Concurrent training remodels the subcutaneous adipose tissue extracellular matrix of people living with HIV: a non-randomized clinical trial

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Concurrent training remodels the subcutaneous adipose tissue extracellular matrix of people living with HIV

a non-randomized clinical trial

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ABSTRACT

Evaluate the effect of 12wks of concurrent training (CT) in extracellular matrix (ECM) of subcutaneous adipose tissue (SAT) in people living with HIV/aids (PLWHA). To the non-randomized clinical trial 19 participants, 11 healthy (HIV-) and 18 PLWHA under the use of highly active antiretroviral therapy (HAART) for at least 1 year (HIV+). All participants engaged in a moderate-intensity CT program for 12 weeks, three times a week. Before and after CT, aerobic and strength performance were assessed, as well as anthropometry and biochemical blood profile. Also, SAT biopsies were carried out for histologic and morphometric analysis. The statistical analysis was carried out with R Studio, using descriptive and inferential analysis, ANOVA test and mixed-effect model were utilized ($P<0.05$). HIV+ showed higher levels of VLDL, TGL, and lower levels of HDL in baseline than HIV- ($P<0.05$). All groups improved aerobic and strength performance ($P<0.05$). Both groups presented reduced adipocyte sizes after CT ($P<0.05$). Lastly, HIV+ presented smaller adipocytes and higher elastic fiber deposition in baseline and decreased after training only in HIV+, matching similarly to HIV- group. Thus, CT in PLWHA promoted a decrease in size heterogeneity of adipocytes and elastic fiber deposition, remodeling ECM and improving SAT fibrosis profile.

Brazilian Clinical Trials Registry (UTN: U1111-1214-3022)

Novelty

- **Adipose tissue fibrosis is improved by training in people living with HIV.**
- **Concurrent training remodels adipose tissue extracellular matrix.**

Keywords: HIV; AIDS; Adipocyte; Fibrosis; Collagen; Exercise.

Draft

INTRODUCTION

The advance in the understanding of the Human Immunodeficiency Virus (HIV) infection and the treatment of the Acquired Immune Deficiency Syndrome (AIDS) culminated with the introduction of systematic use of the highly active antiretroviral drugs (HAART), prolonging the life span of people living with HIV/AIDS (PLWHA) (Jain, Ramteke, Raparti, & Kalra, 2012). However, one of the main outcomes of the continuous use of HAART is the surge of a series of metabolic alterations, such as insulin resistance, dyslipidemia, and cardiovascular diseases (Agarwal, Fryns, Auchus, & Garg, 2003; Garg, 2000, 2011; Hussain & Garg, 2016). Also, the regular use of HAART prompts a redistribution of adipose tissue (AT), immunometabolic changes, adipocyte apoptosis (Béréziat et al., 2011; Divoux et al., 2010), and local fibrosis (Silva, Peixoto, Luz, Moraes, & Peres, 2019; Tebas et al., 2014; Villarroya, Domingo, & Giralt, 2005).

Fibrosis is associated with the excessive deposition of intercommunicating membrane structural proteins in the extracellular matrix (ECM) (Batzios, Zafeiriou, & Papakonstantinou, 2013; Divoux et al., 2010). The fibrous elements of ECM represent a vast portion of macromolecules in most tissues, which is divided into collagens and a diverse array of non-collagenous proteins (i. e. elastic fibers). Although they are often thought of as inert structures that provide tensile strength, they also have been associated with pro-and anti-inflammatory responses, regulation in cell adhesion, chemotaxis, and tissue development signals (Batzios et al., 2013; Liu et al., 2016; Sun, Tordjman, Clément, & Scherer, 2013).

