Title. A pilot study for the promotion of brisk walking in HIV infected people: health benefits after 12-weeks of training.

INTRODUCTION

Race walking is one of the most famous disciplines in the Olympic games. It is an athletic movement that can be defined as a form of advances and agonistic way of walk (Rule 230 IAAF). Although it is a foot race, it is different from running in that one foot must appear to be in contact with the ground at all times. Brisk walking is the act of walking that comes from race walking and it permits to train at moderate intensity and it has been demonstrated that it could delay all-cause mortality in the general population and reduces the risk of cardiovascular disease (CVD), stroke, type-2 diabetes and some types of cancer [1]. In addition, moderate physical activity reduces blood level of immune activation markers, suggesting that health benefits are mediated by reduction of inflammation [2].

The Human Immunodeficiency Virus (HIV) causes a progressive loss of CD4+ cells, leading to onset of severe opportunistic infections, defining the Acquired Immunodeficiency Syndrome (AIDS), and to premature death. The introduction of combination antiretroviral therapy (cART) in the mid ‘90s, effective in inhibiting HIV replication, was associated with a dramatic increase of survival, transforming a deadly infection into a chronic disease. However, treatment does not fully restore health, in fact HIV-infected adults who have durable treatment-mediated suppression of HIV replication are at risk of developing several non-AIDS disorders including cardiovascular diseases (CVD), cancer, kidney disease, liver disease, osteopenia or osteoporosis. Moreover, patients showed a condition of persistent low-level inflammation that is a feature of treated chronic HIV infection and, like in the general population, is associated with metabolic disorders e.g., CVD diseases and type 2 diabetes [3, 4].

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The aim of this study was to determine whether 12-week of moderate physical activity with or without strength exercises could have effects on metabolic parameters and inflammatory markers in HIV infected people. For this reason we designed a pilot study of physical activity. Our rationale for selecting a recreational kind of race walking (brisk walking), as physical activity was that is a low impact exercise that permits to train at moderate intensity with a lower risk of injuries in lower limbs as these patients usually suffer from osteoporosis.

METHODS

Study design

This was a 12-week study, which enrolled sedentary HIV-infected patients receiving combination antiretroviral treatment (cART). Inclusion criteria were: age ≥18 years; cART for ≥6 months; sedentary lifestyle, defined as physical activity for <2 days per week for <20 minutes per session; either objective evidence of lipodystrophy, a medical condition characterized by abnormal or degenerative conditions of the body's adipose tissue, as established by the visiting physician [5], or of at least one of the criteria of metabolic syndrome [6]. Exclusion criteria included any disease requiring hospitalization in the 6 weeks before enrolment; medical conditions contraindicating exercise as established by a sport medicine specialist; inability to walk at brisk pace; current substance or alcohol abuse. San Raffaele Hospital Ethical Committee approved the study. Written informed consent was obtained from all study participants. This trial was registered retrospectively at Australian New Zealand Clinical Trials Registry (ACTRN12615001258549).

Participant screening and protocol

An infectious diseases specialist and a sport medicine specialist screened subjects for eligibility, after performing electrocardiogram at rest and during sub-maximal cycle ergometer test. Patients who met the above inclusion criteria and with no contraindications to exercise were offered to either join the 'brisk walk' group, consisting of brisk walking only, or the 'strength- brisk walk' group, where each walking session was preceded by a strength exercise session. Briefly, brisk walking involves a technique similar to race walking, with a great movement in the three planes of motion of pelvis and arm, which are necessary to increase the walking speed; on the other hand, subjects have not to strictly adhere to the straight advanced knee rule. Overall, four groups of 10-15 subjects trained three times a week for 12 weeks. The walking sessions were performed outdoor on measured tracks in green areas in Milan for 60 min at an intensity of 65-75% of maximal HR (HR_{max}) [7]. Each subject was equipped with a personal HR monitor (Polar FT4, Polar Electro 2011, Kempele, Finland) with an acoustic warning if HR was below or exceeded the predetermined range, which was maintained throughout the study. Mean HR (HR_{mean}) was recorded during each session, with values captured every 5 seconds. Strength exercise was carried out before walking in a gym by circuit training, including crunch, lat machine, chest press, leg press, leg extension, sitting calf. Each exercise was repeated 12 times for three sets at 65% of 1-Repetition Maximum Test (1-RM). Professional coaches followed all the sessions providing technical instruction, supervision and encouragement. Participants received generic dietary advice, consisting of a total food intake of ≤ 2000 Kcal/day (corresponding to ≤ 8374 Kj), including 50-60% carbohydrates, 15-30% proteins and 20-30% fat. Study variables were assessed at baseline (BL) and at the end (W12) of the program.

Physical Fitness Evaluation

6 Minutes Walking Test (6MWT). Participants were instructed to walk as fast as possible for six minutes on a 400 m outdoor athletic track [8], HR_{mean} was recorded during the test.

Strength measurements. 1-RM test assessed the maximal load lifted in one repetition, and the 30-seconds crunch test the number of crunches performed in 30 seconds.

