Homocysteine-lowering exercise effect is greater in hyperhomocysteinemic people living with HIV: A randomized clinical trial

<table>
<thead>
<tr>
<th>Journal:</th>
<th>Applied Physiology, Nutrition, and Metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID</td>
<td>apnm-2018-0734.R1</td>
</tr>
<tr>
<td>Manuscript Type:</td>
<td>Article</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>30-Jan-2019</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
<td>Oliveira, Vitor; Universidade Estadual de Londrina, Rosa, Flavia; Universidade Filadélfia Wiechmann, Susana; Universidade Estadual de Londrina Narciso, Argéria; Universidade Estadual de Londrina Webel, Allison; Case Western Reserve University Franzói de Moraes, Solange; University State of Maringá, Human Physiology Department Deminice, Rafael; Department of Physical Education, Faculty of Physical Education and Sport, State University of Londrina, Londrina, PR, Brazil., Physical Education</td>
</tr>
<tr>
<td>Keyword:</td>
<td>Folate, Antioxidants, Lipid Peroxides, Reactive Oxygen Species, AIDS Virus, resistance training &lt; exercise</td>
</tr>
<tr>
<td>Is the invited manuscript for consideration in a Special Issue? :</td>
<td>Not applicable (regular submission)</td>
</tr>
</tbody>
</table>
Homocysteine-lowering exercise effect is greater in hyperhomocysteinemic people living with HIV: A randomized clinical trial

Vitor HF Oliveira¹; Flávia Troncon Rosa²; Susana Wiechmann³; Argéria Maria Serraglio Narciso³; Allison R Webel⁴; Solange Marta Franzói de Moraes⁵; Rafael Deminice¹

Vitor Hugo Fernando Oliveira¹; vitorhfo@hotmail.com
Flávia Troncon Rosa²; flaviatrosa@yahoo.com.br
Susana Lilian Wiechmann³; swiechmann@sercomtel.com.br
Argéria Maria Serraglio Narciso³; argeria@sercomtel.com.br
Allison Webel⁴; arw72@case.edu
Solange Marta Franzói de Moraes⁵; smfmoraes@gmail.com
Rafael Deminice¹; rdeminice@uel.br

¹ Department of Physical Education, State University of Londrina, Londrina, PR, Brazil.
² Filadélfiia University, Londrina, PR, Brazil
³ University Hospital, Institute of Health Science, State University of Londrina, Londrina, PR, Brazil.
⁴ Frances Payne Bolton School of Nursing, Case Western Reserve University, Cleveland, Ohio, United States.
⁵ Department of Physiology, State University of Maringá, Maringá, PR, Brazil.

*Corresponding Author: Rafael Deminice, Department of Physical Education, Faculty of Physical Education and Sport - State University of Londrina. Rodovia Celso Garcia Cid | Pr 445 Km 380 | Campus Universitário, Londrina, Paraná, Brazil. Phone: 05543 36024564, e-mail: rdeminice@uel.br
ABSTRACT

Elevated concentration of homocysteine has been identified as an independent risk factor for the development of cardiovascular disease and is frequently associated with oxidative stress. Moreover, studies have shown that people living with HIV (PLHIV) present elevated concentration of homocysteine and oxidative stress compared to people without HIV. Our purpose was to describe blood homocysteine and oxidative stress markers in PLHIV and those without HIV infection, and to examine the effects of a 16-week combined training exercise program (CTE) on oxidative stress and homocysteine concentrations of PLHIV. We included 49 PLHIV (21 men, 28 women) and 33 people without HIV infection (13 men, 20 women). After baseline evaluations, 30 PLHIV were randomized to either CTE (trained group TG, n=18) or the control group (CG, n=12); CTE consisted of aerobic and strength exercise sessions during 16 weeks, three times a week. Plasma homocysteine, oxidative damage markers, folate and vitamin B12 were assessed pre- and post-training and by hyperhomocysteinemia (homocysteine≥15 µmol/L) status. At baseline, PLHIV had higher levels of homocysteine and malondialdehyde, as well as reduced circulating folate when compared to people without HIV infection. CTE resulted in a 32% reduction ($p<0.05$) in homocysteine concentration and a reduction in lipid hydroperoxide in PLHIV with hyperhomocysteinemia which was not observed in those without hyperhomocysteinemia. Hyperhomocysteinemic participants experienced a 5.6±3.2 µmol/L reduction in homocysteine after CTE. In summary, sixteen weeks of CTE was able to decrease elevated homocysteine concentration and enhance redox balance of PLHIV with hyperhomocysteinemia, which could improve their cardiovascular risk.

