J. Phys. Ther. Sci. 28: 983–991, 2016

The Journal of Physical Therapy Science

Original Article

Exercise intervention as a protective modulator against metabolic disorders in cigarette smokers

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Abstract. [Purpose] assess the impact of exercise intensity on desire to smoke, serum cotinine, stress hormones, total antioxidant capacity, and oxidative free radicals as potential markers of cardiopulmonary metabolic disorders were measured.in cigarette smokers. [Subjects and Methods] The participants (150 randomly selected healthy men, aged 18–55 years) were classified into 4 smoking groups: control (non-smokers; N= 30); mild (N = 33); moderate (N = 42), and heavy (N = 45). The participants were assigned to either moderate (8 weeks) or short-term (20–45 min) exercise training. The desire to smoke, Mood and Physical Symptoms Scale, and Subjective Exercise Experiences Scale scores, cotinine, stress hormones (cortisol and testosterone), free radicals (malondialdehyde, nitric oxide), and total antioxidant capacity were evaluated. [Results] Significant increases in serum cotinine, cortisol, testosterone, nitric oxide, and malondialdehyde levels and a reduction in total antioxidant capacity activity were observed in all smoker groups; heavy smokers showed a higher change in metabolites. In all smoker groups, both short and moderate- intensity exercises significantly reduce cotinine, cortisol, testosterone, and malondialdehyde and increased nitric oxide levels and total antioxidant capacity activity; further, the desire to smoke, Mood and Physical Symptoms Scale, and Subjective Exercise Experiences Scale scores were reduced. This supports the ability of exercise to increase nitric oxide bioavailability, enhance of blood vessels function and ultimately decrease the incidence of cardiopulmonary disorders. [Conclusion] Exercise interventions with varying intensities may be used as nicotine replacement therapy or protective aids against smoking-related cardiopulmonary disorders.

Key words: Physical activity, Cigarette smoking, Cardiopulmonary metabolic disorders

(This article was submitted Nov. 19, 2015, and was accepted Dec. 12, 2015)

INTRODUCTION

Cigarette smoking is considered one of the most common causes of premature death, mortality, and morbidity in heavy smokers in developed and industrial countries. The risks of cancer, particularly lung cancer and cardiopulmonary diseases are much greater in smokers than in non-smokers[1–4)]. Smoking was reported to promote the initiation and acceleration of pathological diseases via an oxidative stress free radical mechanism[5–7)].

The cotinine level is considered one of the most reliable serum markers refers to the level of nicotine uptake, because cotinine increases according to the number of cigarettes smoked per day in a dose-dependent manner. Therefore, cotinine levels might be a reliable marker of measuring the risk of smoking-related diseases from heavy cigarette smoking especially, lung cancer[8)].

Further, nicotine and tobacco were reported to have negative effects on endocrine function in heavy smokers Nicotine binds to acetylcholine receptors and causes the release of dopamine in the central nervous system (CNS). Several studies showed that nicotine has ability to alter hormonal homeostasis in both men and women. Smoking affects pituitary, thyroid,

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adrenal, testicular, and ovarian functions, calcium metabolism, and insulin action[9].

Acutely, smoking increases cortisol and testosterone levels at a rate that depends mainly upon nicotine exposure and inhalation. This action produces nitric oxide (NO) mediators which are responsible for inhibition of hypothalamic- pituitary-adrenocortical (HPA) activity, resulting in hormonal disorders[10, 12].

The changes in the levels of stress hormones in smokers are associated with nicotine uptake, whereas the function of the HPA axis is involved in the addictive process. Further, the levels of cortisol and testosterone were shown to have drastic effects on biological systems, with long term smoking associated with the promotion of cardiovascular disease, metabolic syndrome, and psychological stress[13].

Regarding smoking cessation, which is a painful process for smokers, stress hormones such as cortisol and testosterone are markers that are directly correlated with smoking status, and the changes the levels of these hormones may be a good indicator to assess the response to smoking cessation[13, 14]. Although combination therapy, such as a nicotine alternative along with behavioral support, showed remarkable effect in trials, others promising treatments for helping smokers who are unable to quit are still needed[8, 15]. Besides smoking, physical inability is considered quit harmful for human health, because it promotes chronic diseases and premature death[16].