Body composition

Anthropometric variables included weight, body mass index (BMI), waist, hip, and thigh circumference on dominant side. Total and % fat mass, lean mass and body mineral content (BMC) at arms, limbs, trunk and as total body was measured by dual-energy X-ray absorptiometry (DEXA)(Lunar Prodigy, version 8.8, GE Medical Systems, Madison, WI).

Laboratory analysis

Blood examination included complete blood count; standard biochemical exams with fasting total, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, triglycerides, glucose, insulin, HbA1c; CD4+ and CD8+ T-cell counts, HIV-1-RNA plasma level (Abbott RealTime HIV-1 assay).

Inflammatory markers

Soluble markers. Soluble biomarkers were measured in cryopreserved plasma samples, drawn at BL and W12, by commercially available enzyme-linked immunosorbent assays according to manufacturers' recommendation. These included high-sensitivity C-reactive protein (hsCRP, Catalog Number DCRP00), interleukin-6 (IL-6, Catalog Number D6050) and soluble CD14 (sCD14, Catalog Number DC140, R&D Systems, Minneapolis, MN), D-dimer (Asserachrom,

Diagnostica Stago, Asnieres-Sur-Seine, France), interleukin-18 (IL-18) (Medical and Biological Laboratories, Nagoya, Japan).

Statistical Analysis

Quantitative variables were expressed as median and 25-75% interquartiles (Q1-Q3). The Kolmogorov-Smirnov test was performed to test normality of distribution. Since many of the tested variables were not normally distributed, nonparametric tests were used. Changes between BL and W12 were assessed by Wilcoxon matched-pairs signed rank test, BL values and % change differences between groups by Mann-Whitney test, and correlations of continuous variables by the Spearman's test. Statistical analysis was performed using Graph Pad Prism Software, version 6.0 for Macintosh (Graph Pad Software, San Diego, CA). Level of significance was set at 0.05.

RESULTS

Patient disposition and baseline characteristics

Fifty-nine subjects underwent a screening visit and 49 were eligible: 29 joined the 'brisk walk' group and 20 the strength-walk group. Fourteen subjects (29%) dropped out and were not included in the analyses (Figure 1). Thirty-five subjects were evaluated at W12, including 21 in the walk group and 14 in the strength-walk group. BL patients' characteristics are shown in Table 1. Except from gender, there was no difference between the two training subgroups and in clinical variables.

*** Figure 1 here***

*** Table 1 here***

Physical Fitness

Performance during the training sessions. Median overall adherence to the sessions was 67% (dropout subjects were not considered). Participants walked a median distance of 122 km in 12 weeks (5040 m each session) at a median exertion of 66% HR_{max} (Table 2). Participants in the strength-brisk walk group walked longer distances than those in the walk group, both in each session and as a total. No different performances were observed between women and men within the brisk walk group.

*** Table 2 here ***

6MWT. At W12 6MWT, participants walked for a significantly longer distance compared to BL both in the overall sample and in the two subgroups, in parallel with significant increases of HR_{mean} (Table 3). Overall, better distance improvement correlated with higher adherence ($r=0.580$; $p=0.0003$) and longer brisk walked distance ($r=0.555$; $p=0.0005$) during the 12 weeks of training (Spearman's correlation).

*** Table 3 here ***

1-RM and 30-seconds crunch tests. In the strength-brisk walk group, training was followed by significant improvement of performance for all strength exercises.

Body composition

Significant reductions were observed of weight, BMI and waist and hip circumference in the whole group. Weight, BMI reduction and waist circumference reductions were maintained in the walk group only (Table 4). However, changes of these parameters from BL did not differ between the two training groups. No significant changes were observed by DEXA of fat and lean mass (Table 4).

*** Table 4 here ***

Laboratory examinations

At W12, significant reductions were observed of total and LDL cholesterol values in the whole sample. Both were also decreased in the brisk walk group, and LDL cholesterol in the strength-brisk walk group (Table 5). Changes from BL did not differ between groups. No significant changes were observed of the other laboratory variables (Table 5).

*** Table 5 here ***

Inflammatory markers

Significant reductions were observed of hsCRP, IL-6, D-dimer and IL-18, but not of sCD14 (Figure 2). HsCRP decreased significantly in both training groups, and IL-6 and D-dimer in the brisk walk group only. Changes from BL did not differ between training groups.

*** Figure 2 here ***

DISCUSSION

This pilot study explored the efficacy of a 3-day per week, 12-week program of brisk walking, with or without strength exercise in order to promote a recreational kind of race walking as physical activity in HIV infected people. In parallel with improvement of physical fitness and of some morphometric measures, we observed substantial improvements of cholesterol profiles and inflammatory markers.

This study showed that a 12-week exercise program was feasible and associated with acceptable discontinuation rate (24%) and adherence (67%), likely favoured by coach supervision during all sessions [3]. Our results are in agreement with the systematic review of Gomes et al. [9] that indicated that dropout rate could vary from 4% to 54%. Our good discontinuation rate was related with the fact that all sessions were completely supervised by professional coaches and training was tailored on functional capacity and individual symptoms presented by each patient. In addition, none of the participants experienced physical injuries or other medical problems directly related to exercise. In addition, this type of physical activity was well tolerated from the immune system as indicated by CD4+, which is the common marker to indicate the severity of HIV infection.

