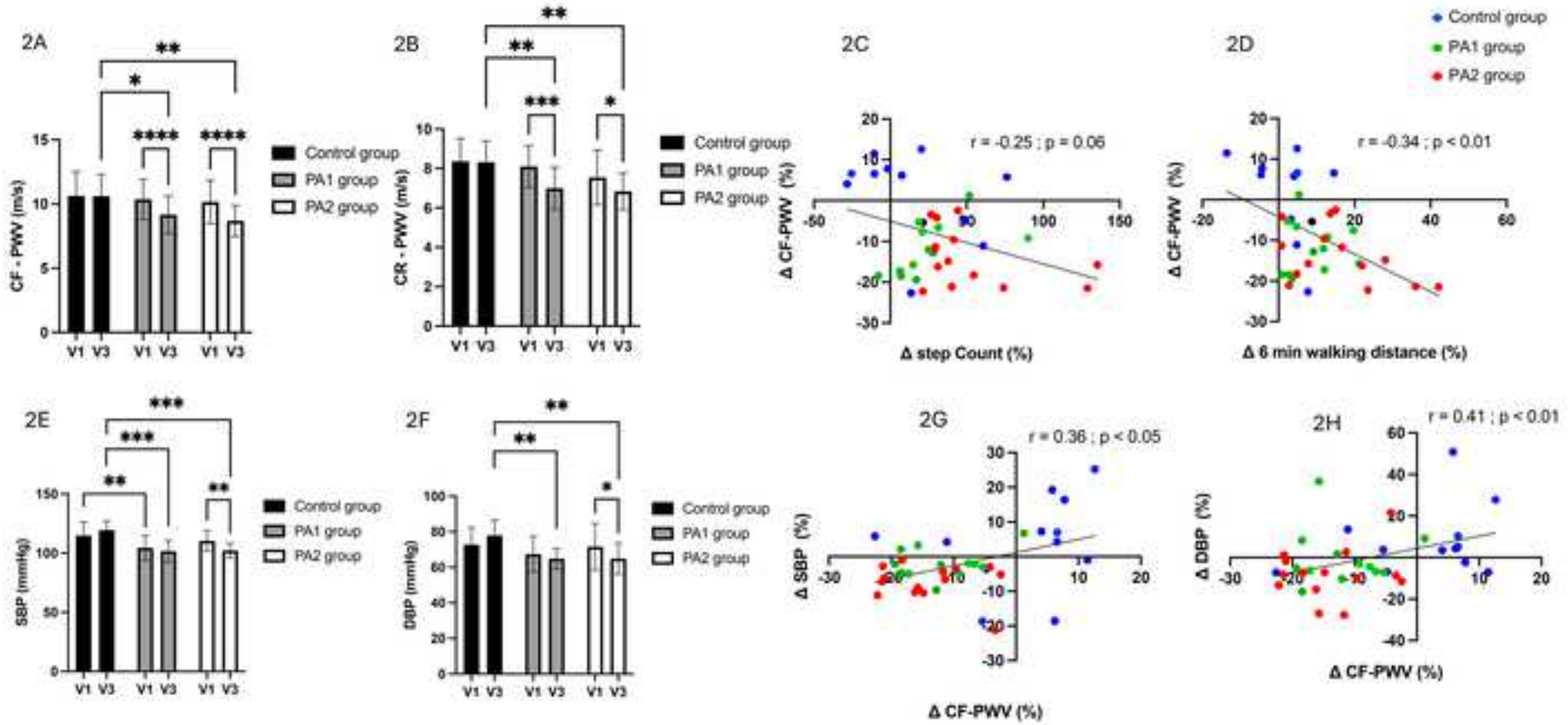

1C
r = 0.30 ; p < 0.05
Δ 6 min walking distance (%)
60
40
20
-20
-50
50
100
150
Δ step Count (%)


1D
*
**
Frequency of daily pain (%)
0.6
0.4
0.2
0.0
V1 V3 V1 V3 V1 V3
Control group
PA1 group
PA2 group