Keywords: Folate, Antioxidants, Lipid Peroxides, Reactive Oxygen Species, AIDS Virus, Resistance Training.
INTRODUCTION

The Human Immunodeficiency Virus (HIV) is the etiological agent of Acquired Immune Deficiency Syndrome (AIDS), a chronic and progressive disease that weakens the immune system, leaving one susceptible to opportunistic infections. Antiretroviral therapy (ART) can restore immune function and prevent HIV replication (Hogg et al. 2008). Advancements in ART has led to fewer AIDS-related deaths, but a corresponding increased incidence of mortality from chronic diseases including cardiovascular disease (CVD) (Smit et al. 2015; Eyawo et al. 2017). Indeed, ART turned a fatal disease into a chronic and manageable, although not yet curable condition.

In the past few years, elevated concentrations of plasmatic homocysteine (Hcy) in the blood have been identified as an independent risk factor for the development of CVD (Lentz 2005; Steed and Tyagi 2011). Several studies have also related hyperhomocysteinemia (HHcy) to neurodegenerative disease, diabetes mellitus, renal dysfunction, fatty liver disease, and others (Hu et al. 2013; Zhang et al. 2014; Kumar et al. 2017). Because of its pro-oxidant activity, HHcy is frequently associated with oxidative stress and damage (Brosnan and Brosnan 2006; Glesby et al. 2009); this pro-oxidant activity may explain the relationship between HHcy and the numerous diseases mentioned before. Recently, studies have shown prevalence of mild to moderate HHcy (Deminice et al. 2015) and oxidative stress (Mandas et al. 2009; Vassimon et al. 2010; Blas-Garcia et al. 2011) in PLHIV. Studies from our laboratory previously demonstrated elevated plasma Hcy and oxidative damage markers in PLHIV compared to people without HIV infection (Deminice et al. 2015). We also demonstrated that lipodystrophy in men living with HIV exacerbates disturbances in Hcy metabolism and oxidative stress due long-term ART usage (Vassimon et al. 2010).

Exercise has been recognized as an important tool to prevent and to treat cardiovascular and other diseases. However, few studies have examined the effects of exercise training on Hcy levels. A recent meta-analysis demonstrated that strength, but not aerobic exercise training, decreases Hcy levels in people without HIV infection (Deminice et al. 2016). Among PLHIV, physical exercise has beneficial effects on cardiorespiratory fitness, muscle strength, and overall health (Gomes-Neto et al. 2013; Gomes Neto et al.
2015). However, whether resistance exercise mitigates HHcy and oxidative stress in PLHIV taking ART is unknown. Thus, we aimed to 1) compare blood Hcy and oxidative stress markers in PLHIV and people without HIV infection and 2) evaluate the effects of a 16-week combined training exercise program (CTE) on oxidative stress and elevated Hcy plasma concentrations of PLHIV under ART. We hypothesized that CTE may mitigate elevated oxidative damage and HHcy in PLHIV under ART.

METHODS

Participants

The study included 49 PLHIV (21 men and 28 women; mean age 44.7 ± 7.5 years; body mass index 26.2 ± 5.9 kg/m²) that were recruited at the Hospital of the State University of Londrina and the Integrated Center for Infectious Diseases of Londrina, Brazil. To be included participants had to be between 18 and 60 years of age and be on ART for at least one year. We excluded those who reported participating in a physical exercise program in the last six months, used hormones or anabolic steroids, those who had a systemic infection within 30 days prior to the start of testing, or any other medical contraindication.

We also enrolled 33 people without HIV infection meeting the same eligibility criteria, keeping the same proportion of sexes and in the same age and body mass index range of the PLHIV group (13 men and 20 women; mean age 47.1 ± 10.2 years; body mass index 26.2 ± 4.5 kg/m²) who were recruited among university employees. People without HIV infection were used for baseline comparisons and were not randomized to the exercise training program.