Physical exercise has been regarded as a useful strategy for smoking cessation, and evidence indicates that exercise dramatically reduces craving and withdrawal symptoms in adults[17, 18]. Nevertheless, smokers attempting to quit do not usually receive any counseling on the benefits of exercise from their physicians, who do not know how to prescribe exercise[19]. Following smoking cessation, acute tobacco withdrawal symptoms such as stress, anxiety, mood changes, depression, weight gain, and sleep abnormalities may be ameliorated by increasing physical activity through exercise[19–21].

To explore further the importance of physical activity as a modulator against the occurrence of metabolic disorders and cardiopulmonary diseases, the impact of exercise intensity on the desire to smoke, serum cotinine, stress hormones, total antioxidant capacity (TAC), and oxidative free radicals as potential markers of cardiopulmonary metabolic disorders were assessed in a cross-sectional survey of healthy cigarette smokers.

SUBJECTS AND METHODS

A total of 150 healthy adult men (age range, 18–55 years) were included in this study after electoral roll randomized selection. The participants were classified into 4 groups according to smoking habit: control (non-smokers; N=30); mild (pack-years 10–15; N = 45); moderate (pack-years 16–20; N = 40), and heavy (pack-years≥20; N = 35). Health and physical activity statuses were assessed according to pre-validated questionnaires. Participants who had health-related problems such as recent musculoskeletal injuries, metabolic disease, and current illness were excluded.

The participants were subjected to both short-term and regular physical exercise programs after written informed consent was obtained. The study protocol followed the principles of the Helsinki Declaration and the Ethics Committee of Rehabilitation Research Chair (RRC), King Saud University, Riyadh, KSA, approved the protocol, under file number (ID: RRC-2013-014). All demographic parameters of the participants are shown in Table 1.

Table 1. Physiological characteristics of the participants

Variables	Smokers (N=120)	Non-smokers (N=30)
Age (years)	36.9 (6.5)	36.8 (5.8)
Height (cm)	165.7 (5.5)	161.7 (4.3)
Weight (kg)	68.9 (11.6)	58.6 (7.6)
Body mass index (kg/m^2)	24.8 (3.7)	21.6 (2.4)
Smoking behavior :		
Smoking years:		
Smoking volume (pack- years; %)	4.7 (3.5)	None
Mild (10–15 pack- years)	45 (37.5%)	None
Moderate (16–20 pack- years)	40 (33.3%)	None
Heavy (≥20 pack- years)	35 (19.2%)	None
Educational level:		
Less than primary and primary	47 (39.2%)	25 (20.8%)
Secondary	51 (42.5%)	35 (29.2%)
University	22 (18.3%)	60 (50.0%)
Estimated VO_2 max, (mL kg^{-1} min^{-1})	26.8 (8.5)	35.5 (9.3)
Self-determined morbidity and medical history	None	None

The values are expressed as the mean (SD)

The smoking history was elicited in detail from the data collected from the questionnaires regarding smoking habits, and the smoking pack year was then calculated by using the following formula, {(Number of cigarettes smoked per day × Number of years smoked)/20}. Withdrawal symptoms such as irritability, depression, tension, restlessness, difficulty concentrating, and stress, as well as happiness and energy were measured by using the Mood and Physical Symptoms Scale (MPSS; score 1–7 as previously reported)[22, 23].

Each subject was provided with a general explanation of the cardiopulmonary exercise test procedure, a brief description about the exercise test environment, and education regarding the signs and symptoms related to the termination of exercise test at least one day before the exercise test. All participants signed a written consent form before undergoing the exercise test and the exercise programs. The exercise test was performed by all subjects on an electronic treadmill at a moderate intensity (40–60%) of heart rate reserve according to the Karvonen formula[24]. The exercise test was performed to give the participants physical activities corresponding to 30–45% of VO2max uptake.

