Abstract. – OBJECTIVE: The aim of the study was to identify the possible benefits of physical activity program in improving the antioxidant enzymes activity and lipid profile among smokers.

PATIENTS AND METHODS: Fifteen cigarette smokers (CS), 14 hookah smokers (HS), and 14 non-smokers (NS) participated in the low-intensity continuous training (LCT). Eleven CS, 12 HS, and 12 NS participated in the moderate-intensity intermittent training (MIT). The LCT groups performed a 20 to 30-minute continuous exercise at 40% of the VO$_{2\text{max}}$. The MIT groups performed 6 to 10 sets of 2-minute sprint at 70% of the VO$_{2\text{max}}$ interspersed by a 1-min recovery period. At baseline and after 12 weeks of intervention, the antioxidant defense activity and lipid profile were assessed.

RESULTS: The improvement in antioxidant capacity under the effect of MIT program is statistically more significant than after LCT. The increase of glutathione peroxidase (GPx), superoxide dismutase (SOD), glutathione reductase (GR), malondialdehyde (MDA) and α-tocopherol was higher in smoker subjects participating in the MIT program compared to those participating in the LCT. In contrast, the LCT program has favorably altered lipid and lipoprotein profile of smokers and thus reduced their cardiovascular risk.

CONCLUSIONS: The combination of the two training methods may have major implications in both defense and prevention programs.

Key Words: Training, Oxidative stress, Lipids, Smokers, Physical activity.

Introduction

Smoking is a crucial problem of public health; it is a very important risk factor for cardiovascular diseases, respiratory, and lung cancer$^{1,2}$. Also, inactive lifestyle was believed to be a risk factor$^3$ and oxidative stress was found matched to the evolution of several diseases, including cardiovascular disease (CVD), which were recently presented$^{4,5}$. Cigarettes or hookah smokers have a higher risk of CVD, possibly through increased production of free radicals (RL). Smoking aggravates the formation of RL and poses a significant oxidative stress$^6$. Existing surveys show that smokers have oxidative stress rates higher than non-smokers$^7,8$. It might be described in part by the decreased ability of blood antioxidants.
Smoking cigarettes or hookah causes almost the same decrease amounts of glutathione reductase (GR), glutathione peroxidase (GPx) and α-tocopherol unlike the non-smoking subjects. In addition, as for smoking cigarettes, hookah consumption causes a significant elevation of LDL-C, a high concentration of triglyceride (TG) and reduced levels of HDL-C.

Anaerobic training has been shown able to increase the antioxidants (SOD, GPx and GR) and strengthen the body opposed to new oxidative attacks. In contrast, a number of studies have found a decrease in antioxidant defense, which can be caused by the excessive creation of free radicals by increasing the effect of anaerobic exercise. Other different studies have shown that organized physical activity can alter the balance prooxidant/antioxidant and rise the activity of endogenous antioxidants and the resistance of LDL to oxidation; and that severe physical activity rises the use of oxygen and the creation of free radicals and could induce lipid peroxidation.

The response of the antioxidants activity to exercise is a subject for discussion. Different experimental studies have shown an increase, while others have shown no change even a decrease. The differences in results can be described in part, by the variety in the training protocols, lifestyle, smoking habits and age of subjects.

Physical training plays a major role in increasing the lipid profile. Previous studies suggest that normal aerobic exercise has been linked to the favorable changes in lipid and lipoproteins level. Conversely, intensive physical training can even provoke the development of atherosclerosis by delivering catecholamines that damage blood vessel walls which will lead to lipid deposition. However, MacFarlane et al. showed that training periods at intermittent exercise leads to a significant improvement in cardiovascular fitness and improves plasma lipoproteins compared to continuous physical training session.

Moreover, not so many studies proved evidence on the effects of resistance training on lipid levels. Results from Shaw et al. proved that the training resistance was not linked to a favorable change in lipid levels in sedentary male smokers. On the other hand, Pate et al. recommended to exercise moderate-intensity physical activity 30 minutes at least for the most days of the week. Similarly, prior guidelines recommended endurance exercises for 20 minutes at least, at least three more times in a week.

These results lead us to think of the idea of recognizing the potential benefits of both intermittent and continuous training program in order to avoid smoking dangers and explore the most suitable activity method for most smokers.

Thus, the purpose of this study is to determine whether the continuous training of low intensity (LCT) and/or the moderate-intensity interval training (MIT) could improve the antioxidants activity and lipid profile of adult smokers; and to identify the difference of individual training effects in cigarette smokers compared with hookah consumer.

**Patients and Methods**

The experimental protocol lasted four months, from October 15, 2019, to February 20, 2020.