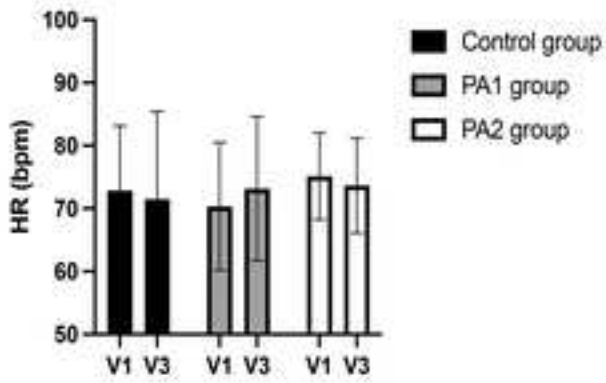

1E
*
*
Pain intensity
2.5
2.0
1.5
1.0
0.5
0.0
V1 V3 V1 V3 V1 V3
Control group
PA1 group
PA2 group


1F
Medication per day (n)
2.0
1.5
1.0
0.5
0.0
V1 V3 V1 V3 V1 V3
Control group
PA1 group
PA2 group

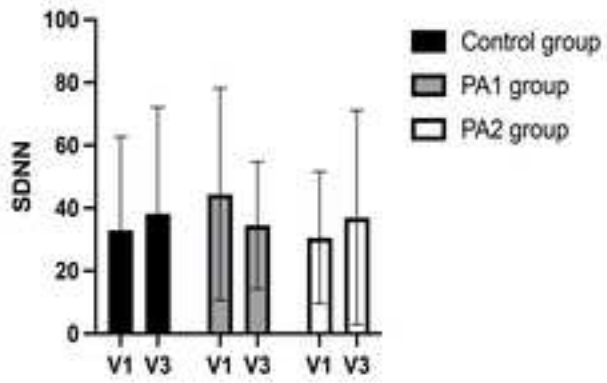

1G
*
**
Interference with daily activities
2.0
1.5
1.0
0.5
0.0
V1 V3 V1 V3 V1 V3
Control group
PA1 group
PA2 group


2A
CF - PWV (m/s)
V1 V3 V1 V3 V1 V3
Control group
PA1 group
PA2 group
2B
CR - PWV (m/s)
2C
Δ CF-PWV (%)
r = -0.25 ; p = 0.06
Δ step Count (%)
2D
r = -0.34 ; p < 0.01
Δ 6 min walking distance (%)
2E
SBP (mmHg)
2F
DBP (mmHg)
2G
Δ SBP (%)
r = 0.36 ; p < 0.05
Δ CF-PWV (%)
2H
Δ DBP (%)
r = 0.41 ; p < 0.01
Δ CF-PWV (%)