To study the effects of the short- term exercise intervention, the participants were required to exercise for 20–45 min. Measurements were taken just before the intervention, during exercise, just after the effort, and 5 to 30 minutes after completion of the exercise period. To measure the effects of the regular exercise intervention, subjects participated in 8 weeks of supervised walk-training. Training was conducted 6 days per week for 8 weeks. Following a warm up, the subjects performed walking (50 m/min for 25 min with 1–5 min of rest between sessions) was performed on a motor-driven treadmill.

Blood samples were collected from all participants (pre- and post-exercise) following an overnight fast. The serum samples were separated and stored at −80 °C until reuse. All samples were taken at the same time of day for each participant. The serum sample was used to estimate levels of the nicotine metabolites (cotinine), and cortisol by using enzyme-linked immunosorbent assay (ELISA) kits.

Cotinine was measured as a parameter of the nicotine level in the serum of all participants by using the immunoassay cotinine ELISA Direct Kit (catalog no. BQ 096D; BIO-QUANTCO. San Diego, CA, USA). The optical density (OD450) of the test wells was read at a wavelength of 450 nm with an automated microplate ELISA reader within 15 minutes after adding the stopping solution. Apparent cotinine concentrations of each sample were calculated by using a standard curve known cotinine concentrations.

Saliva samples were obtained from each subject during a rest day (24 hours without training). All saliva samples were collected mid-morning (9:00–11:30 AM), because cortisol levels are high in the early morning and diminish to optimum control levels thereafter. ELISA kits were used to measure the concentrations of salivary cortisol (Diagnostics Biochem Canada, Inc.) and testosterone (Cat no., 11-TESHU-E01-SLV, ALPCO Diagnostics, Inc.) in human saliva in duplicate according modified procedures suggested by the manufacturer.

Plasma total antioxidant capacity (TAC), malondialdehyde (MDA), and NO free radical concentrations were measured as the antioxidant profile in all participants. Plasma TAC was measured by using a colorimetric assay kit (Catalog #K274-100; BioVision, CA, USA). The data are reported according to the manufacturer recommendation.

The plasma NO concentration was estimated as nitrate and nitrite by using HPLC technology as previously reported[25]. The concentration of nitrite was estimated by using Griess reagent and the absorbance of the developed azochromophor compound was measured at 540 nm, which is directly related to the concentration of nitrite in samples.

Plasma MDA was estimated by using a colorimetric assay kit (Cat. No. MAK085, Sigma-Aldrich Co. LLC, USA). The MDA in the sample reacts with thiobarbituric acid (TBA) to generate a MDA-TBA adduct, which can be easily quantified calorimetrically at 532 nm. The level of MDA was calculated from a standard curve. The data are expressed as µmol/l.

Statistical analysis was performed by using SPSS version 17. The data were tabulated as the mean and standard deviation. The comparison between the study groups was calculated with the Student's t-test, and the correlation between studied variables was measured by using Pearson's correlation coefficient. P-values < 0.05 were considered significant.

RESULTS

None of the volunteers reported smoking side-effects like tachycardia or nausea. The participants were classified according to cigarette smoking into 2 groups; 120 smokers with a smoking period of (4.7 ± 3.5) and a mean age of (36.9± 6.5 years), and 30 non-smokers with a mean age of (36.8 ± 5.8 years). The smoker group was further classified according to smoking volume into 3 groups, mild group (10–15 pack-years), moderate group (16–20 pack-years), and heavy group (≥20 pack-years). The frequency distribution of smokers by cigarette consumption, smoking duration, demographic, and baseline is provided in Table 1.

In order to determine whether the levels of serum cotinine, cortisol, and testosterone changed during exercise among the smokers, the participants were subjected to short-term and moderate aerobic exercise (Table 2). Significant changes ($p < 0.001$) in the levels of cotinine, cortisol, and testosterone were observed in subjects treated who performed both short-term and moderate exercise. However, a more significant reduction was found in all parameters was reported in subjects who underwent moderate aerobic exercise training compared with short- term training. The levels of cotinine per cigarette smoked showed substantial variability, as observed in heavy smokers compared with mild and moderate smokers.