**Participants**

Seventy-eight sedentary, non-smokers and healthy male smokers from Tunisia volunteered to participate in this study and were recruited within pharmacology laboratory of the Faculty of Medicine of Sfax, Tunisia. Their mean values of age, height, weight, and BMI are respectively 44.2 ± 3.5 years, 174.9 ± 2 cm, 72.7 ± 3.2 kg and 24.4 ± 1.2 kg.m⁻².

Participants were normolipidemic, not obese. They did not take any kind of nutritional supplements or medications. The participants did not suffer from any disease and were not involved in physical activities or exercise for the last months.

All participants were informed of the nature and progress of the experiment and a consent was signed by all the members as it was required by the Research Ethics Committee of the Faculty of medicine, University of Sfax, Tunisia.

Cigarette smokers who consume 10 pack-years (PY) are all subjects to the experiment. Hookah consumption is quantified, as in the study of Kiter et al. with HY and kg of cumulative tobacco. Hookah tobacco used weights between 10 and 25 grams. Actually, the regular hookah consumption is 5 hookah-years (HY).

**Study Design**

The participants were subject to a continuous or intermittent training program and were divided into six groups.

- Continuous training: Cigarette smokers’ group (CS, n = 15); hookah smokers’ group (HS, n = 14) and non-smokers group (NS, n = 14).
• Intermittent training: Cigarette smokers’ group (CS, n = 11); hookah smokers’ group (HS, n = 12) and non-smokers group (NS, n = 12).

All participants were subjected to test sessions and biochemical analyzes before and after the training program. It includes anthropometric tests, a biochemical analysis and antioxidant status review.

In order to quantify individual loads, effort test was made before the training program.

Blood Samples
Smokers were ordered to stop smoking one hour at least before reporting to the laboratory as proposed by Dietrich et al. The samples were taken two times before and after the training program at 8 am after 12 hours fasting and 9 hours sleep. The blood samples were processed and stored directly in micro centrifuge at -80°C to the time it will be analyzed.

Biochemical Analyses
Total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) were used using the standard techniques presented by Wegge et al. Low-density lipoprotein cholesterol (LDL-C) was calculated with the Friedewald formula: \[ \text{LDL} = \text{TC} - \text{HDL} - \left( \frac{\text{TG}}{2.18} \right) \].

The antioxidant markers used were examined with the use of essay kits available commercially and brought from Randox Laboratories (Randox Laboratories Ltd, place country-region UK). Plasma concentrations of superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and the total antioxidant status (TAS) were measured using standard colorimetric assays, and α-tocopherols was taken out with hexane from plasma and then measured through high performance liquid chromatography (HPLC). Malondialdehyde (MDA) was analyzed using malondialdehyde HPLC procedure. MDA concentrations were performed with the following formula: sample = (Peak height sample × concentration of the calibrator)/Peak height calibrator.

Dietary Program
Participants were told to keep their normal food plan all along the training period. Also, we standardized meals 72 hours before each blood sample. In order to do this, we instructed the participants to be present in the institute during breakfast, lunch and dinner in the 72 hours preceding the blood samples so that we can have the same dietary intake and stay away from alterations in postprandial oxidative stress values.

Physical Fitness Assessment
\( \text{VO}_{2\text{max}} \) measurements during exercise were examined through treadmill maximal exercise test (COSMED Pulmonary-Function Equipment 37 Via dei Piani di monte Savello I-00040, Rome, Italy). This test consists in increasing the speed of 1km.h\(^{-1}\) every 2 min, after warming up of 5 min with a 6 km. h\(^{-1}\) speed until the participant could no longer continue. Heart rate, using (Polar Electro Oy, Kempele, Finland), was monitored throughout the test and was recorded at the conclusion of every two-minute stage. The oxygen consumption (\( \text{VO}_{2}\)) was continually recorded and measured in real time using oxygen analyzer (Fitmate, version 1.2 PRO COSMED). Verbal encouragement was provided throughout the test to ensure that the maximal effort was achieved.

Training Protocol
The training program lasts three months, with 3 periods per week of 20 minutes during the first month, 25 minutes in the second month and 30 minutes during the third month. The intensity was controlled according to time and distance traveled.

All training sessions were conducted at the Higher Institute of Sport and Physical Education of Sfax under the supervision of qualified specialist trainers.

Continuous Training
The training was performed repeatedly, three times/ week at an intensity of 40% of \( \text{VO}_{2\text{max}} \). We ask told the participants to keep their running rhythm continuous and to respect the sound of beeps and the requested time. The increase in the training load was ensured by increasing in working time and the distance covered in each session.

