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Research article

INFLUENCE OF ACUTE EXERCISE ON OXIDATIVE STRESS IN CHRONIC SMOKERS

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ABSTRACT

The relative oxidative insult caused by exercise and smoking on biological systems are well documented, however, their cumulative influence needs to be clarified. In order to examine the collective effects of exercise and smoking on oxidant and antioxidant parameters, young male smokers (n=10) and non-smokers (n=10) made to perform a negative slope (10%) cycling exercise for 30 minutes at individual load equivalent to 60% maximal oxygen consumption (VO_2max). Pre- and post-exercise (post-ex) haematocrit, haemoglobin, white blood cells, plasma malondialdehyde (MDA) levels, protein carbonyl formation and non-HDL oxidation, erythrocyte superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities, serum ceruloplasmin (CER) and urinary cotinine concentrations were evaluated. Pre-ex CER and urinary cotinine concentrations of smokers were significantly higher ($p<0.05$ and $p<0.01$, respectively) compared to that of non-smokers and pre-ex CER concentrations were significantly correlated with cotinine levels in all subjects ($p<0.05$). Significant ($p<0.01$) increases were observed in non-HDL oxidation following the exercise in both groups and the elevations were more pronounced in smokers. Pre-ex SOD and GPX activities were not different between the two groups, however post-ex enzyme activities were significantly reduced in smokers ($p<0.05$). MDA and protein carbonyl concentrations were not different between the two groups and there were not any significant changes due to exercise.In conclusion, according to the results of the present study, we suggest that erythrocyte antioxidants SOD and GPX and plasma non-HDL are more prone to the possible oxidant damage of acute physical exercise in chronic smokers.

KEY WORDS: Exercise, smoking, protein oxidation, non-HDL oxidation, superoxide dismutase, glutathione peroxidase

INTRODUCTION

Oxygen free radicals are highly reactive species that can cause a wide spectrum of cell damage including enzyme inactivation, lipid peroxidation, protein and lipoprotein oxidation; and various factors are reported to cause free radical generation in biological systems (Ji, 1995). Physical exercise, which is associated with an increased free radical generation primarily due to a dramatic increase in oxygen uptake (Sjödin et al., 1990, Khanna et al., 1999, Gul et al., 2003), and cigarette smoking, which causes inhalation of various oxidant and prooxidant compounds (Kalra et al., 1991) are two of these factors. As the number of exercising smokers in the population is quite large, the collective effects of these factors on oxidative injury deserve interest.

Various oxidation indices and antioxidant parameters have been evaluated to investigate the effects of physical activity or smoking in human subjects. However, a precise inference on how these parameters are influenced has not been reached yet. Observing the changes in the activity of antioxidant enzymes is a very widely used way to show the effect of oxidative stress on antioxidant status.

Although erythrocyte superoxide dismutase (SOD) and glutathione peroxidase (GPX) are two of these enzymes which are very frequently used as markers of antioxidant status in human studies, there is still a controversy between the results reporting either increased, unchanged or decreased SOD and GPX activities due to exercise (Sjödin et al.1990; Marzatico et al., 1997; Aslan et al., 1998; Tozzi-Ciancarelli et al. 2001). Studies investigating the antioxidant status in smokers indicate discrepant results as well (Duthie et al., 1991; Kaçmaz et al., 1997; Koçyigit et al., 2001; Kim et al., 2003). Oxidative injury to lipids and lipoproteins has also been reported both in smokers and exercising subjects (Sanchez-Quesada et al., 1995; Sanderson et al., 1995; Sanchez-Quesada et al., 1997; Gouaze et al. 1998; Wetzstein et al., 1998; Benitez et al., 2002; Urso and Clarkson, 2003). However, reports concerning the effects of smoking or exercise on these parameters are not in agreement. Similarly, individual effects of exercise and smoking on protein oxidation, which is usually expressed as protein carbonyl formation, have been studied on various subject groups, and different results have been reported (Panda et al., 1999; Pignatelli et al., 2001; Inayama et al., 2002).