When the mean levels of serum cotinine, cortisol, and testosterone were compared between short-term and moderate exercise training, a greater decline in cortisol, testosterone, and cotinine was found in heavy smokers compared with mild

and moderate smokers; these differences were significantly ($p < 0.001$) related to self-reports indicating a greater number of cigarettes smoked. These results suggest that a positive relationship exists between nicotine involvement, stress hormones, and physical activity level in cigarette smokers.

The effects of short-term and moderate exercise interventions on NO (nitrate and nitrite concentrations), MDA, and TAC as biomarkers of oxidative free radicals were measured in all smoker groups. All groups had a significant reduction in lipid peroxide MDA, an increase in the level of nitrite and nitrates, and a higher TAC level following short-term and moderate exercise interventions. Further, exercise had a positive effect in non-smokers, as shown by significant improvements ($p < 0.01$) in the levels of nitric oxide, MDA, and TAC; these data support the preventive effect of exercise against oxidative stress-related human disorders (Table 3).

In order to evaluate the effect of physical exercise on the behavioral and psychological status of smokers, the desire to smoke and the MPSS and Subjective Exercise Experience Scale (SEES) scores were determined at baseline and after 30 min of both short-term and moderate exercise training. The data for the strength of the desire to smoke, withdrawal symptoms, and the exercise-induced effects in the groups over time (pre- and post-training) are shown in Table 4.

Follow-up independent tests at each time point revealed that those performing moderate exercise reported significantly higher improvement scores of the MPSS and Subjective Exercise Experience Scale (SEES) ($p < 0.001$) during exercise compared with short-term training conditions. Significant differences in all scores were observed between the groups at 30 min after exercise. The data showed a significant improvement in MPSS and SEES scores as well as a reduction in the desire to smoke ($p < 0.001$) in heavy smokers who underwent moderate aerobic training compared with short-term training.

The data showed significant variation when compared to the baseline ratings. Further, physical activity, MPSS and SEES scores, and the desire to smoke were significantly correlated ($p < 0.001$) with the levels of cotinine and stress hormones (cortisol and testosterone) in mild, moderate, and heavy smokers. These data collectively show that cortisol, cotinine, and

Table 2. Effect of exercise intervention on serum cotinine and stress hormone levels in smokers and non-smokers (n=150)

Variables	Short -term exercise in smokers (N=60)						Non-smokers (N=15)	
	Mild (N= 15)		Moderate (N= 20)		Heavy (N= 25)			
	Pre-test	Post-test**	Pretest	Post-test**	Pre-test	Post-test***	Pre-test	Post-test**
Serum cotinine (ng/ml)	95.5 (16.5)	85.5 (9.5)	165 (16)	155 (13.7)	350 (25.3)	310 (18.4)	12.3 (4.5)	11.2 (3.4)
Serum cortisol [C] (pg/ml)	25.6 (11.4)	18.3 (9.1)	31.8 (9.1)	27.9 (5.7)	45.7 (7.8)	35.2 (9.1)	8.9 (2.3)	5.1 (1.8)
Testosterone [T] (pg/ml	22.1 (4.3)	20.7 (3.78)	29.1 (5.7)	25.8 (4.6)	51.7 (5.3)	41.5 (4.8)	11.3 (1.8)	9.4 (1.2)
Variables	Moderate aerobic exercise in smokers (N=60)						Non-smokers (N=15)	
	Mild (n= 18)		Moderate (n= 22)		Heavy (n= 20)			
	Pre-test	Post-test**	Pre-test	Post-test**	Pre-test	Post-test***	Pre-test	Post-test**
Serum cotinine (ng/ml)	100 (18.2)	75.3 (5.8)	172 (12)	135 (10.3)	280 (15.6)	215 (11.7)	13.9 (2.4)	12.9 (3.7)
Serum cortisol [C] (pg/ml)	35.1 (8.6)	22.3 (6.7)	47 (10.8)	37.5 (4.5)	56.2 (8.1)	37.5 (5.8)	10.3 (1.9)	6.1 (0.9)
Testosterone [T] (pg/ml	25.1 (5.7)	18.5 (3.8)	35.4 (4.3)	21.7 (3.7)	65.2 (6.1)	28.7 (3.5)	12.7 (2.9)	7.5 (1.7)