On the other hand, the engagement in regular exercise training is an effective strategy to alleviate the deleterious effects elicited by HAART (Garg, 2011; Montoya et al., 2019), improving cardiorespiratory capacity, muscle strength (Pedro et al., 2016), and body composition (lean mass and fat percentage) (Gomes-Neto, Conceição, Oliveira Carvalho, & Brites, 2013; Guariglia et al., 2018). The intervention reported for exercise training in PLWH preferentially adopt aerobic, resistance, or concurrent training protocols (Gomes-Neto et al., 2013) being the last one recognized for improving body composition, decreasing AT mass, and increasing lean mass (Gomes-Neto et al., 2013; Wilson et al., 2012).

In line with the effects of concurrent training, it is well reported in the literature that exercise training promotes profound changes in the architecture of adipose tissue, modulating the number of adipocytes and vascularization (Hatano et al., 2011). Aerobic

training or concurrent training can decrease the percentage of body fat in individuals with obesity (Monteiro et al., 2015). On the other hand, PLWHA under HAART presents a reduced efficiency to store energy as triglycerides and to undergo the differentiation of new adipocytes (adipogenesis). Cellular toxicity imposed by HAART (Hart, Samuels, & Hulgán, 2012) directly affects the respiratory capacity of adipocytes (Cunha, Maselli, Stern, Spada, & Bydlowski, 2015) and inhibits the expression of genes responsible for the differentiation of preadipocytes (Domingo et al., 2014; Giralt et al., 2006). PLWHA may also develop a lipotrophic adipose tissue phenotype characterized by smaller adipocytes and irregular distribution of fat depots compared to non-infected individuals (lipodystrophy) (Bastard et al., 2002).

Therefore, the present clinical trial aimed at investigating the effects of 12 weeks of concurrent training on AT remodeling in PLWHA under HAART.

METHODS

Experimental design

The present report is a non-randomized, parallel, two arms clinical trial that compared the effects of concurrent training in adults living with HIV/AIDS undergoing HAART, with a control exercise group. The experimental approach followed the guidelines described by the CONSORT statement for nonpharmacological treatment interventions (Boutron, Moher, Altman, Schulz, & Ravaud, 2008a, 2008b). The professional involved in group selection was not the same included in assessment and intervention, enabling a reduction in the risk of bias.

Subjects

The present study was approved by the Ethical Committee in Human Research of the State University of Maringá (n: 910.562). The recruitment of all participants was conducted at the Specialized Attendance Service in HIV/aids (SASHIV) by direct invitation to participate in the study. All participants who agreed to participate in the study provided a written consent form. The control group was composed of sedentary non-seropositive individuals that participated in any attendance of SASHIV and showed interest in starting a physical exercise program. The intervention was realized at the Center for Excellence in Physical Activity of the State University of Maringá (CEAF/UEM).

Inclusion Criteria

All individuals infected by HIV undergoing HAART for at least one year with correct follow-up of therapy that demonstrated interest in training were included in the sample; and non-HIV-infected (HIV-) not engaged in the regular practice of systematic exercise and without any physical limitations that would impair the realization of the exercises proposed were also included (**Fig. 1**).

Exclusion criteria

- Cardiac disease;
- Systematic physical training during the previous six months;
- Mioarticular disorders that impaired the ability to perform exercise;
- No medical clearance for physical exercise.

Characterization of the sample

All individuals that complied with the demands of the protocol were intentionally distributed into two groups: 1. non-infected (HIV-) and 2. HIV seropositive (HIV+). The groups were characterized for age, height, body composition by bioimpedance (In Body® - R20, Seoul, KO) and blood plasmatic profile (glucose, total cholesterol, high-density lipoproteins (HDL), low-density lipoproteins (LDL), very low-density lipoproteins (VLDL), and triglycerides). The clinical condition of the HIV+ group is provided in table 1.

Blood sample

Blood samples were collected in the morning after 12-hour fasting, with tubes containing EDTA + sodium fluoride and gel clot activator. The samples were kept at room temperature for 2 h before glucose and lipid analysis using an enzymatic automatic method using commercial kits (Abbott®, Lake Forest, USA).