While the differences between the results of exercise-studies can be partly explained by the differences in exercise models used and by the different training backgrounds and ages of the subjects in different studies, variations in the results of smoking-studies can be due to the differences in age, lifestyle or smoking habits of the subjects. These methodological and subject differences limit the comparison and interpretation of data from different reports.

Thus far, a comprehensive picture regarding the collective effects of exercise and smoking on oxidative injury is not clear. Taking into consideration the discrepancies between various studies and that the number of studies examining the collective effects of exercise and smoking on oxidation are very limited (Sanchez-Quesada et al., 1997; Sürmen-Gür et al., 1999), we aimed to examine their effects on oxidant and antioxidant parameters in the same subject group. Accordingly, in this report we investigated the oxidant damage due to acute oxidative stress that occurs upon exercise in subjects that are under chronic oxidant stress of cigarette smoking, and to our knowledge, the present study is the first in evaluating the cumulative influence of exercise and smoking on protein oxidation.

METHODS

Subjects and experimental groups

A group of 20 healthy, sedentary, male subjects volunteered for the study. The physical characteristics of the subjects in the two groups, who were aged between 18 and 34, were statistically alike (Table 1). All subjects showed normal body mass for height, had no unusual dietary habits, and none of the above was taking any kind of medication. After being informed about the study and test procedures, and the possible risk and discomfort that might ensue, they gave their written consent to participate; the investigation was in accordance with Helsinki Declaration of 1975, as revised in 1983 (Forty-First World Medical Assembly Declaration of Helsinki, 1990). The volunteers were classified as smokers (those who had smoked at least ten cigarettes a day for at least 1 year; n=10) and non-smokers (those never have smoked; n=10).

Table 1. Characteristics of subjects. Values are means (median; SEM).

	Non-smokers (n=10)	**Smokers (n=10)**
Age (years)	21.2 (20.0; 1.2)	21.1 (20.0; 1.6)
Height (cm)	175.4 (175.0; 1.8)	176.4 (176.5; 1.3)
Body mass (kg)	70.0 (72.7; 52.4)	71.0 (70.0; 2.6)
Cigarettes per day	NA	20.5 (20.0; 2.4)
Smoking years	NA	4.8 (4.5; 1.0)
Cotinine (nmol·mg^{-1} creatinine)	3.97 (2.86; 1.46)	17.78 (18.08; 2.90)**

NA, not applicable
** $p<0.01$, compared to non-smokers.

Exercise protocol

Submaximal test: Each subject performed an incremental submaximal test lasting 16 min in order to establish the relationship between oxygen consumption (VO_2) and work rate. Testing load on the cycle ergometer (Monark 814-E, Sweden) was increased every 4 min from an initial 60 W according to heart rate and rating perceived exertion of the subjects. A regression equation for VO_2 (dependent) and workload (independent) was calculated for each subject from the results of the 16 minutes submaximal test. If the "r" value of the equation was less than 0.97, the test was repeated.

Maximal test: On a subsequent visit to the laboratory, in order to evaluate maximal oxygen consumption (VO_2max), the subjects performed a maximal test following a 5min of warm-up by cycling (Monark 814E, Sweden) at 75 watt. The workload during the test was changed every 3min

from an initial 100 W by 60 W until the subjects informed that they could not exercise any more. The subjects were instructed to maintain the pedaling rate as close to 60 rpm as possible and the actual pedaling rate detected was 60±3 rpm for the tests. When the pedaling rate fell to 55 rpm, subjects were verbally encouraged to pedal faster. If the subject could not return to the required rpm, the test was terminated. During the submaximal and maximal tests, ventilatory parameters were continuously measured breath-by-breath using a metabolic analyzer of SensorMedics 2900C system (USA). Heart rates were recorded continuously from 4 chest electrodes and monitored over an oscilloscope (Cardiovit, Switzerland). The criteria for achieving VO_2max was evaluated as maximum heart rate with respect to age (220-age), V_E/VO_2 value close to 30 $L.min^{-1}$ and respiratory exchange ratio (RER) greater than 1.15. All test results were in accordance with the criteria. Individual workload equivalent to 60% of VO_2max was then calculated using individual regression equation and VO_2max.