After the baseline assessment, PLHIV were then invited to enroll in a CTE. Forty-six participants agreed to participate and were randomized to either a CTE intervention or an attention control, and 30 completed the intervention (trained group, TG, n=17; control group, CG, n=13) and were included in the analysis. The main drop-out causes were related to personal and professional issues, health problems not related to the exercise training and moving to another city. Figure 1 presents a flow diagram of participant recruitment and allocation. The study was conducted at the State University of Londrina and was initiated only after approval by the Ethics Committee for Research Involving Human Subjects of this university (#319.837) and Clinical Trials registering (#RBR-7hf8jw). All participants completed written informed consent.
Experimental design

After initial contact at health services where they were attended, PLHIV were invited to enroll in a CTE program at the Physical Education and Sports Center of the University. Those agreeing to participate were randomized to either the trained group (TG), which participated of CTE (aerobic training + strength training) three times a week, or control group (CG), which participated of light recreational sessions two times a week. The randomization codes were generated by a computer, blinded and reveled only during the scheduling of the pre-training tests. We randomized men and women separately, in order to balance the sexes through the groups. The intervention lasted 16 weeks and anthropometric, biochemistry, nutritional and physical fitness evaluations were performed pre (in the week prior the beginning) and post intervention (in the week after).

Combined training exercise protocol

CTE consisted of 16 weeks of aerobic and strength exercises organized in four modules of four weeks each. CTE was chosen because it has been identified as a promising exercise model for PLHIV (Farias et al. 2016) and because it generates cardiovascular and muscle strength adaptations at same time (Garcia et al. 2013; Gomes Neto et al. 2015). CTE was held three times a week on nonconsecutive days, always supervised by experienced exercise physiologists who were responsible for a maximum of three participants. The order of execution (aerobic or strength training) was not controlled, due to the equipment availability.

Aerobic training was performed on a treadmill for 15 min and participants used a heart rate monitor during exercise to ensure that they keep the desired intensity of 50-60% of heart rate reserve (HHR). At week nine, aerobic training was increased in volume and intensity (20 min at 60-65% of HHR), and participants were encouraged to start jogging when it was necessary to keep the desired cardiovascular intensity. The HHR was calculated at the beginning of each stage of the training (weeks 1, 5, 9 and 13) and was used to adjust aerobic training loads. The strength training component initially included the following exercises: bench press, leg press, pulldown, leg curl, arm curl and sit-ups, performed in that order.
week nine, the pulldown and leg curl exercises were replaced by seated cable rows and hack squat, and we modified the order of strength exercise (participants performed lower limbs exercises first and then upper limb exercises) to increase training intensity (Ratamess et al. 2009). Strength training loads were adjusted weekly, based on the results obtained through the application of maximum repetitions tests (Rodrigues and Rocha 2003). Total duration of the session was approximately 50 to 70 min. Participants were instructed to not carry out any other type of exercise during the duration of the study.

During the same time period, the CG participated in a recreational session held twice a week. The sessions were composed of stretching, dancing, walking and recreational activities. This CG was designed to retain participants without promoting significant physical fitness improvements. Heart rate was monitored during the exercise sessions, and the mean heart rate of the CG was 58% of maximal heart rate vs 74% in the TG, demonstrating a greater intensity in the TG activities.

**Medical records**

The medical records were obtained from the health services where the participants were attended and were consulted for the following data extraction: the year of HIV diagnosis; latest measures of HIV viral load, CD4+, and CD8+ lymphocyte counts; the year of initiation ART; and the composition of the current ART regimen. These data were used descriptively.

**Anthropometric and dietary intake data**

Total body weight was measured with an electronic scale (Urano, Canoas, RS, Brazil) with resolution of 0.1 kg and height was measured with a stadiometer (Urano, Canoas, RS, Brazil) with 0.1 cm resolution. A 3-day food record was used to assess food intake, including two weekdays and one weekend day. The records were filled during interviews conducted by a dietitian. The software DietPro 5.1 (A.S. Sistemas, Viçosa, MG, Brazil) was used to quantify the mean intake of energy, macronutrients, Hcy metabolism related vitamins as folic acid and vitamin B12, and antioxidants vitamins A, C and E. The Estimated Average Requirement (EAR) values from Dietary Recommendations Intakes were used to estimate the prevalence of inadequate intakes from folate and B12 micronutrients (Padovani et al. 2006).