Intervention

The supervised concurrent training (CT) followed the recommendations of the American College of Sports Medicine of volume and intensity, with a frequency of three times per week during twelve weeks and was supervised by specialized instructors (**fig. 2**) (Abreu & Freitas, 2012; Garber et al., 2011; Montoya et al., 2019). The training program was composed of aerobic plus

resistance training; both types were performed in the same training section following the order of 20 minutes of aerobic training plus resistance training during all weeks of training. The intensity of aerobic training was controlled using a rating of perceived effort (RPE) scale (BORG, 1992), with each section lasting 20 minutes. The individuals were oriented to keeping the treadmill speed equivalent to a perceived effort ranging from 13-15 on the scale (Foster et al., 2001).

The resistance training was designed to target the major muscle groups and was composed of the following exercises: 1) barbell bench press; 2) lat pull down; 3) barbell shoulder press (SP); 4) deadlift (DL); 5) leg press 45°; 6) seated leg curl; and 7) sit-ups. The participants performed three sets of 10-12 maximal repetitions for each exercise and the load adjustments were done weekly following the 12 maximal repetition protocol described by Pedro et al. (Pedro et al., 2016). During the experimental protocol, the participants were familiarized with the routine of exercises for 2 weeks.

Performance analysis

The aerobic performance was assessed by an incremental peak velocity (V_{peak}) test carried on a treadmill. The test began at 7 km.h⁻¹ with increments of 1 km.h⁻¹ every 3 minutes, after a previous warm-up of 5 minutes at a speed of 5 km.h⁻¹, as described by Machado et al. (Machado, Higino, Peserico, Mezzaroba, & Moraes, 2011). The strength performance was assessed by determining the total weight lifted by participants in two exercises, lower limbs (DL) and upper limbs (SP), estimated by the sum of the total of kilograms lifted in all repetitions of each exercise as described by Tan (Tan, 1999).

Adipose tissue biopsies

After asepsis of the skin, the suprailiac region was anesthetized with 2% lidocaine hydrochloride plus epinephrine. A small incision of 10 mm in the skin allowed the removal of approximately 300 mg of subcutaneous adipose tissue. The material collected was rinsed in saline solution and immediately transferred to a tube containing HistoChoice® (Amresco, Solon, USA) for histological analysis.

Histological analysis

Adipose tissue samples fixed with HistoChoice® for 3 hours were kept in ethylic alcohol (70%) until histologic processing for inclusion in paraffin. The semi-serial histological sections of 5 μm were stained with: (a) Hematoxylin and Eosin (HE) to determine adipocyte area or (b) Sirius Red F3B200 (0.1%) to determine total collagen type fiber or (c) Weigert/VanGieson to determine the elastic fiber area. The images of histologic sections were captured with a picture system (Nikon® digital Sight DS-Fi1c, Tokyo, Japan) coupled to a microscope Nikon® Eclipse E110 (Tokyo, Japan).

Adipocyte area: 10 images of each section stained with HE were captured in a 20x objective to determine adipocyte area (μm^2) (Image-Pro Plus®, Cybernetics®, Silver Spring, Maryland, USA). Eight to twelve adipocytes were measured in each field, totalizing one hundred cells. Besides, the adipocytes measured were organized in the class range to identify the predictive effect of health and training as described in statistical analysis.

Qualitative analysis of the fibrotic status of adipose tissue: the occurrence of fibrosis in adipose tissue was determined by the area occupied by total collagen, collagen type I, and collagen type III as mentioned in "*adipocyte area*" (10 images by sample). The analysis of collagen was realized under light microscopy (polarized light), with a 20x objective. Classification of type I (orange to red) and type III (green to yellow) collagen was determined by its birefringence (Cuttle et al., 2005). To determine the morphometric analysis of the occupied area by collagen, standardized images of 277.190 μm^2 were captured and digitalized by software Image-Pro Plus® (Media Cybernetics®, Silver Spring, Maryland, USA). The stained areas were identified and quantified automatically, and the results were expressed as mean (μm^2) $\pm$ standard deviation. To evaluate the elastic fibers, content tissue sections were stained with the Elastica Weigert/ van Gieson staining kit for connective tissue (Merck, Buchs, Switzerland) according to the manufacturer's protocol and was determined equally in a standard procedure as well as morphometric analysis.