At W12 we observed a significant improvement of physical fitness as indicated with a longer distance walked during 6MWT. An improvement of physical fitness is associated with a decrease of all-cause mortality in the general population. In fact, exercise and physical activity decrease the risk of developing CVD, stroke, type-2 diabetes, and some forms of cancer [1]. Our results indicated this type of supervised physical activity could be a prophylactic intervention to reduce the incidence of chronic degenerative diseases also in this population.

HR_{mean} during the training session was prescribed in the so-called fat-burning zone, and then the aim was to observe a reduction of body weight. BMI and waist circumference reductions were less marked in the strength-brisk walk group participants, despite they brisk walked longer distances than those in the brisk walking group, likely resulting from increase of muscle mass. Indeed, there was a median increase of lean mass of 1.35 Kg by DEXA examination in the strenght-brisk walk group, differently from what observed in the brisk walk group, in which no substantial change of lean mass was observed.

From a clinical standpoint, a remarkable observation was the general reduction of total and LDL cholesterol in both training groups. Total, HDL and LDL cholesterol are each independent strong predictors of CVD in the general population and elevated LDL is the primary target for cholesterol-lowering therapy [10, 11]. Only a few studies have examined the effects of exercise on blood lipids in HIV infection, with inconsistent outcomes, likely resulting from large variability of populations and exercise interventions [12-16]. Our findings indicate that brisk walking as moderate exercise may reduce blood cholesterol in HIV infection, supporting exercise interventions including prior to use of cholesterol-lowering drugs [17].

At the end of the training program we assessed an effect of exercise on plasma hsCRP, IL-6 and IL-18. In addition, we disclose a beneficial effect of exercise on plasma D-dimer. In contrast to the above markers, we did not observe changes of sCD14, a microbial translocation marker and independent predictor of mortality in chronic HIV infection [18]. cART-controlled chronic HIV infection is associated with increased inflammation and coagulation [20-22], and higher plasma levels of hsCRP, IL-6 and D-dimer strongly predicted higher overall mortality and cardiovascular events [23, 24]. In particular we observed that subjects with hsCRP above 2 mg/L, i.e., the value

considered to confer higher risk for cardiovascular disease in the general population, were 13 of 25 (52%) at BL and 8 of 25 (32%) after 12 week of exercise. The anti-inflammatory effect of exercise in HIV infection has been rarely addressed in clinical studies. Reductions of hsCRP, IL-6, and IL-18, a cytokine released by adipocytes and other cell types, were observed in a 16-week study of aerobic or resistance training performed at variable intensity [19-21], but no of IL-6 after 6 weeks of aerobic plus resistance moderate intensity exercise. More in general, two main mechanisms have been suggested to mediate the effect of exercise on inflammation. First, the reduction of fat mass following physical activity may promote an anti-inflammatory environment via reduced infiltration of immune cells in the adipose tissue and release of adipokines, including pro-inflammatory cytokines [20-24]. In addition, contracting skeletal muscle secretes molecules with immunomodulatory effects, including the so-called myokines, most notably IL-6, which mediates metabolic changes during exercise. While single bouts of exercise induce an increase of IL-6 and other cytokines, regular exercise with repeated bouts may induce an anti-inflammatory environment, with lower basal levels of inflammatory markers over time [21]. Compared to the general population, inflammation in HIV infection may be caused or enhanced by specific conditions, including persisting low-level HIV replication, chronic co-infections, and ART-induced altered lipid and metabolic profiles [5], suggesting that additional mechanisms may mediate and perhaps enhance the effects of exercise on inflammation.

CONCLUSION

The practice of brisk walking was feasible and associated with acceptable discontinuation rate and adherence and it was associated with significant improvement of cholesterol profile and soluble and cell inflammatory markers in sedentary patients with treated HIV infection and metabolic problems.

RECOMMENDATIONS

Like for the general population a comprehensive program of exercise including cardiorespiratory and/or resistance exercises of sufficient volume and target intensity for HIV cART-treated people should carry out. The exercise prescriptions have to be adjusted according to the individual responses because of the considerable individual variability in the response to an exercise program. We would like to give some advice to perform physical activity in HIV infected subjects. Generally, before staring training program patients have to assess a general health check-up with a consultation with a health professional and diagnostic exercise testing as medically indicated. Moreover, should be bringing attention to several elements with exercise prescription including a proper warming-up, cooling down, a gradual progression of exercise, volume, intensity ad proper training technique. It is important that these parameters are properly defined because exercise training should be enjoyable and not represent an additional source of stress that could be added to the professional life. To be effective training should be practiced everyday with regularity and constancy. Therefore, general guidelines for aerobic exercise and resistance training exercises are presented in (Table 6). As we demonstrated in our study, the supervision of experienced fitness professionals can enhance the adherence to exercise.

Table 6 here

Figures, Tables and References are in APPENDICES