**Blood sampling and biochemical analyses**

https://mc06.manuscriptcentral.com/apnm-pubs
Blood samples for all analyses were collected (5 mL in heparinized tube and 5 mL in EDTA vacutainer tube) from patients after a 12-hour overnight fasting. The blood sample tubes were kept refrigerated at 4°C until the end of collection, then centrifuged at 1000 g for 15 min, plasma and red blood cells separated and stored in Eppendorf® tubes at -80°C for future analysis.

Hcy and relative amino acids were determined in plasma by gas chromatography (GC-17A Shimadzu®, Kyoto, Japan) using a commercially available kit EZ:Faast Amino Acid Analysis (Phenomenex®, Torrance, CA, USA). The determination of plasma Hcy levels involved preliminary pretreatment of the sample with SAH hydrolase and dithiothreitol to release protein-bound Hcy. Plasma vitamin B12 and folate were determined by chemiluminescence using commercially available kits from IMMULITE® (Siemens, Los Angeles, CA, USA).

Plasma advanced oxidation protein products (AOPP) concentrations were determined using the method previously described by Witko-Sarsat et al. (Witko-Sarsat et al. 1996). Plasma Malondialdehyde (MDA) and total lipid hydroperoxide (TH), markers of lipid peroxidation, were determined as previously described by Spirlandeli et al. (Spirlandeli et al. 2013) and Costa et al. (Costa et al. 2006). Reduced (GSH) and oxidized glutathione (GSSG) were determined in red blood cell with the method of Rahman et al. (Rahman et al. 2006).

Statistical analysis

Given that sample size was not previously calculated, an a posteriori power analysis was performed using G*Power version 3.1.9.2 (Franz Faul, Universität Kiel, Germany). Considering α=0.05, the statistical power was 75% for detecting differences in homocysteine levels pre- and post-training.

The data normality was checked using the Shapiro-Wilk test, and the Levene test was used to analyze the homogeneity of variances. Independent t-test for continuous variables and chi-square test for categorical variables were used to compare PLHIV and people without HIV infection. To determine the effects of exercise training, the post-training values were compared between groups using an analysis of covariance (ANCOVA), where the post-training values were considered dependent variables, the group allocation was the independent variable, and the baseline values were the covariates. The magnitude of the differences in the variables was calculated from the effect sizes using the Cohen method, where a Cohen’s d value lower than 0.2 was classified as ‘trivial’, between 0.2 and 0.49 was ‘small’, between 0.5 and 0.79 was
‘medium’, and 0.80 and higher was ‘large’ (Cohen 1988). Pearson correlation coefficients were used to test associations between variables. The statistical significance was set at $p \leq 0.05$ and the data was analyzed using a SPSS for Windows Version 22.0 (SPSS Inc., Chicago, IL, USA).

**RESULTS**

A comparison of biochemical biomarkers and dietary intake data between PLHIV and people without HIV infection found that PLHIV presented significant ($p<0.05$) higher levels of plasma Hcy (47%) and MDA (30%), as well as significant ($p<0.05$) lower levels of plasma GSH (34%) and folate (47%), when compared to those without HIV infection. Table 1 lists baseline data comparison of Hcy, B12 vitamin, folate and oxidative stress biomarkers.

**INSERT TABLE 1**

Nutritional evaluation demonstrated no differences between total energy, carbohydrates, protein, total folate, vitamins B12, A, C and E intake between PLHIV and those without HIV infection. Both groups presented vitamin B12 intake according to the estimated average requirement recommendation; only PLHIV had folate intake according to EAR recommendation of 400 µg/d. Also, vitamin A and E intake were below EAR recommendations for both groups. PLHIV presented significantly lower ($p>0.05$) lipid intake than people without HIV infection (Table 2).