Real time RT-PCR

Collagen I (COL I) and III (COL III) gene expression were determined in subcutaneous adipose tissue by PCR-real time. The mRNA quantification of the COL I and COL III was realized using SYBR Green PCR Master mix reagent (AppliedBiosystems, Foster City, CA), the specific pairs of primers (direct and reverse) for the target genes, and the ABI PRISM 7700 sequence detection System (AppliedBiosystems, Foster City, CA, EUA). The gene RPL19 was used as an internal

control (housekeeping) for the normalization of samples. The relative expression of data was calculated by the delta-delta CT method ($\Delta\Delta^{CT}$) (Livak & Schmittgen, 2001).

Statistical analyses

Data analysis was performed by the statistical software R Studio, version 3.3.5. The *Shapiro-Wilk* test was applied to determine the normality of data, and the *Independent Student T-test* to establish comparisons between groups in the baseline condition. *Analysis of Variance (ANOVA)* for *repeated measures* was used to compare the conditions (groups *versus* moments), considering the assumptions of homogeneity of the variances (*Levene's test*) and equality of matrices of covariance (Box M test) followed by *Bonferroni's post hoc*. The *Sturges equation* was used to categorize adipocyte size frequencies distributed in 13 class ranges ($i \approx 1 + 3.3 \cdot \log n$) determined by the amplitude of adipose tissue size data ($h \approx \frac{TA}{i}$). Finally, the mixed effect model for predictive analysis of the serological status and training time in the class range, as well as, the previous assumptions and statistical significance were adopted ($P < 0.05$).

RESULTS

General characteristics of participants

The characteristics of all participants are shown in table 1. The HIV+ group showed lower levels of HDL and higher levels of VLDL and triglycerides ($P < 0.05$) compared to the HIV- group. The clinical profile did not show any case of immunosuppression grade for the HIV+ group ($CD4 > 500$ cells/mm³). The clinical stage can be determined using the CD4 test to determine the degree of immunocompromise, where < 500 cells/mm³ represents significant immunosuppression for HIV adults and adolescents (World Health Organization (WHO), 2005). The mean time of aids diagnostic was 61.63 months and HAART utilization was 53.40 months.

Performance of concurrent training

The aerobic performance of HIV- and HIV+ groups during the training period was determined by the V_{peak} test (**fig. 3, A**). After 12 weeks, both groups improved aerobic capacity by the same magnitude (training: $F = 20.44$, $P < 0.05$) (**fig. 3, B**).

To analyze the efficiency of the strength training we measured the total weight lift (TWL) during the execution of a lower member exercise (DL) (training: $F = 52.28$, $P < 0.0001$) and an upper member exercise (SP) (training: $F = 26.52$, $P < 0.0001$) (**fig. 3, C e D**). Both groups showed an increase in TWL between the first and the third week of

training (DL: HIV-, 544.0 ± 98.8 kg vs. 1386.6 ± 358.8 kg; HIV+, 478.2 ± 76.0 kg vs. 1534.2 ± 423.9 kg, $P < 0.0001$. SP: HIV-, 248.0 ± 27.7 kg vs. 537.5 ± 194.8 kg; HIV+, 220.4 ± 58.6 kg vs. 614.2 ± 162.4 kg) and first week vs. twelfth week (DL: HIV-, 544 ± 98.8 kg vs. 2042.0 ± 522.3 ; HIV+, 478.2 ± 76.0 kg vs. 1875.5 ± 547.4 kg, $P < 0.0001$. SP: HIV-, 248.0 ± 27.7 kg vs. 719.5 ± 293.2 kg; HIV+, 220.4 ± 58.6 kg vs. 724.4 ± 195.1 kg), but only the HIV- group showed a significant increase in the third week vs. twelfth week (DL, $1386.6 \pm$ vs. $2042.0 \pm$ and SP, 614.2 ± 162.4 kg vs. 724.4 ± 195.7 kg, $P < 0.05$). However, between the third vs. tenth week, the HIV+ group significantly improved TWL in DL (1534.2 ± 423.9 kg vs. 2028.0 ± 362.6 , $P < 0.05$). We did not find any differences between the groups.