Mild smokers (10–15 pack-years); Moderate (16–20 pack-years); heavy (≥20 pack-years). All values represent the mean (SD).
*p < 0.05; **p < 0.01; ***p < 0.001

Table 3. Effect of exercise intervention on the levels of NO, MDA and TAC in smokers and nonsmokers (n=150)

Variables	Short-term exercise in smokers (N=60)						Non-smokers (N=15)	
	Mild (N= 15)		Moderate (N= 20)		Heavy (N= 25)			
	Pre-test	Post-test**	Pretest	Post-test**	Pre-test	Post-test***	Pre-test	Post-test**
Nitrate and nitrite (µmol/L)	12.9 (2.5)	28.5 (3.7)	18.6 (1.6)	31.7 (3.7)	10.9 (2.6)	35.9 (3.8)	32.3 (4.5)	39.6 ±3.7
MDA (µmol/l)	15.4 (7.6)	11.2 (2.4)	22.8 (8.3)	15.9 (2.6)	31.2 (3.8)	21.2 (3.4)	9.6 (2.3)	6.3 ±2.5
TAC (nmol / mM Trolox eq.)	9.5 (2.7)	15.2 (3.8)	6.9 (6.8)	18.2 (3.9)	7.9 (2.7)	22.3 (3.9)	16.7 (4.8)	19.8 ±3.7
Variables	Moderate aerobic exercise in smokers (N=60)						Non-smokers (N=15)	
	Mild (N= 18)		Moderate (N= 22)		Heavy (N= 20)			
	Pre-test	Post-test**	Pre-test	Post-test**	Pre-test	Post-test***	Pre-test	Post-test**
Nitrate and nitrite (µmol/L)	10.5 (1.2)	25.3 (3.2)	12.3 (2.1)	28.4 (3.7)	11.8 (5.6)	29.4 (3.2)	33.7 (2.7)	42.8 (3.2)
MDA (µmol/l)	15.4 (3.6)	11.2 (3.7)	21 (3.8)	12.3 (4.5)	26.5 (8.7)	12.9 (4.5)	8.9 (1.9)	7.2 (1.1)
TAC (nmol / mM Trolox eq.)	10.4 (1.8)	18.5 (4.9)	8.2 (4.6)	26.9 (4.8)	6.5 (6.1)	21.3 (2.5)	12.7 (4.2)	22.4 (2.5)

Mild smokers (10–15 pack/years); Moderate (15–20 pack /years); heavy (≥20 pack/years). All values represent the mean (SD).
*p < 0.05; **p < 0.01; ***p< 0.001

testosterone concentrations are strongly and positively correlated with MPSS and SEES scores and the type of physical activity (Table 5).

DISCUSSION

Smoking is associated with a markedly increased risk of ischemic stroke, myocardial infarction, and aortic aneurysm. It is also associated with increases in cholesterol, arterial stiffness, and blood pressure[26]. Clinical evidence shows that physically active smokers live longer than inactive smokers; therefore, the main reason for advising smokers to exercise is that physical activity has a protective effect against some of the main diseases observed in heavy smokers.

Sedentary smokers are at an even higher risk of cardiovascular disease compared with physically active smokers, and epidemiological studies have confirmed that the signs of cardiovascular disease can be attenuated by exercise[27].

In this regard, the impact of exercise intensity on the desire to smoke, serum cotinine, stress hormones, TAC, and oxidative free radicals as potential markers of cardiopulmonary metabolic disorders were investigated in a cross-sectional survey of healthy cigarette smokers in this study.

Recently, serum cotinine levels were shown to have a direct quantitative correlation with the number of cigarettes smoked; therefore, cotinine is considered the principle metabolite of nicotine and its concentration is feasibly used as a biomarker in epidemiological studies[28].