**INSERT TABLE 2**

We did not observe any differences in the immunological, virologic and ART regimen between CG and TG at baseline (Supplementary data 1). Overall, CTE does not change Hcy plasma concentration, Hcy metabolites or oxidative stress markers of PLHIV (Table 3). However, because hyperhomocysteinemic subjects may respond better to the treatment and exercise training [15], we categorized PLHIV in hyperhomocysteinemic (HHcy=initial Hcy plasma concentration $\geq 15$ µmol/L) and non-hyperhomocysteinemic (initial Hcy plasma concentration < 15 µmol/L). After categorization, we found that CTE promoted a 32% reduction in plasma Hcy concentration in PLHIV with HHcy (pre 17.4 ± 1.9 vs post...
11.8 ± 3.3 µmol/L; (*p*<0.05)), which was not observed in PLHIV without HHcy (Figure 2A). CTE decreased Hcy plasma concentration under 15 µmol/L of 5 from 7 HHcy PLHIV (71% of the participants). Hyperhomocysteinemic PLHIV experienced a 5.6 ± 3.2 µmol/L reduction in plasma Hcy after 16 weeks of CTE. In addition, the reduction of Hcy plasma concentration in trained hyperhomocysteinemic PLHIV was followed by a reduction in plasma lipid hydroperoxide, which was not observed in trained non-hyperhomocysteinemic PLHIV (Figure 2B). Lastly, a significant negative correlation was found between pre-training plasma Hcy concentration and changes in Hcy and lipid hydroperoxides promoted by CTE (Figure 2C).

**INSERT TABLE 3**

**INSERT FIGURE 2**

**DISCUSSION**

The major findings of our investigation were that 16 weeks of CTE decreased elevated Hcy plasma concentration only for the PLHIV who had HHcy, normalizing the Hcy concentration. CTE also reduced lipid peroxidation and enhanced redox balance only in hyperhomocysteinemic PLHIV. These results demonstrated the selective potential effects of exercise training in ameliorate important metabolic disturbances experienced by PLHIV under ART.

Although it has been previously demonstrated that PLHIV have elevated Hcy plasma concentration (Deminice et al. 2013, 2015), to the best of our knowledge, this is the first study demonstrating exercise training decreased elevated plasma Hcy concentration of PLHIV. Interestingly, the lowering Hcy effects of exercise were observed only in hyperhomocysteinemic PLHIV. Indeed, our data demonstrated that participants with the highest pre-training plasma Hcy concentration experienced a larger Hcy reduction post exercise (Figure 2C). HHcy has been associated with a spectrum of diseases demonstrated in different populations (Wijekoon et al. 2007; Seshadri 2012; Hu et al. 2013). In a recent meta-analysis, Deminice et al. (Deminice et al. 2015) demonstrated that PLHIV had an average plasma Hcy ~2 µmol/L higher than people without HIV infection. Our data demonstrated Hcy plasma levels were increased by 4.2 µmol/L in PLHIV compared to people without HIV infection. In contrast, participating in CTE resulted in a 5.6 µmol/L (~32%)
reduction in Hcy plasma levels of hyperhomocysteinemic PLHIV. This result is particularly relevant since each additional 5 µmol/L in Hcy levels have been demonstrated to increases the risk of cardiovascular events by approximately 20% (Humphrey et al. 2008). It is also relevant considering that the folate and B12 vitamin used as Hcy-lowering therapy do not efficiently prevent CVD (Marti-Carvajal et al. 2017), as does the exercise training. In other populations, the effect of the exercise on Hcy levels appear to be related to the type of the exercise. For example, regular strength training can decrease plasma Hcy concentration, whereas similar effects have not been observed after aerobic exercise training (Silva and Da Mota 2014; Deminice et al. 2016). For this reason, combined exercise can be considered a novel and interesting Hcy-lowering strategy that is likely to effectively prevent CVD and others HHcy associated diseases.

Even though Hcy levels are inversely related to the daily intake of folate and vitamin B12 in general population, recent studies demonstrated that disturbances in Hcy metabolism during HIV infection may not be due to nutritional deficiences (Deminice et al. 2013). Our results demonstrated PLHIV have adequate folate and vitamin B12 intake. We also did not find any differences in folate and vitamin B12 intake between PLHIV and people without HIV infection, despite presenting with reduced plasma folate concentration. This suggests that HIV and/or ART complications are likely causes of elevated Hcy plasma concentration in this population. HIV and/or ART may impair Hcy remethylation pathway, where folate have a key role in Hcy removal metabolism (Selhub et al. 2008). It is also be that reduced Hcy plasma levels and changes in folate and vitamin B12 demonstrated in our study were due to metabolic changes promoted by CTE, and not related to food intake. Although some studies demonstrated reduced plasma Hcy concentrations after an exercise training program, the mechanistic explanation for this effect remains poorly investigated.