Adipocyte area

The mean adipocyte area and the different mean ranges before and after training in both HIV- and HIV+ groups are presented in **fig. 4, B**. Adipocyte area decreased in both groups after 12 weeks of training (training: $F = 5.011$; HIV-: $6917.2 \pm 1082.0 \mu\text{m}^2$ before vs. $4808.8 \pm 1461.2 \mu\text{m}^2$ after training, $P < 0.05$; HIV+: $5275.9 \pm 1602.1 \mu\text{m}^2$ before vs. $4132.1 \pm 1164.7 \mu\text{m}^2$ after training, $P < 0.05$). The analysis of adipocyte size showed a significant difference between HIV- and HIV+ (groups: $F = 22.73$, $P < 0.0002$). In addition, the analysis of predictive heterogeneity of adipocytes in class ranges (**fig. 4, C**) demonstrated that the infection caused by HIV, proportionally predispose the HIV+ group before training to present a heterogeneous distribution of smaller adipocytes compared to HIV- ($\beta_{\text{exp}} = -1620.93$; $\sigma = 515.11$; $P < 0.05$) (**fig. 4, A: 1-3**). Time of intervention also predisposes both groups to decrease the size of the adipocytes by frequency of class range ($\beta_{\text{exp}} = -2054.265$; $\sigma = 94.99$; $P < 0.05$) (**fig. 4-C**). However, *post hoc* analysis of mean adipocyte size comparing groups (HIV- vs. HIV+) was significant only before the training ($P < 0.05$).

Collagen analysis

The quantification of total collagen, collagen type I, and type III subtypes, presented in **figure 5 - B, C, and D**, did not show any statistical differences. However, the HIV- group showed a significant decrease in collagen III: I ratio after training (**fig 5, E**) (training: $F = 4.589$; $P < 0.05$; HIV-: 0.801 ± 0.466 AU before vs. 0.462 ± 0.389 AU after training: $P < 0.05$).

Elastic fiber analysis

The quantification of elastic fibers is presented in **fig. 6 (A and B)**. Before the training program, the HIV+ group showed a higher deposition of elastic fibers compared

to HIV- (interaction: $F= 33.3$, $P<0.05$; Groups: $F= 15.2$, $P<0.05$; $153.7\pm48.5\mu\text{m}^2$ before HIV- vs. $447.7\pm90.2\mu\text{m}^2$ before HIV+: $P<0.05$). After training, the area occupied by the elastic fibers decreased in HIV+ when compared to baseline (training: $F= 27.0$, $P<0.05$; HIV+: $447.7\pm90.2\mu\text{m}^2$ before vs. $242.8\pm112.0\mu\text{m}^2$ after: $P<0.05$), matching the area of the control group.

PCR-Real time

In PCR-Real time for collagen I and III were not observed significant modifications at expression on the target genes for training period (COL I: $F= 0.00$, $P= 0.40$; COL III: $F= 0.03$, $P= 0.85$) and groups comparison (COL I: $F= 0.17$, $P= 0.68$; COL III: $F= 0.05$, $P= 0.82$). The collagen I for HIV- group was 1.89 ± 2.03 -fold before vs. 1.27 ± 1.03 -fold after the training and the HIV+ group showed 1.69 ± 1.84 -fold before vs. 2.18 ± 2.33 -fold after training. In the same view, collagen III for HIV- group showed 1.91 ± 1.99 -fold before vs. 1.11 ± 0.53 -fold after training and for the HIV+ group were 1.06 ± 0.44 -fold before vs. 1.62 ± 1.63 -fold after training.