The data in the present study clearly show higher serum cotinine levels in cigarette smokers compared to control non-smokers. Serum cotinine levels are a consequence of tobacco exposure, and a significantly higher cotinine concentration was

Table 4. Effect of exercise intervention on the strength of the desire to smoke, withdrawal (MPSS), and mood (SEES) in smokers (n=120)

	Short-term exercise in smokers (N=60)					
	Mild (N= 15)		Moderate (N= 20)		Heavy (N= 25)	
	Pre-test	Post-test**	Pre-test	Post-test**	Pre-test	Post-test***
Desire to smoke	5.65 (1.17)	5.9 (0.27)	4.56 (0.57)	4.2 (0.13)	6.5 (0.32)	5.1 (0.16)
Irritability	2.75 (1.45)	2.45 (0.45)	3.9 (1.23)	3.0 (0.35)	4.6 (1.7)	4.0 (0.45)
Depression	1.85 (0.85)	1.35 (0.35)	2.45 (0.65)	2.15 (0.55)	3.84 (0.75)	3. 4 (0.65)
Tension	2.56 (1.36)	2.16 (0.65)	3.46 (1.21)	3.16 (0.21)	5.4 (1.4)	4.9 (0.41)
Restlessness	3.5 (1.67)	2.75 (0.15)	3.5 (1.31)	3.1 (0.38)	4.2 (1.41)	3.7 (0.47)
Difficulty concentrating	2.81 (1.1)	2.1 (0.18)	3.9 (1.20	3.4 (0.26)	4.5 (1.170	4.1 (0.27)
Stress	3.12 (1.38)	2.95 (0.29)	4.15 (1.41)	3.75 (0.49)	5.3 (1.3)	4.3 (0.24)
Happiness	4.15 (1.28)	5.82 (0.21)	3.95 (1.43)	4.95 (0.53)	2.85 (1.32)	4.85 (0.42)
Energy	3.55 (1.28)	3.81 (0.31)	4.6 (1.31)	4.9 (0.36)	3.6 (1.5)	3.9 (0.85)
SEES-PWB	4.12 (0.74)	4.85 (0.27)	4.7 (0.84)	4.92 (0.64)	3.7 (0.75)	4.21 (0.42)
SEES-PD	2.45 (0.53)	2.15 (0.21)	3.5 (0.730	3.18 (0.53)	3.9 (0.93)	3.3 (0.53)
SEES-fatigue	2.51 (1.13)	2.53 (0.26)	3.81 (1.16)	3.95 (0.45)	4.81 (1.6)	4.85 (0.87)
	Moderate aerobic exercise in smokers (N=60)					
	Mild (N= 18)		Moderate (N= 22)		Heavy (N= 20)	
	Pre-test	Post-test**	Pre-test	Post-test**	Pre-test	Post-test***
Desire to smoke	6.4 (1.30)	5.3 (0.29)	4.9 (0.61)	3.9 (0.32)	7.6 (0.47)	4.8 (0.22)
Irritability	3.45 (1.2)	3.0 (0.21)	4.1 (1.3)	3.4 (0.42)	5.3 (1.5)	3.5 (0.42)
Depression	2.9 (1.5)	2.1 (050)	3.1 (1.2)	2.8 (0.25)	3.9 (0.98)	2.8 (0.48)
Tension	3.6 (1.4)	3.0 (0.41)	4.5 (1.3)	4.1 (0.35)	5.9 (1.75)	4.5 (0.61)
Restlessness	3.7 (1.8)	3.1 (0.25)	3.9 (1.9)	3.0 (0.71)	4.5 (1.5)	3.5 (0.35)
Difficulty concentrating	3.9 (1.3)	3.0 (0.21)	4.2 (1.5)	3.8 (0.50)	5.1 (1.2)	4.1 (0.21)
Stress	3. 8 (1.4)	3. 1 (0.51)	4.2 (1.3)	3.7 (0.34)	5.7 (1.9)	4.3 (0.31)
Happiness	3.5 (1.3)	4.9 (0.71)	2.5 (1.1)	3.7 (0.34)	1.6 (0.85)	3.7 (0.91)
Energy	3.9 (1.5)	4.7 (0.75)	2.9 (1.3)	3.9 (0.21)	2.0 (0.97)	3.7 (0.67)
SEES-PWB	3.7 (0.8)	3.9 (0.64)	3.2 (0.7)	3.6 (0.61)	2.85 (0.42)	3.28 (0.71)
SEES-PD	2.7 (0.64)	2.0 (0.23)	3.7 (0.74)	3.0 (0.31)	4.2 (0.65)	3.82 (0.51)
SEES-fatigue	3.7 (1.4)	3.51 (0.51)	4.3 (1.5)	3. 9 (0.31)	5.4 (1.7)	5.1 (0.52)