Recent studies have demonstrated PLHIV experience elevated oxidative stress markers when compared to people without HIV infection (Gil et al. 2003; Ngondi et al. 2006; Ibeh and Emeka-Nwabunnia 2012). Oxidative stress in PLHIV favors disease progression with increased viral replication, immune dysfunction, and carcinogenesis (Stehbens 2004). Recently, oxidative damage has been associated with HIV disease progression, because it can exacerbate CD4 cell decline (Ibeh and Emeka-Nwabunnia 2012), and it may predict all-cause mortality in PLHIV (Masià et al. 2016). Our data demonstrated increased lipid peroxidation (MDA) between PLHIV and people without HIV infection but did not find differences in other oxidative stress markers largely used in HIV studies such as TH, AOPP and GSH/GSSG ratio. This was unexpected, since several studies demonstrated elevated oxidative stress markers in PLHIV due increased
superoxide anion formation in mitochondria induced by HIV viral proteins and ART (Aukrust et al. 2003; Deresz et al. 2010). However, differences in ART regimen may directly change oxidative stress markers (Ngondi et al. 2006; Lagathu et al. 2007; Masiá et al. 2007), and contribute to our observations. Masia et al. (2007), demonstrated that protease inhibitors-based ART regimens increases TH plasma concentration, when ART regimens based on non-nucleoside reverse transcriptase inhibitors were associated with lower TH plasma concentrations. Our sample size, especially after CTE, limited our ability to demonstrate specific interaction between ART regimen and oxidative stress. In addition to the antiretroviral class, oxidative stress in PLHIV is usually associated with the presence of lipodystrophy syndrome (Vassimon et al. 2010), and with the duration of infection and ART use (Ibeh and Emeka-Nwabunnia 2012), wherein the oxidative stress is directly proportional to the time of infection and ARV use.

Studies have demonstrated that exercise training upregulates the endogenous antioxidant system which decreases susceptibility to oxidative damage in healthy subjects (Powers et al. 2011). Our data demonstrated modest effects of CTE on oxidative stress markers such as decrease in lipid peroxidation and a marginal up-regulation in redox balance demonstrated by elevated GHS/GSSG ratio. Again, as demonstrated for Hcy plasma concentration, the protective effect of exercise was demonstrated only in hyperhomocysteinemic PLHIV compared to non-hyperhomocysteinemic ones. Garcia et al. (Garcia et al. 2013), demonstrated that 20 weeks of combined exercise reduced lipid peroxidation, but did not increase antioxidant enzymes in PLHIV. Taken together, these data demonstrate that 16 to 20 weeks of CTE promotes modest changes in oxidative stress markers in PLHIV taking ART. Therefore, is reasonable to speculate that exercise training may impose different antioxidant and oxidative stress changes between PLHIV and people without HIV infection. Thus, future studies should determine the appropriated relation among exercise training intensity, volume, duration and frequency for PLHIV’s best metabolic benefits.

Our study has limitations that should be considered. The small sample size is the main limitation. This was due to the elevated number of drop-outs (39%) during the exercise training intervention. The final sample size made it hard for us to stratify the sample in subgroups related to ART drug class, time of ART use and infection, and presence or absence of lipodystrophy syndrome, all conditions that are directly associated with oxidative stress. Similar limitations have been cited by other authors that experienced drop-out rates from 20 to 50% (O’Brien et al. 2016) and concerned about the difficulties to motivate PLHIV to be involved in an exercise program. Thus, strategies to increase adherence to exercise programs for this
population must be investigated. Finally, considering that the effect of exercise training on oxidative stress and Hcy plasma levels is related to the intervention duration, the 16-week period of our study may not be enough to promote significant changes in the oxidative stress markers studied.

In conclusion, our results demonstrated that 16 weeks of CTE decreased elevated Hcy plasma concentration and enhance redox balance of PLHIV. This lowering Hcy and lipid peroxidation effect promoted by exercise however is restrict for hyperhomocysteinemic PLHIV. These results suggest the potential, but selective, effects of combined exercise training in ameliorate important metabolic disturbances experienced by PLHIV under ART.

Acknowledgements: The authors thank all the staff from the University Hospital services for helping to recruit participants and providing the medical records, the staff that helped with the training, and also a special thanks to all the participants of the study. This study was supported by grants from Seti (Secretaria da Ciência, Tecnologia e Ensino Superior do Paraná) in Brazil.