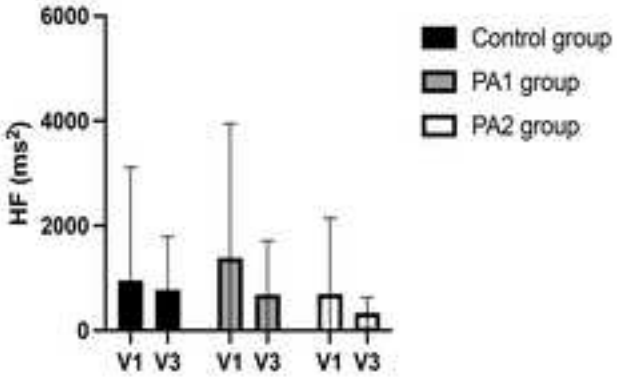
3A


HR (bpm)
100
90
80
70
60
50
V1 V3 V1 V3 V1 V3
Control group
PA1 group
PA2 group

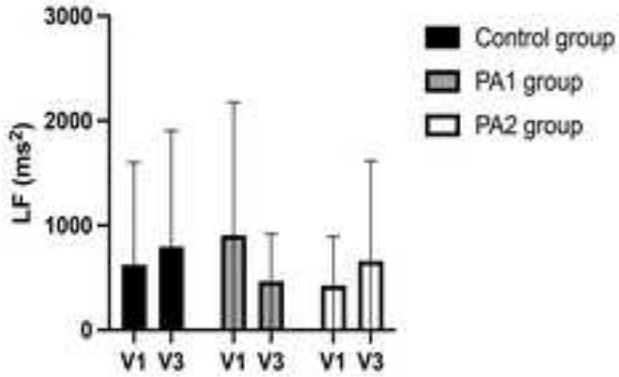
3B


SDNN
100
80
60
40
20
0
V1 V3 V1 V3 V1 V3
Control group
PA1 group
PA2 group

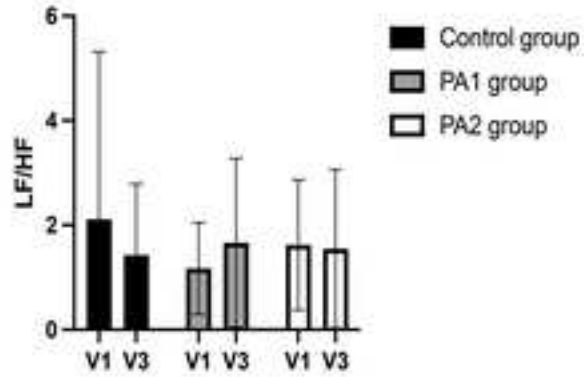
3C

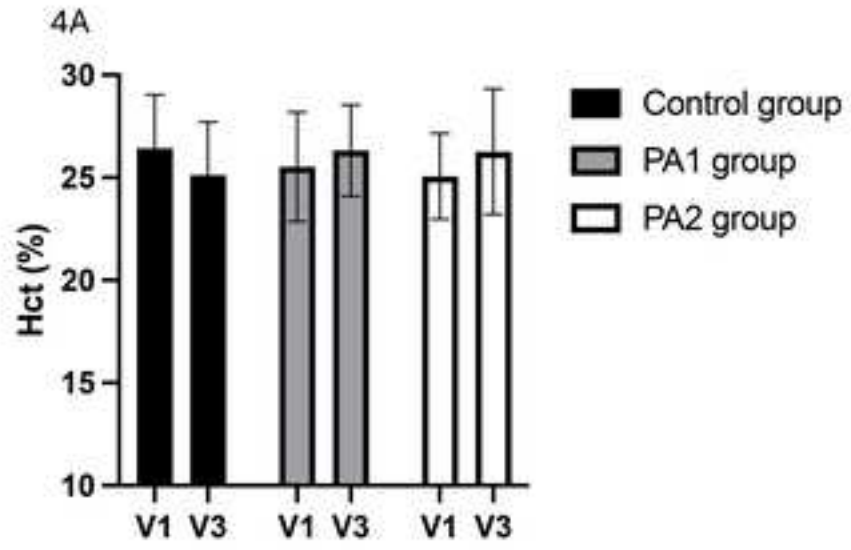

HF (ms²)
6000
4000
2000
0
V1 V3 V1 V3 V1 V3
Control group
PA1 group
PA2 group

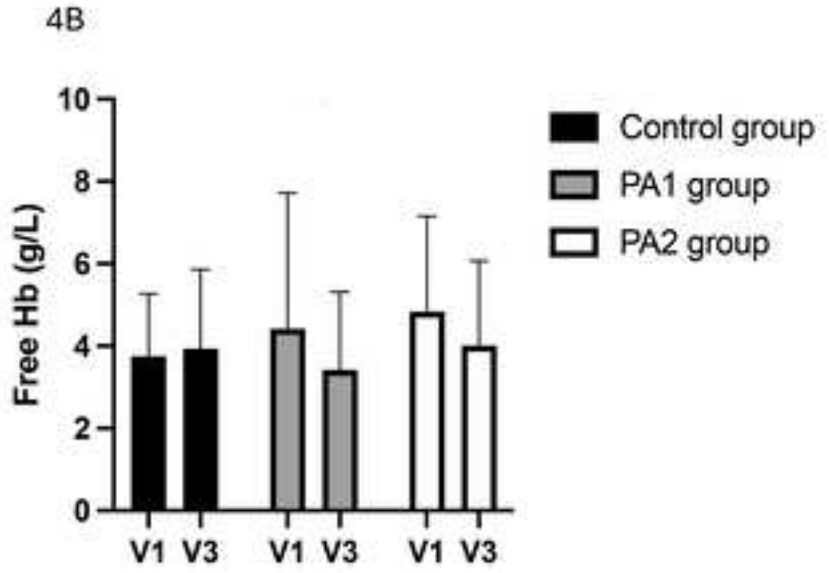
3D

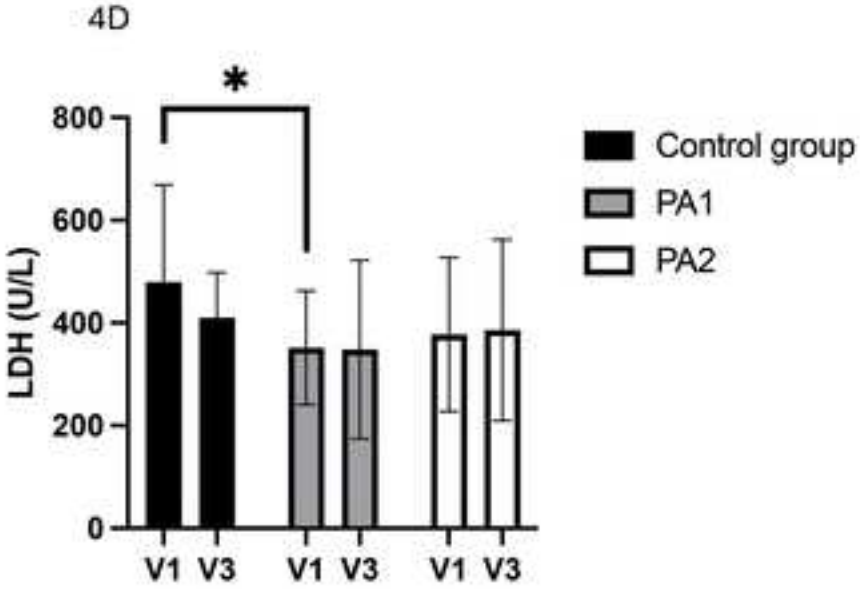

LF (ms²)
3000
2000
1000
0
V1 V3 V1 V3 V1 V3
Control group
PA1 group
PA2 group

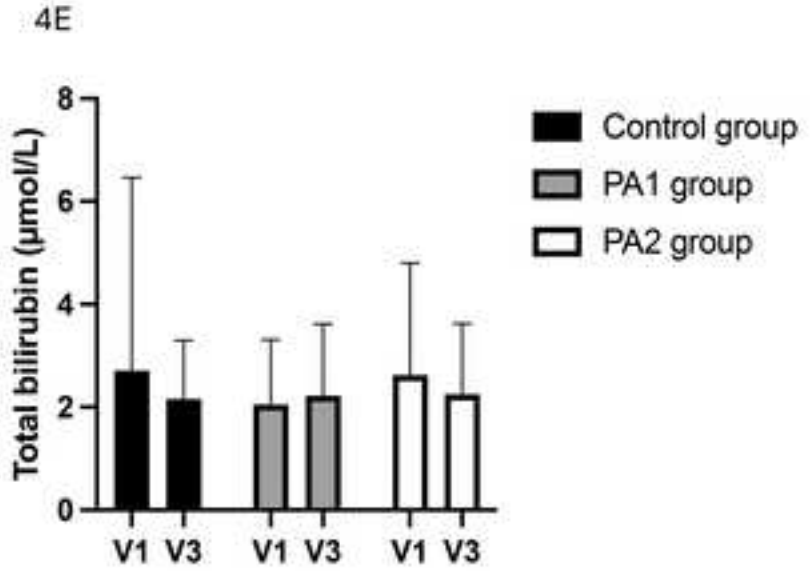