For all of the above variables, responses were rated on a 7-point scale ranging from 1 (low amount of the variable) to 7 (high amount of the variable). All values represent the mean (SD).* $p < 0.05$; **$p < 0.01$; *** $p < 0.001$

observed in heavy smokers (i.e., > 20 pack-years) compared to the mild and moderate smokers who participated in this study.

These data are in accordance with other studies showing significant correlations between serum cotinine concentrations and the quantity of cigarettes smoked. A previous study suggested that the salivary cotinine concentration increases in a non-linear manner according to the increase in the number of cigarettes smoked[29].

Further, the effect of smoking on stress hormones was evaluated in this study, and a significant increase ($p = 0.001$) in both serum testosterone and cortisol concentrations was reported in all smoking groups compared with the non-smoker group. Smoking activates the HPA, stimulates adrenocorticotropic hormone (ACTH) secretion, and enhances the effect of ACTH on the adrenal cortex, which results in stimulation of cortisol production. Among other effects, this activation is associated with the lower sensitivity of nicotine receptors in the CNS. Further, an elevated serum testosterone level was reported in both males and females, and the increase was positively correlated with the number of smoked cigarettes[30].

The release of NO and oxidative free radicals such as MDA in biological systems plays a significant role in the maintenance of vascular normal homeostasis and reduction of vascular disease[31]. The colorimetric assay estimations of MDA and the plasma levels of inorganic nitrites and nitrates, which are the final stable metabolites of the NO metabolic pathway, provide more details about the drastic effects of cigarette smoking[32–34].

In the current study, a significant increase in lipid peroxide MDA free radical ($p = 0.001$) and a decrease in NO and TAC levels ($p = 0.001$) was found in all smoker groups compared with the non-smoker group. These data are consistent with a previous study[35], which reported a significant increase in MDA and lower NO levels in smokers. The basic mechanism of cigarette smoking in producing cardiovascular diseases depends on the generation of oxidative free radicals and a decrease in the bioavailability of NO, which plays a vital role in the regulation of endothelium structure and function[36].

Regarding the effect of smoking on antioxidant status, many studies reported a significant decrease in antioxidant status and higher MDA values in participants with different smoking intensities[37, 38]. Further, after cessation of cigarette smoking, antioxidant capacity and its effect against oxidative free radical damage increase significantly[39, 40].

Generally, interventions for smokers willing to quit use similar approaches: exercise programs that last 8 to 12 weeks and contain 1 to 3 sessions per week, with a focus on some kind of aerobic activity that lasts about an hour[41, 42].

In this study, the effect of exercise on the concentrations of serum cotinine and stress hormones (i.e., cortisol and testosterone) was measured in cigarette smokers with various smoking intensities. Mild, moderate, and heavy smokers who performed moderate exercise for 8 weeks showed significant decreases ($p = 0.001$) in the concentrations of serum cotinine, cortisol, and testosterone compared with baseline. Although similar results were observed in subjects who performed short-term aerobic exercise, the changes were smaller than those observed when the subjects performed moderate training. Different intensities of physical exercise were shown to have a positive impact on smoking habits via changes in the levels of cortisol and testosterone concentrations among smokers[43], whereas moderate-intensity exercise interventions were shown to reduce cravings in smokers via reduction in stress hormones[44].