Conflict of Interest: The authors declare that they have no conflict of interest.

REFERENCES


Rahman, I., Kode, A., and Biswas, S.K. 2006. Assay for quantitative determination of glutathione and


Table 1. Plasma initial levels of homocysteine, B12 vitamin, folate and oxidative stress biomarkers for people living with HIV (HIV+) and people without HIV infection (HIV-).

<table>
<thead>
<tr>
<th>Variables</th>
<th>HIV- (n=33)</th>
<th>HIV+ (n=49)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocysteine (µmol/L)</td>
<td>8.9 ± 2.8</td>
<td>13.1 ± 3.6*</td>
</tr>
<tr>
<td>Vitamin B12 (pg/mL)</td>
<td>470.3 ± 213.9</td>
<td>395.2 ± 161.9</td>
</tr>
<tr>
<td>Folate (ng/mL)</td>
<td>13.5 ± 4.8</td>
<td>7.2 ± 4.4*</td>
</tr>
<tr>
<td>Malondialdehyde (µmol/L)</td>
<td>4.3 ± 2.9</td>
<td>5.6 ± 2.9*</td>
</tr>
<tr>
<td>TH (µmol H2O2/L)</td>
<td>31.7 ± 14.4</td>
<td>37.7 ± 16.6</td>
</tr>
<tr>
<td>AOPP (µmol chloramine-T/L)</td>
<td>638.4 ± 137.9</td>
<td>668.2 ± 110.3</td>
</tr>
<tr>
<td>GSH (µmol/g hgb)</td>
<td>90.9±35.7</td>
<td>59.5±25.7*</td>
</tr>
<tr>
<td>GSH/GSSG</td>
<td>2.6±1.0</td>
<td>2.5±0.8</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation. *Indicates significant difference (p<0.05) compared to people without HIV infection by independent t-test. TH=Total Hydroperoxide; AOPP=Advanced Oxidation Protein Products; GSH=Reduced Glutathione; hgb=Hemoglobin; GSH/GSSG=Reduced/Oxidized glutathione ratio.
Table 2. Comparison of initial habitual macronutrients and selected vitamins intake between people living with HIV (HIV+) and people without HIV infection (HIV-).

<table>
<thead>
<tr>
<th>Variables</th>
<th>HIV- n=33</th>
<th>HIV+ n=49</th>
<th>Reference Intakes (EAR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caloric intake (kcal)</td>
<td>2269.6 ± 652.7</td>
<td>2419.6 ± 1001.7</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates (%)</td>
<td>45.9 ± 9.4†</td>
<td>49.4 ± 9.7†</td>
<td>50-60%</td>
</tr>
<tr>
<td>Protein (g/kg)</td>
<td>1.4 ± 0.6†</td>
<td>1.7 ± 0.8†</td>
<td>0.8-1 g/d</td>
</tr>
<tr>
<td>Lipids (%)</td>
<td>36.2 ± 6.7†</td>
<td>31.3 ± 8.2*†</td>
<td>20-25%</td>
</tr>
<tr>
<td>Folate (µg)</td>
<td>362.9 ± 214.5†</td>
<td>424.8 ± 202.1</td>
<td>400 µg/d</td>
</tr>
<tr>
<td>Vitamin B12 (µg)</td>
<td>7.2 ± 7.9</td>
<td>6.2 ± 5.3</td>
<td>2.4 µg/d</td>
</tr>
<tr>
<td>Ascorbic acid (mg)</td>
<td>141.2 ± 82.5†</td>
<td>353.0 ± 799.8†</td>
<td>75-90 mg/d</td>
</tr>
<tr>
<td>Vitamin E (mg)</td>
<td>5.9 ± 2.6†</td>
<td>6.4 ± 3.7†</td>
<td>15 mg/d</td>
</tr>
<tr>
<td>Retinol (Eq)</td>
<td>411.1 ± 224.9†</td>
<td>553.7 ± 374.4†</td>
<td>900 Eq/d</td>
</tr>
</tbody>
</table>

*Indicates significant difference (p<0.05) compared to people without HIV infection by independent t-test.
†Indicate inadequate intake according to Dietary Reference Intakes (DRIs-2005)(Padovani et al. 2006).
EAR=Estimated Average Requirement.
**Table 3.** Plasma concentration of homocysteine, B12 vitamin, folate and oxidative stress biomarkers before and after 16 weeks of combined training exercise.