Intermittent Training
The intermittent training program consists of 3 sessions per week and the period lasts three months. Participants were asked to run 2-minute periods at an intensity of 70% of \( \text{VO}_{2\text{max}} \) interspersed with recovery periods of 1 minute. The increase in training load was ensured by increasing in number of repetitions and traveled distance.
Physical activity reduces oxidative stress and improves lipid profile made by smoking

Statistical Analysis
We used STATISTICA software from StatSoft, Franceto process the tests. We expressed data as mean ± SD (standard deviation). We performed the parametric tests after normality verification with the Shapiro-Wilk’s w test, and homogeneity of variances with Levene’s test. In order to inter group differences in baseline subjects’ characteristics, we used ANOVA. Inter and intra-group comparisons of the variables were made by two-way ANOVA (group vs. training) with repeated measurements. Least Significant Different (LSD) post-hoc analysis was used to identify significant group differences that were indicated by one-way and two-way ANOVA. A probability level of 0.05 was selected as the criterion for statistical significance.

Results

Changes Intra and Inter-Group of Antioxidant Status
The differences in the antioxidant values (Δ) of the subjects of the six groups in Pre vs. Post program are summarized in Table I.

In CS and HS groups, the increased of GPx is significant after the two training methods. However, the improving after the MIT was greater that after the LCT program and was better in smokers compared with non-smokers. ANOVA showed a significant main effect for exercise type \[F (1, 62) = 30.78; p < 0.001, \eta^2 = 0.332\] (Figure 1).

Similarly, the increased GR follows the same trend under the effect of MIT; it is more pronounced among groups CS and HS compared to that of NS group \(p = 0.01\). In contrast, the effect of the LCT program on MDA is less important and revealed a single significant difference in CS subjects. ANOVA showed a significant main effect for exercise type \[F (1, 62) = 65.37; p < 0.001, \eta^2 = 0.513\] (Figure 2).

Concerning blood concentrations in SOD, the increase is significant for all participants, and it is more marked after MIT that after the LCT program. ANOVA showed a significant main effect for exercise type \[F (1, 62) = 62.15; p < 0.001, \eta^2 = 0.501\]. In addition, this increase is higher in HS group compared to the CS and NS groups under the main effect of MIT (Figure 4).

Table I. Changes (Δ) in the antioxidant values in Pre vs. Post program.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Continuous NS</th>
<th>Continuous CS</th>
<th>Continuous HS</th>
<th>Intermittent NS</th>
<th>Intermittent CS</th>
<th>Intermittent HS</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPx (U.gHg⁻¹)</td>
<td>0.65 ± 0.7**</td>
<td>0.5 ± 0.8*</td>
<td>0.44 ± 0.5*</td>
<td>2.8 ± 5.2</td>
<td>6.5 ± 5***</td>
<td>7.23 ± 4.8***</td>
</tr>
<tr>
<td>SOD (U.gHg⁻¹)</td>
<td>5.7 ± 7.8</td>
<td>9.6 ± 17.3*</td>
<td>9.6 ± 13.5*</td>
<td>190.8 ± 129.2***</td>
<td>167.4 ± 191.0***</td>
<td>300.8 ± 126.9***</td>
</tr>
<tr>
<td>GR (U.gHg⁻¹)</td>
<td>0.13 ± 0.3*</td>
<td>0.16 ± 0.2*</td>
<td>0.05 ± 0.1</td>
<td>1.24 ± 1.7***</td>
<td>2.49 ± 0.9***</td>
<td>2.44 ± 1.3***</td>
</tr>
<tr>
<td>MDA (µmol.l⁻¹)</td>
<td>-0.03 ± 0.1</td>
<td>-0.11 ± 0.2**</td>
<td>-0.06 ± 0.1</td>
<td>0.16 ± 0.2+++</td>
<td>0.25 ± 0.2+++</td>
<td>0.19 ± 0.1+++</td>
</tr>
<tr>
<td>TAS (mmol.F⁻¹)</td>
<td>0.03 ± 0.4*</td>
<td>0.05 ± 0.4**</td>
<td>0.03 ± 0.4*</td>
<td>0.01 ± 0.03</td>
<td>0.04 ± 0.02**</td>
<td>0.02 ± 0.03*</td>
</tr>
<tr>
<td>α-Tocopherol (µmol)</td>
<td>0.06 ± 0.2</td>
<td>0.3 ± 0.4*</td>
<td>0.05 ± 0.6</td>
<td>0.48 ± 0.8</td>
<td>1.05 ± 1.5*</td>
<td>1.45 ± 0.8**</td>
</tr>
</tbody>
</table>

*, **Significant difference in Pre vs. Post program at \(p < 0.05, p < 0.01\) respectively. †, ††, †††Significant difference compared with the continuous exercise at \(p < 0.05, p < 0.01, p < 0.001\) respectively. Abbreviations: GPx = Glutathione peroxidase; SOD = Superoxide dismutase; MDA = Malondialdehyde; GR = Glutathione reductase; TAS = Total antioxidant status.