Submaximal exhausting exercise: On a separate day, subjects came to the laboratory following overnight (8 hours) fasting and abstinence from smoking. They were also instructed to abstain from strenuous activity before testing. Subjects had a light breakfast consisting of 60 g lemon-cream cake (Eti Pop™; energy: 482 kcal, protein:5,32 g, carbohydrate:54,52 g, lipid:28,30 g in 1000 g) and 200 ml fruit juice (Aroma™: 51 kcal/100ml) 3 hours before the tests and were asked not to consume any other food or drink during tests except water. After resting for 20 minutes lying down on a bed, resting blood samples (pre-exercise) were collected. Following the rest, subjects performed a negative slope (10%) cycling exercise for 30 minutes at individual load equivalent to 60% VO_2max using pedaling rate as close to 60 rpm as possible.

Collection and treatment of blood samples

Pre- and post exercise blood samples were kept on ice until assayed. Haematocrit (Hct), was measured on the day of the experiment as were haemoglobin (Hb), and leukocyte (white blood cell, WBC) counts. Whole blood samples separated for SOD and GPX measurements were kept at +4 °C and studied within 48 hours, plasma separated for MDA and protein carbonyl content (PCC) measurements and sera separated for ceruloplasmin (CER) measurements were kept at –40 °C until assayed in one month. Plasma processed for non-HDL oxidation was studied on the day of the experiment. Except cotinine, all parameters were evaluated in both pre- and post-exercise blood samples.

Biochemical analyses

The Hct and Hb concentrations, and WBC counts were determined using Abott Cell-Dyn (USA). As an index of plasma lipid peroxidation, plasma malondialdehyde (MDA) concentrations were determined by measuring the thiobarbituric acid reactive substances according to the spectrophotometric method of Kamal et al. (1989), using 1,1,3,3-tetraethoxypropane (Fluka, Switzerland) as the external standard, and expressed as nanomoles per milliliter. The isolation and oxidation of non-HDL fraction was studied according to Zhang et al. (1994). MDA levels of the non-HDL fraction were measured at 0 and 180th minutes of incubation and expressed as nanomoles MDA per mg cholesterol, and their difference (Δ MDA) was used to evaluate susceptibility of non-HDL to oxidation. Plasma PCC was determined to evaluate protein oxidation as described by Reznick and Packer (1994), and was expressed as nanomole carbonyls per mg protein.

To evaluate blood antioxidant status erythrocyte SOD and GPX activities were measured using test kits (Randox, UK) and expressed as units per gram Hb. Serum CER concentrations were measured using the method described by Schosinsky et al. (1974) and expressed as units per milliliter.

Urinary cotinine values were determined by the spectrophotometric method of Barlow et al. (1987) and were expressed as nanomoles per milligram urinary creatinine, which was determined by using picric acid (Bauer 1982).

All plasma and serum post-exercise values were adjusted for haemoconcentration using the following equation, derived according to the suggestion of Van Beaumont et al. (1981) and used in evaluating the results:

$$post\text{-}exercise_{adjusted} = post\text{-}exercise\ [Hb_{pre}\ (100\text{-}Hct_{post})]\ /\ [Hb_{post}(100\text{-}Hct_{pre})]$$

Statistics

Selected physical characteristics of the patients were compared by Mann-Whitney U test. Mann-Whitney U test was also used to test the differences in baseline values between two groups. Comparison of pre- and post-ex values was made by nonparametric Wilcoxon signed rank test. The individual differences (% changes) between pre- and post-ex values for the variables were calculated by the formula: $\Delta S = Post - Pre / Pre \times 100$, and then the differences (% changes) between groups were compared by Mann-Whitney U test. Linear curve estimation regression analysis was used to calculate regression equation between VO_2 and workload; and between cotinine and CER values. The significance level was set at $p < 0.05$. Data presented in figure and tables are means (median; SE).

Table 2. Pre- and post-ex haematocrit (Hct), haemoglobin (Hb) and leucocyte (WBC) values of non-smokers and smokers. Data are means (median; SEM).