<table>
<thead>
<tr>
<th>Variables</th>
<th>CG (n=13)</th>
<th>TG (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td><strong>Hcy metabolism</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hcy (µmol/L)</td>
<td>12.3 ± 2.6</td>
<td>10.7 ± 4.5</td>
</tr>
<tr>
<td>B12Vitamin (pg/mL)</td>
<td>407.1 ± 163.7</td>
<td>389.9 ± 153.1</td>
</tr>
<tr>
<td>Folate (ng/mL)</td>
<td>8.8 ± 6.9</td>
<td>8.5 ± 6.4</td>
</tr>
<tr>
<td><strong>Oxidative stress markers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TH (µmol H₂O₂/L)</td>
<td>38.1 ± 9.3</td>
<td>30.3 ± 16.7</td>
</tr>
<tr>
<td>Malondialdehyde (µmol/L)</td>
<td>4.3 ± 2.4</td>
<td>4.8 ± 2.9</td>
</tr>
<tr>
<td>AOPP (µmol clor-T/L)</td>
<td>630.1 ± 140.5</td>
<td>657.5 ± 143.4</td>
</tr>
<tr>
<td>GSH (µmol/g hgb)</td>
<td>74.9 ± 28.9</td>
<td>132.1 ± 74.1*</td>
</tr>
<tr>
<td>GSH/GSSG ratio</td>
<td>3.7 ± 1.1</td>
<td>4.1 ± 1.9</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation. *Indicates significantly difference (p<0.05) compared to pre-training values by ANCOVA. CG=Control Group; TG=Trained Group; ES=Effect Size (Cohen’s d); Hcy=Homocysteine; TH=Total Hydroperoxide; AOPP=Advanced Oxidation Protein Products; GSH=Reduced Glutathione; GSSG=Oxidized Glutathione; hgb=Hemoglobin; GSH/GSSG ratio=Reduced/Oxidized glutathione ratio.
**Figure 1.** CONSORT flow diagram of participant recruitment and allocation.

**Figure 2.** Plasma concentrations of Hcy (A) and lipid hydroperoxides (B) measured before and after 16 weeks of combined training exercise for trained HHcy compared to trained non-HHcy people living with HIV. (C) Correlation between pre-training plasma Hcy concentration and changes in Hcy and lipid hydroperoxides promoted by combined training exercise. $p<0.05$ by ANCOVA.
People living with HIV invited to take part (n=226)

Excluded (n=177)
- Did not meet inclusion criteria (n=140)
- Declined (n=37)

HIV+ (n=49)
- Men = 21
- Women = 28

Excluded (n=3)
- Declined (n=3)

Randomized (n=46)
- Men = 21
- Women = 25

Allocation

Allocated to Control group (n=21)
Allocated to Trained group (n=25)

Follow-Up

Lost to follow-up or discontinued intervention (n=8)
- Did not give reason (n=3)
- Personal issues (n=2)
- Moved to a new city (n=2)
- Health problems (n=1)

Analysed (n=13)
- Men = 4
- Women = 9

Analysis

People without HIV infection invited to take part (n=50)

Excluded (n=17)
- Did not meet inclusion criteria (n=14)
- Withdrawals (n=3)

HIV- (n=33)
- Men = 13
- Women = 20

Baseline Assessment

Excluded (n=3)
- Declined (n=3)

People living with HIV invited to take part (n=226)

Lost to follow-up or discontinued intervention (n=8)
- Professional reasons (n=3)
- Health problems (n=2)
- Personal reasons (n=2)
- Pregnancy (n=1)

Analysed (n=17)
- Men = 9
- Women = 8
A) Plasma Hcy (μmol/L)

- Pre: Trained
- Post: Trained HHcy

B) Total Hydroperoxides (μmol H2O2/L)

- Pre: Trained
- Post: Trained HHcy

C) Pre-training plasma [Hcy] (μmol/L)

Training-induced changes in plasma [Hcy] (μmol/L)

- Trained
- Trained HHcy

Training-induced changes in hydroperoxides (μmol H2O2/L)

- Trained
- Trained HHcy

Statistical Analysis:

- $P = 0.02$
- $P < 0.01$
- $r = -0.55$; $P < 0.05$
- $r = -0.61$; $P < 0.05$