DISCUSSION

The present study aimed at investigating the effects of concurrent training on adipose tissue remodeling in PLWHA undergoing HAART. In summary, 12 weeks of concurrent training elicited an improvement in aerobic and strength performance, in adipocyte area in both groups reducing the size of adipocytes and making them more homogeneous, and reduction of elastic fibers deposition in SAT of PLWH reducing the fibrosis development. The literature is scarce when assessing the effects of HIV on the remodeling of adipose tissue associated with fibrosis, as observed in our study, which showed an increase in elastic fibers in the SAT may be a pathological grade of fibrosis response. Elastic fibers have been described as the unique mechanical properties that provide flexibility to the tissue and resilience to deformation, and a scaffold for the macrophages of adipose tissue, in addition to metabolic and inflammatory differences between deposits (Martinez-Santibanez et al., 2015).

Considering the general baseline characteristics of PLWHA, lower levels of high-density lipoproteins (HDL), increased levels of very low-density lipoproteins (VLDL) and triglycerides (TGL) were observed, which is in accordance with the literature and the diagnosis of dyslipidemia (National Institutes of Health, 2001). In PLWHA, such metabolic changes are mainly associated with the use of HAART, as virgin therapy patients treated for 48 weeks

with HAART have higher levels of total cholesterol, low-density lipoprotein (LDL), APO A-I, TGL, and HDL (Saumoy et al., 2014). The genetic component of dyslipidemia accounts for a lower proportion of cases, as it is an APOCIII gene polymorphism (Cunha, Morganti, Maselli, Bydlowski, & Spada, 2015).

Concurrent training is characterized by a combination of resistance and aerobic training and is recognized as an efficient model to decrease body fat, improving dyslipidemia, and reducing cardiovascular diseases (Gomes-Neto et al., 2013; Monteiro et al., 2015; Simonik et al., 2016). Previously published data demonstrated the effectiveness of concurrent training in improving muscle strength and aerobic capacity in PLWHA (Pedro et al., 2016). Several studies have shown that chronic use of HAART affects not only the immune system, but also the musculoskeletal, endocrine, cardiovascular system among others, decreasing the ability to perform activities of daily living including exercise (Chaparro et al., 2018; Gomes-Neto et al., 2013; Grace, Semple, & Combrink, 2015; Ibeneme et al., 2019). In our work, both HIV- and HIV + showed similar cardiorespiratory fitness and strength in the initial tests, but after 2 weeks of simultaneous training, strength and aerobic performance improved, as seen in fig. 03. Concurrent training, when applied to sedentary PLWHA, produced similar responses for uninfected sedentary people, in addition to improving physical fitness.

Interestingly, in our study, we observed the presence of a higher number of smaller adipocytes and a heterogeneous distribution of cells in PLWHA compared to the control group, which is suggestive of the development of lipodystrophy (fig. 4 – A, 3-4, and C). The lipodystrophy in HIV people is characterized after approximately 2 to 4 years of HAART use consisting of IP and INTR drugs that may induce the loss of subcutaneous adipose tissue and fibrosis, reduced potential of adipocyte differentiation and storage capacity (Gorwood et al., 2019; Hussain & Garg, 2016; Sun et al., 2013). Finally, after training the distribution of adipocytes in PLWHA became more homogenous, and with adipocytes of smaller sizes compared to the baseline condition. This condition represents a favorable remodeling of adipose tissue where adipocytes acquire a phenotype associated with a better metabolic profile (Segatto et al., 2011).

One of the several outcomes of obesity is the drastic increase in the content of connective fibers, mainly collagen (Khan et al., 2009) and in the content of elastic fibers, both in AT (DeMarsilis et al., 2014). The condition increases the stiffness of AT, characterizing a fibrotic status (Khan et al., 2009), which correlates positively with the BMI and negatively with insulin sensitivity (DeMarsilis et al., 2014; Spencer et al., 2010). Concomitantly, a similar scenario is found in PLWHA and is associated with inhibition of

adipogenesis, decreased adipocyte size, fibrosis, and increased apoptosis (Pérez-Matute, Pérez-Martínez, Blanco, & Oteo, 2013). Our work confirmed this pathologic condition of adipose tissue in the HIV+ group, where we observed a considerable deposition of elastic fibers.