Figure 1. Improvement rate (Δ) of glutathione peroxidase (GPx) in Pre vs. Post program. *\(p < 0.05\); **\(p < 0.01\); ***\(p < 0.001\). Significant difference between groups compared with continuous training. Abbreviations: GPx = Glutathione peroxidase; NS = Non- smokers; CS = Cigarettes smokers; HS= Hookah smokers.
The continuous or intermittent training program induces an increase in α-tocopherol in all participants; however, it varies according to the groups. It is significant only for smokers who participated in the MIT protocol and subjects of CS group who underwent the LCT program. The result is a significant main effect for exercise type \[F (1, 62) = 17.88; p < 0.001; \eta_p^2 = 0.224\].

Finally, the subjects of the six groups showed increased values of SAT under the effect of two training methods. Statistical analysis revealed no significant difference of the effects of MIT compared to the LCT.

### Changes Intra and Inter-Group of the Lipid and Lipoproteins Profile

The training period of three months in intermittent or continuous exercises, induces varying changes on the lipid and lipoprotein profile; nevertheless, these changes vary according to the groups (Table II).

These changes showed significant differences in all lipid profile parameters of smokers’ subjects measured after the period of ECF \((0.05 < p < 0.001)\), except TG and HDL-C / TG report. In contrast, after the EIM, all measured variables showed a small \((\Delta)\) positive but did not reach statistical significance, except in smokers, significant changes were found for HDL-C and CL / HDL-C report \((p < 0.05 \text{ and } p < 0.01 \text{ respectively})\).

However, statistical analysis showed no significant difference of the effects of two training methods, except for the CL / HDL-C report \([F (1, 62) = 5.99; p = 0.017; \eta_p^2 = 0.088]\) of cigarette smokers (Figure 5 and Figure 6 respectively).

### Discussion

This study demonstrates that a training program with sessions of intermittent or continuous exercises, as part of cardiovascular prevention, provides substantial improvements in plasma antioxidants and lipid profile. The improvements were accompanied by favorable changes of all measured parameters of antioxidants occurring after our MIT program.
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In addition, the comparison of the LCT effect in comparison to MIT program on the antioxidant status showed that the two training methods induce enhancements in a different magnitude. However, our analysis has recorded increases in antioxidant activity and a decreased in the MDA concentration after 12-weeks training. These results are consistent with the findings of Alessio and Franzoni et al which revealed an improvement in antioxidant status after different training protocols. In this context, Bloomer et al suggest that anaerobic exercises could help increase the antioxidant defense capacity and reduce the production of oxidants.

Moreover, the increase in GPx, and SOD was statistically significant in smokers. However, the improvement following the MIT is greater and statistically different compared to LCT program ($p<0.001$). This finding has also been reported in two other studies.

The improving of GR, MDA and α-tocopherol recorded in this study varies according to groups; it is higher in subjects smoking participating in MIT program compared to those participating in the LCT. These results are similar to those published by Cuevas et al. In contrast, the GR and α-tocopherol level was not changed in the HS group following the LCT protocol. These results are in contrast to the studies of Criswell et al, Powers et al and Elosua et al which demonstrated an improvement in GR activity after training programs.

This divergence can be clarified, partially, by the diversity of realized protocols (Training methods, participants’ age, smoking duration, etc.), the response to physical exercise of each subject, and the food intake rich in fat that could lead exaggerated reactions of oxidative stress. Our main conclusion is that both MIT and LCT methods have shown increases in antioxidant ca-

### Table II. Lipid improvement rate (Δ) after training program.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Continuous</th>
<th>Intermittent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NS</td>
<td>CS</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>0.07 ± 0.08**</td>
<td>0.08 ± 0.08**</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>-0.09 ± 0.07***</td>
<td>-0.14 ± 0.09***</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>-0.02 ± 0.03</td>
<td>-0.05 ± 0.07**</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>-0.01 ± 0.03</td>
<td>-0.02 ± 0.05</td>
</tr>
<tr>
<td>H/DL-C</td>
<td>0.09 ± 0.09**</td>
<td>0.04 ± 0.08</td>
</tr>
</tbody>
</table>

*, **, ***Significant difference in Pre vs. Post program at $p<0.05$, $p<0.01$ and $p<0.001$ respectively. †Significant difference compared with the continuous training at $p<0.05$. Abbreviations: HDL-C= Height density lipoprotein cholesterol; LDL-C = Low density lipoprotein cholesterol; TC = Total cholesterol; TG = Triglycerides.