		Non-smokers (n=10)	Smokers (n=10)
Hct (%)	Pre-ex	42.47 (41.65; 1.02)	46.31 (46.5; 0.94) $^{\#}$
	Post-ex	45.16 (45.0; 0.9) **	47.93 (48.6; 0.86) *$^{\#}$
Hb(g· dl^{-1})	Pre-ex	14.03 (13.8; 50.31)	15.73 (16.05; 0.34) $^{\#\#}$
	Post-ex	14.93 (14.95; 0.29) **	16.36 (16.35; 0.33) *$^{\#\#}$
WBC (X10^{3}· µl^{-1})	Pre-ex	5.92 (5.93; 0.33)	5.89 (6.07; 0.35)
	Post-ex	7.13 (6.98; 0.47) **	6.85 (6.55; 0.41) *

* p<0.05 and ** p<0.01, significantly different from the pre-ex value.
$^{\#}$ p<0.05 and $^{\#\#}$ p<0.01, significantly different from non-smokers.

RESULTS

Hct and Hb concentrations at rest were significantly higher in smokers, and significant post-ex elevations in both groups indicated haemoconcentration effect due to exercise (Table 2). Pre-ex urinary cotinine concentrations were significantly higher in smokers compared to that of non-smokers (p<0.001) (Table 1). While pre-ex WBC counts were the same in both groups, post-ex values showed significant elevations compared to pre-ex levels and the difference remained to be significant after the adjustment for haemoconcentration (Table 2).

Plasma MDA concentrations and PCC did not show any significant difference between groups either before or after the exercise. Pre-ex and post-ex ΔMDA in non-HDL oxidation were not different between groups, as well. However, significant elevations in post-ex non-HDL oxidizability were observed in both smokers and non-smokers (Table 3); and the difference (%change) between pre- and post-ex non-HDL oxidizability was significantly greater in smokers (Fig. 1).

Erythrocyte SOD and GPX activities are given in Table 3. These antioxidant enzymes were not statistically different in two groups before the exercise, however post-ex SOD and GPX activities were significantly reduced in smokers. Also, pre- and post-ex GPX activities of smokers displayed significantly greater differences. On the other hand, significantly higher CER concentrations were measured in smokers compared to non-smokers at rest (Table 3) and pre-ex CER concentrations were found to be significantly correlated with the pre-ex urinary cotinine concentrations (r=0.579; p=0.01, Fig. 2). Post-ex CER values were not altered in either smokers or non-smokers.

DISCUSSION

Various exercise models have been conducted to study the effect of acute physical activity on various oxidative stress indices and antioxidant parameters, and different results have been reported on different models (Sanchez-Quesada et al., 1995; Marzatico et al., 1997; Leaf et al., 1997; Vasankari et al., 1997;

Table 3. Pre- and post-ex plasma malondialdehyde (MDA), plasma protein carbonyl content (PCC), susceptibility of non-HDL to oxidation (non-HDL ox.), serum ceruloplasmin (CER) values and erythrocyte superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities in non-smokers and smokers. Data are means (median; SEM).

		Non-smokers (n=10)	Smokers (n=10)
MDA	Pre-ex	9.72 (9.64; 0.52)	7.96 (7.85; 0.65)
(nmol· ml^{-1})	Post-ex	9.86 (9.69; 0.46)	8.38 (8.03; 0.61)
PCC	Pre-ex	2.45 (2.37; 0.20)	2.28 (2.05; 0.20)
(nmol· ml^{-1}plasma)	Post-ex	2.63 (2.58; 0.29)	2.59 (2.37; 0.25)
non-HDL ox.	Pre-ex	303.75 (329.35; 36.75)	261.35 (289.90; 22.90)
(nmol MDA· mg^{-1}cholesterol)	Post-ex	329.90 (336.65; 35.70) **	309.65 (325.40; 21.50) **
CER (U· L^{-1})	Pre-ex	35.23 (29.95; 5.46)	57.73 (60.23; 6.74) $^{\#}$
	Post-ex	35.55 (28.47; 5.52)	58.02 (64.62; 6.60) $^{\#}$
SOD (U· g^{-1}Hb)	Pre-ex	988.89 (933.00; 114.83)	967.50 (880.00; 141.14)
	Post-ex	832.67 (763.00; 94.02)	847.90 (792.00; 102.49) *
GPX (U· g^{-1}Hb)	Pre-ex	34.03 (32.62; 2.84)	38.14 (29.56; 6.32)
	Post-ex	33.13 (31.10; 2.50)	27.54 (20.46; 5.65) **$^{\#}$