Stress hormones, especially cortisol, may be linked to nicotine metabolism because sensitivity to nicotine tends to be reduced under conditions of enhanced corticosteroid activity[11, 13]. Cortisol interacts with several neurotransmitters that mediate the effects of nicotine[45, 46]; this explains the positive effect of moderate aerobic exercise, which plays a significant role in decreasing the level of stress hormones, thereby minimizing the cotinine level in cigarette smokers. Similarly, in the

Table 5. Association between smoking desire, withdrawal (MPSS), mood (SEES), and physical activity with cotinine and stress hormone levels in cigarette smokers (n = 120)

	Cotinine level (ng/ml)	Cortisol level (pg/ml)	Testosterone (pg/ml)
Desire to smoke	0.52**	0.73**	0.46**
Irritability	0.53**	0.57**	0.47**
Depression	0.75**	0.65**	0.61**
Tension	0.67**	0.48**	0.58**
Restlessness	0.02**	0.05**	0.03**
Difficulty concentrating	0.10* *	0.09* *	0.14**
Stress	0.18**	0.28**	0.13**
Happiness	0.15**	0.11**	0.13**
Energy	0.14**	0.15**	0.45**
SEES-PWB	0.11**	0.17**	0.36**
SEES-PD	0.25**	0.56**	0.25**
SEES-fatigue	0.14**	0.63**	0.46**
Physical activity level			
Active PA	0.16**	0.42**	0.28**
Moderate	0.13***	0.32***	0.31***

* $p < 0.05$; **$p < 0.01$; *** $p < 0.001$

current study, we observed a significant improvement in antioxidant status in smokers following exercise interventions. Significant increases in NO concentrations and TAC activities along with a reduction in MDA oxidative lipid peroxide were found. These data are in accordance with many reports that demonstrated the importance of exercise training in protecting against oxidative stress via an improvement in antioxidant status[46–48]. Regarding NO concentrations, our data are consistent with reports showing a significant increase in NO level following exercise training. The NO radical molecule can inhibit lipid peroxidation by scavenging propagatory lipid peroxyl radicals[49]; this function may add to the importance of exercise training as a modulator against oxidative damage because an increase in NO levels decreases the chance of vascular diseases in smokers via the dilatation of blood vessels[50].

Moderate-intensity exercise has been shown to produce both a greater psychological benefit and higher adherence rates in comparison with vigorous exercise; further, moderate-intensity exercise is more effective than light-intensity exercise in reducing withdrawal symptoms. Therefore, moderate-intensity activities should be prescribed when adding exercise to a smoking cessation program[51]. At least 30 min of moderate intensity physical activity should be undertaken at least 5 days a week for health benefits[52]. In the present study, compared with short-term aerobic training, moderate exercise resulted in a significant reduction in the strength of the desire to smoke and in the withdrawal symptoms reported in acutely abstaining adult smokers[53].

Moderate exercise resulted in a marginally significant greater reduction in cravings from pre- to post-exercise compared with short-term training. The present study demonstrated favorable acute (pre- to post-exercise) changes in affect and cigarette cravings. Previous studies showed that exercise counseling for smokers resulted in an increase in exercise levels from sedentary status, even in the long term, and an improvement in some psychological symptoms[51–55]. Similarly, it was shown that smokers who exercise find it easier to cope with withdrawal symptoms during and after intervention programs and a higher level of exercise participation during the intervention phase is related to a greater likelihood of achieving smoking cessation at the end of treatment[41].

In order to determine whether the changes that occur during exercise are related to changes in symptom ratings, correlations were calculated between all of the ratings regarding smoking withdrawal symptoms. Significant correlations were found between the serum cotinine, cortisol, and testosterone levels and the desire to smoke a cigarette, negative affect, irritability, depression, tension, restlessness, difficulty concentrating, stress, and physical activity for both groups that performed short-term and moderate aerobic exercise. Common withdrawal symptoms and the desire to smoke were rated lower during and immediately after exercise relative to the baseline ratings. These results are consistent with previous research concerning exercise and smoking[41, 51, 53, 54].

In conclusion, significant improvements in the level of serum cotinine, NO, MDA, TAC, stress hormones, and withdrawal symptoms in cigarette smokers were found following short-term and moderate exercise interventions. Further, the data suggest that exercise interventions play a significant role as a preventive measure against cardiovascular diseases and as nicotine replacement therapy among cigarettes smokers.

ACKNOWLEDGEMENT

The project was full financially supported by King Saud University, through Vice Deanship of Research Chairs, Rehabilitation Research Chair.

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