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**Figure 5.** Improvement rate (Δ) of TC/HDL-C report in Pre vs. Post program. *$p<0.05$, Significant difference compared with continuous training. Abbreviations: TC = Total cholesterol; HDL-C= High density lipoprotein; NS = Non-smokers; CS = Cigarette’s smokers; HS= Hookah smokers.

**Figure 6.** Improvement rate (Δ) of high density lipoprotein (HDL-C) in Pre vs. Post program. *$p<0.05$, Significant difference compared with continuous training. Abbreviations: HDL-C= High density lipoprotein; NS = Non-smokers; CS = Cigarette’s smokers; HS= Hookah smokers.
capacity; but improvement under the effect of MIT is more transparent and statistically more significant and different to the LCT.

Some studies 51-56 that examine the effects of different training methods on lipid profile, showed controversial results; but to our knowledge, it has not been demonstrated whether continuous or intermittent training would have more favorable effects on blood lipid profile, in particular among adult smokers unable or unwilling to quit. Indeed, a broad consensus in the literature 51,54 and the conclusions of this study have shown that MIT has no effect on the TC rate and LDL-C levels. However, other studies have shown that interval training can effectively decrease the TC rate 55,57 and LDL-C 56, but, it has no effect on HDL-C levels 56,58. In contrast, in some studies 53,54, including our present study, the MIT program is found to increase HDL-C. A possible explanation underlines why some studies have found a decrease in HDL-C levels have used, perhaps, samples with low basal levels of HDL-C with study periods over short. In contrast, the HDL-C was significantly increased in subjects of the three groups under the effect of the LCT. Our results are almost equivalent to those reported by Donnelly et al 53 that showed an increase in HDL-C after a continuous training. These results tend to be in the same line with the preceding findings on the effects of continuous training with low-intensity exercises on lipid profile 59.

Following the LCT Protocol, the TC rate showed a significant decrease only for smokers’ group (p<0.01), but it did not change in the non-smoking group. The HDL-C/TG report did not change significantly in the two smoker groups. Also, no significant differences were observed in the concentrations of lipids and lipoproteins between smoker groups. Nevertheless, there was a notable difference between the CS and NS groups concerning TC (p<0.05) and between smokers and non-smokers for HDL-C/TG report (p<0.05).

Similar to this case study, which significantly show a drop in the TC / HDL-C report, there are many other studies showed a drop in this variable 55,68. Furthermore, the report of smokers has showed that following a regular or irregular activity has no influences on triglycerides and HDL-C/TG. These findings agreed with those of Frey et al 58.

In conclusion, this study showed that the MIT was not related with favorable changes in the lipid’s levels in all smokers’ participants whatsoever of cigarette or hookah. In contrast, the LCT program has favorably altered lipid and lipoprotein profile of sedentary smokers and thus reduced their cardiovascular risk.

The variety of different training methods make it easy the attempt to determine if such type of training can favorably affect lipids and oxidative stress. Therefore, in our study the MIT and LCT programs could be used as two effective methods to respectively ameliorate antioxidant status and lipid profile for smokers. The current study stressed another demonstration concerning the most effective method of training that can be advised and recommended for smokers.

We suggest that a varied training method (with combined continuous and intermittent exercises), could be considered as a solution for smokers at increased risk of cardiovascular disease and oxidative stress.

Conclusions

The results of this study indicate that cigarette or hookah smokers have lowered levels of antioxidant defense before training protocol with impaired lipid profile.

Quitting smoking is with no doubt the best approach to minimize smoking caused diseases, but the success rates among those who try to quit are dismal. Nevertheless, the moderate-intensity intermittent training was linked to the improved antioxidant defense capacity, much better than the LCT program. In contrast, this last modified more favorably lipids profile and therefore minimizing the risk of cardiovascular diseases.

The combination of both training methods (mixed training: continuous and intermittent exercises combined), could have major implications in the prevention and defense programs. Smokers who are unable to quit could focus to improve their leisure time with physical activities (mixed training) to mitigate the smoking harms.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

The writers want to express their sincere appreciation to all collaborators and volunteers for their contribution in implementation of the experimental protocol of this study. We are thankful to all the members who were involved so willingly in the study.
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