* p<0.05 and ** p<0.01, significantly different from the pre-ex value.
$^{\#}$ p<0.05, significantly different from non-smokers.

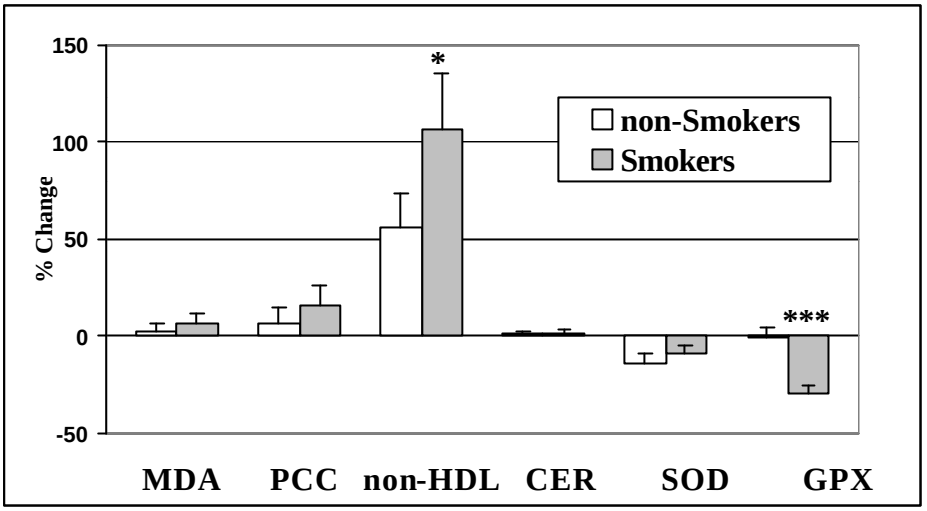

Figure 1. Differences (ΔS, % Change) between pre- and post-ex plasma malondialdehyde (MDA), plasma protein carbonyl content (PCC), susceptibility of non-HDL to oxidation (non-HDL), serum ceruloplasmin (CER) values and erythrocyte superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities in non-smokers and smokers. Bars represent means and standard errors of means.
* $p<0.05$ and *** $p<0.001$, compared to non-smokers.

Aslan et al., 1998 ; Wetstein et al., 1998; Khanna et al., 1999 ; Sürmen-Gür et al., 1999; Tozzi-Ciancarelli 2001, Benitez et al., 2002; Gül et al., 2003). The acute submaximal exercise model used in the present study, caused significant elevations in Hct and Hb levels, indicating haemoconcentration, and hence the necessary corrections in plasma and serum parameters were made to eliminate the haemoconcentration effect. Pre-ex Hct and Hb values from smokers were significantly higher than those of non-smokers, indicating the presence of a compensatory mechanism against chronic hypoxia smokers have been exposed to.

According to the results of the present study, acute submaximal exercise significantly reduced erythrocyte SOD and GPX activities in smokers, compred to their pre-ex values. The pre-ex activities of these enzymes were not significantly different between the two groups, and although decreased, the post-ex changes remained to be non-significant in non-smokers. The decrements in antioxidant activities observed in the present study are in agreement with the results of Aslan et al. (1998), Tozzi-Ciancarelli et al. (2001) and a previous study that was carried out in our laboratory (Akova et al., 2001). The loss of enzyme activities after exercise may be explained by an exercise-induced oxidative damage to the enzyme proteins that modify the catalytic activity of the molecules (Ji et al., 1988). Since the activity losses are only significant in smokers, we suggest that the oxidant damage of acute exercise is more pronounced in individuals that are under chronic exposure of cigarette smoking. Differences (% change) between pre- and post-ex GPX activities, being significantly greater in smokers, support this comment.