The main hypothesis of our work was based on the concept that concurrent training would be an efficient non-pharmacological tool to revert or ameliorate AT fibrosis in PLWHA. Concurrent training decreased the deposition of elastic fibers in the AT of the HIV+ group, therefore contributing to the reduction of fibrosis. Interestingly, Ezure and Amano (2015) have reported a negative correlation of the elastic fibers in the human dermis with adipocyte size and BMI. Concomitantly, the increment of subcutaneous adipose tissue was associated with the decrease of dermal elastic fibers in the human obese abdominal skin.

Finally, concurrent training did not promote a significant modification in total, type I or III collagen deposition in the HIV+ group, including mRNA expression (**Fig. 5**). Only the control group (HIV-) seemed to respond to exercise training, as denoted by a lower collagen stimulation (collagen III: I ratio) in AT. It is conceivable that a longer period of training engagement could promote a deeper remodeling of AT, which would possibly also affect collagen as well other ECM structures.

The current study presented some limitations such as the absence of a control group without exercise, which would highlight the efficacy of the training protocol. Also, the role played by macrophages, adipokines, adipogenic factors, metalloproteases and their relationship with the modifications caused by training in HIV patients would shed some light on the mechanisms involved in ECM fibrosis.

CONCLUSION

In summary, concurrent training in PLWHA improved physical fitness and promoted a decrease in the heterogeneity of the size of the adipocytes and the deposition of elastic fiber, improving adipose tissue stiffness and fibrosis profile.

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Conflicts of interest

No conflict of interest was declared.

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Word count: 719

Figure 1. Flow chart of the study.

Figure 2. Representative timeline during 12 weeks of concurrent training intervention (CT) for HIV- and HIV+ groups. IA= initial assessment; FA= final assessment.

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Figure 3: Performance of concurrent training. (A) Comparison between the V_{peak} (Km.h⁻¹) before and after concurrent training for HIV- (n=13) and HIV+ (n=10). (B) V_{peak} (%) average difference before and after training by for both groups. (C) Evolution of total load lifted over 12 weeks of training for Dead Lift (DL) and (D) Shoulder Press (SP) . *Statistical difference in HIV- before x after (P<0.05). **Statistical difference in HIV+ before x after (P<0.05). In C and D, the letters are symbolizing the statistical difference in the respective line (a≠b≠c; P<0,05). The letters in black are symbolizing time effect in HIV- group and the letters in red symbolizing time effect in HIV+ group. Data are shown as mean ± SD. There is not reported statistical differences in groups analysis in any cut points.

Figure 4: Adipocyte area (staining: *Haematoxylin and Eosin*). (A) Photomicrograph of adipocytes HIV- before and HIV- after training; HIV+ before and HIV+ after training (200x magnification). (B) Average adipocyte area before and after training. (C) Histogram of absolute frequencies of the different class ranges (μm^2). Data are shown as mean $\pm$ SD. HIV- (n=11) and HIV+ (n=8). Mixed liner model: interaction ($\beta = 6913.71$, $\sigma = 334.08$, $P < 0.0001$); HIV ($\beta = -1620.93$, $\sigma = 515.11$, $P < 0.001$); training ($\beta = -2054.16$, $\sigma = 94.99$, $P < 0.0001$). **Post hoc* statistical difference in HIV- before x after ($P < 0.05$). ***Post hoc* statistical difference in HIV+ before x after ($P < 0.05$). #*Post hoc* statistical difference in Before HIV- x HIV+ ($P < 0.05$). Letters represent statistical differences of each respective line ($a \neq b \neq c \neq d$, $P < 0.05$).