3E

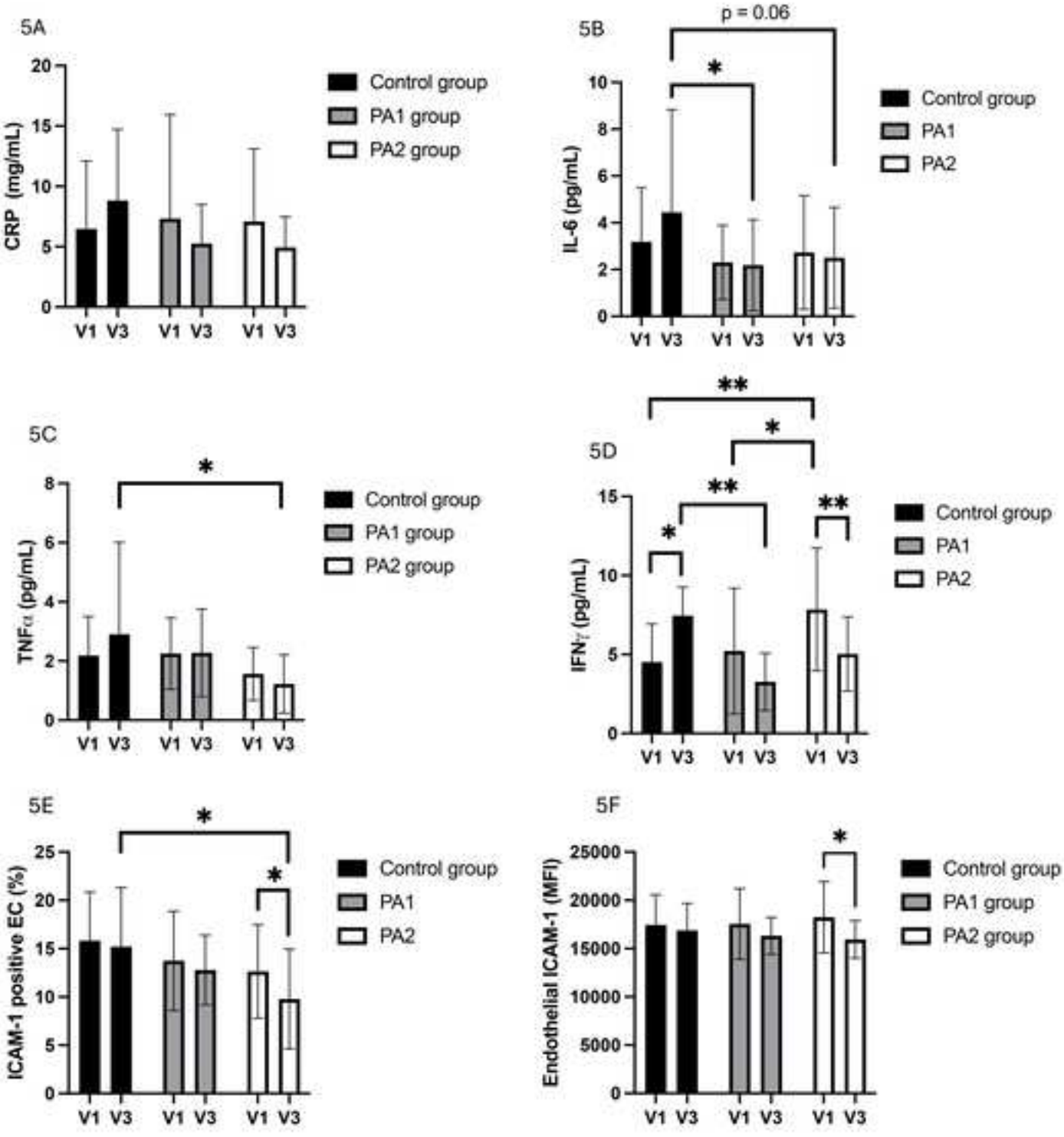

LF/HF
6
4
2
0
V1 V3 V1 V3 V1 V3
Control group
PA1 group
PA2 group


4A
Hct (%)
30
25
20
15
10
V1 V3 V1 V3 V1 V3
Control group
PA1 group
PA2 group


4B
Free Hb (g/L)
10
8
6
4
2
0
V1 V3 V1 V3 V1 V3
Control group
PA1 group
PA2 group


4D
*
LDH (U/L)
800
600
400
200
0
V1 V3 V1 V3 V1 V3
Control group
PA1
PA2


4E
Total bilirubin (μmol/L)
8
6
4
2
0
V1 V3 V1 V3 V1 V3
Control group
PA1 group
PA2 group


5A
CRP (mg/mL)
20
15
10
5
0
V1 V3 V1 V3 V1 V3
Control group
PA1 group
PA2 group
5B
p = 0.06
*
IL-6 (pg/mL)
10
8
6
4
2
0
V1 V3 V1 V3 V1 V3
Control group
PA1
PA2
5C
*
TNFα (pg/mL)
8
6
4
2
0
V1 V3 V1 V3 V1 V3
Control group
PA1 group
PA2 group
5D
**
*
**
*
**
IFNγ (pg/mL)
15
10
5
0
V1 V3 V1 V3 V1 V3
Control group
PA1
PA2
5E
*
*
ICAM-1 positive EC (%)
25
20
15
10
5
0
V1 V3 V1 V3 V1 V3
Control group
PA1
PA2
5F
*
Endothelial ICAM-1 (MFI)
25000
20000
15000
10000
5000
0
V1 V3 V1 V3 V1 V3
Control group
PA1 group
PA2 group

Supplemental material

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

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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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