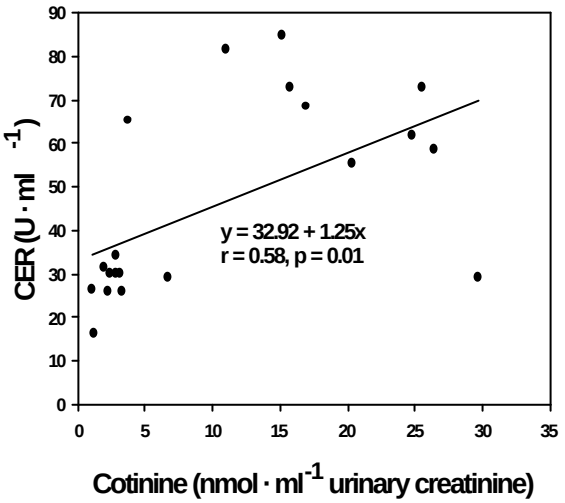

Figure 2. Relationship between pre-exercise urinary cotinine and serum ceruloplasmin (CER) concentrations in all subjects.

Susceptibility of non-HDL to oxidation was significantly increased in both smokers and non-smokers in the present study. According to these results one can state that while the submaximal exercise model used in the present study affects the susceptibility of non-HDL to oxidation, cigarette smoking does not have any further influence on non-HDL oxidizability. However, when the differences (ΔS) between pre- and post-ex values were compared, the differences were found to be significantly greater in smokers. These findings give

evidence of a collective effect for smoking and exercise. It has been previously reported that smoking and exercise separately have oxidative effects on lipoproteins (Sanchez-Quesada et al. 1995; Sanderson et al 1995; Gouaze et al. 1998; Wetstein et al. 1998; Benitez et al. 2002). Our results are in accordance with these findings and provide additional information on the collective effects of smoking and exercise on lipoprotein oxidizability.

Pre-ex serum CER concentrations were significantly higher in smokers compared to that of non-smokers. Duthie et al. (1991) have reported elevated CER activity in smokers, and found that plasma copper concentrations were correlated with CER concentrations, and suggested that higher CER concentrations in smokers might reflect an adaptive response to a sustained oxidant stress or might merely reflect increased copper intake from the tar component of cigarettes (Duthie et al., 1991). The significant positive correlation observed between pre-ex urinary cotinine levels and serum CER concentrations support this interpretation. There was not any additional change in CER concentrations after the exercise, suggesting that it was not affected by the present exercise model.

Plasma PCC and MDA levels were not significantly affected by either smoking or exercise, in the present study. Although there are reports that indicate increased MDA (Kalra et al., 1991; Kaçmaz et al., 1997) or PCC (Panda et al., 1999; Pignatelli et al., 2001) levels in smokers, these differences may be due to the differences in ages, smoking habits and smoking years of the subjects or the methodologies used in different studies. Similarly there are discrepant results about the effects of exercise on these parameters (Maxwell et al., 1993; Leaf et al. 1997; Aslan et al. 1998; Sürmen-Gür et al. 1999; Tozzi-Ciancarelli et al. 2001; Inayama et al. 2002). Our results are in agreement with those of Leaf et al. (1997), Sürmen-Gür et al. (1999) and Maxwell et al. (1993). On the other hand, although statistically non-significant, more pronounced increases in the differences between the pre- and post-ex values of these parameters suggest the presence of a collective oxidant effect of cigarette smoking and acute exercise. However, further studies should be conducted to make a precise judgment on this matter.

CONCLUSION

This is the first study in our knowledge examining the individual and collective effects of cigarette smoking and exercise on some oxidation indices and antioxidant parameters in the same subject group. According to the results of the present study we can propose that smokers are more prone to the possible oxidant damage of physical exercise.

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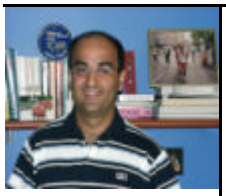

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