HIV+ Before

HIV+ After

Figure 5: Collagen analysis (staining: *Sirius Red*). Photomicrograph of adipose tissue from HIV + and HIV- individuals before and after training. Under polarization, note the fibrosis (white arrows), collagen type I (red arrows), collagen type III (green arrows) and non-polarized image (inset). (B) Area occupied by collagen III. (C) Area occupied by collagen I. (D) Area occupied by total collagen. (E) Collagen III to I ratio (III:I). (F) Collagen I (COL I) and III (COL III) mRNA expression ($\Delta\Delta_{ct}$ Method). *Statistical difference in HIV-before x after (Interaction: $F=1.957$, $P<0.05$; Training: $F=4.589$, $P<0.05$). Data are shown as mean $\pm$ SD. HIV- ($n=11$) and HIV+ ($n=8$).

Figure 6: Elastic fiber analysis. (A) Photomicrograph of adipocyte of subcutaneous adipose tissue stained for elastic fibers. The elastic fibers appear stained in black by Weigert staining, and some fibers are indicated by the arrowheads. (staining: Weigert/Van Gieson). (B) Area occupied by elastic fibers in SAT for HIV- (1 and 2) and HIV+ (3 and 4) before and after concurrent training, respectively. Note the elastic fibers remodeling after concurrent training in HIV+ group (black arrowheads). Statistical difference between HIV- before X HIV+before (interaction: $F_{33.3}$, $P < 0.05$; Groups: $F = 15.24$, $P < 0.05$); **Statistical difference between HIV+ before X after (Training: $F = 27.09$, $P < 0.05$). Data are shown as mean $\pm$ SD. HIV- ($n = 11$) and HIV+ ($n = 8$).

Table 1. Baseline general characteristics, biochemical and clinical profile of the participants who completed the study.

	HIV - n=13 (♂ = 6)	HIV + n=10 (♂ = 7)	Δ%	P
GENERAL CHARACTERISTICS				
Age (years)	45.31±8.15	40.10±6.57	-11.50	0.155
Height (m)	1.66±0.11	1.71±0.07	3.01	0.411
Weight (kg)	73.47±11.87	71.39±8.71	-2.83	0.608
BMI (kg.m ⁻²)	26.62±3.43	24.42±3.52	-8.26	0.241
Body Fat (%)	27.18±6.18	26.08±7.55	-4.05	0.480
BLOOD BIOCHEMICAL PARAMETERS (mg/dl)				
Glucose	89.08±8.19	87.36±6.17	-1.93	0.795
Total Cholesterol	198.80±27.71	185.60±39.96	-6.64	0.440
HDL	44.92±10.74	33.80±5.18	-24.76	0.017*
LDL	134.5±22.39	117.20±34.34	-12.86	0.347
VLDL	19.38±8.54	32.33±12.86	66.82	0.028*
TGL	97.00±42.64	185.9 ±98.64	91.65	0.026*
CLINICAL PROFILE (months)				
Diagnosis time	-	61.63		
Treatment time	-	53.40		
CD4 count (cell/mm ³)	-	530.80±126.40		
HAART TYPE - n(%)				
NRTI	-	10(100%)		
NNRTI	-	5(50%)		
PI	-	6(60%)		
II	-	1(10%)		
VIRAL CHARGE OF HIV n(%)				
Not detectable	-	6 (60%)		

BMI=Body Mass Index, HDL=High Density Lipoprotein, LDL=Low Density Lipoprotein, VLDL=Very Low Density Lipoprotein, TGL=Triglycerides, HIV=Human Immunodeficiency Virus, HAART=Highly Active Antiretroviral Therapy, NRTI=Nucleoside Reverse Transcriptase Inhibitors, NNRTI=Non-Nucleoside Reverse Transcriptase Inhibitors, IP=Protease Inhibitors, II=Integrase Inhibitors, CD4=Auxiliary T Lymphocytes; m=Meter, kg=kilo, kg/m²=kilogram/meter², %G=Fat percentage, mg/dl=milligram/deciliter, n(%)=number(Percentage), Δ%=percentage difference. *P<0.05. Data are shown as mean ± SD.

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