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MODERATE EXERCISE DECREASES NITRIC OXIDE EXHALATION IN PATIENTS WITH STABLE CORONARY ARTERY DISEASE

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Nitric oxide (NO) is present in exhaled air in humans and its level may decrease in heart diseases. Nitrates are metabolized to NO. In the present study we prospectively investigated how coronary disease treated with oral nitrates and physical exercise influence the exhaled NO concentration (exNO). The study was performed in 44 patients with stable coronary artery disease (CAD) treated with oral nitrates (31 nonsmokers and 13 smokers) and 34 healthy volunteers (21 nonsmokers and 13 smokers). End-tidal concentration of exhaled NO was measured by the use of a chemiluminescence method. The Bruce protocol of an exercise test was performed in 21 coronary patients and 11 volunteers. NO was measured before and 2-5 min after the test. We found no significant differences in the exNO level between healthy controls and CAD patients as analyzed either for the whole groups or non-smoker and smoker subgroups (6.01 parts per billion (ppb) vs. 4.91 ppb; 7.02 ppb vs. 5.89 ppb; 3.62 ppb vs. 3.33 ppb, respectively). However, the coronary patients group, as a whole, had lower exNO after exercise (4.22 ppb vs. 3.84 ppb, $P<0.01$). The difference persisted after division of this group into non-smokers and smokers: 5.19 ppb vs. 4.79 ppb, $P<0.05$ and 3.63 ppb vs. 3.27 ppb, $P<0.05$, respectively). The level of exNO changed inappreciably after exercise in control subjects. We conclude that coronary disease and oral nitrates, in themselves, do not influence the exhaled NO concentration. Physical exercise, on the other side, lowers the exhaled NO level in coronary patients.

Key words: *coronary artery disease, exercise, exhaled nitric oxide, nitrates*

INTRODUCTION

Nitric oxide (NO) is present in exhaled air; in amounts of several parts per billion (ppb) in healthy humans. Exhaled nitric oxide (exNO) is mainly produced by the endothelial cells in microvessels of airways and alveoli, and only a small portion of it is produced by pulmonary vessels (1, 2). There are many potential sources of NO in the human body, but it remains unclear whether we can record any disorders of NO production by exNO measurement. In addition, it has been confirmed that patients with heart disease may present decreased levels of exNO in comparison with healthy volunteers (3, 4). The influence of coronary disease on the exNO concentration has not yet been determined. There is no information about oral nitrates in this regard either. However, the potential effect of intravenous nitrates on the exNO level has been mentioned in some animal studies (5, 6). Persson et al (6) have observed an increase in exNO concentration in mechanically ventilated rabbits after intravenous nitroglycerin. Although the doses of nitroglycerin were 20 times higher than those used in human studies, only a 15% increase of exNO was observed (6). Agvald et al (5) reported similar data, with different intravenous vasodilators.

It is known that physical exercise increases the amount of NO produced in the lungs, but how this increase is reflected in the level of exNO is controversial. Some authors report a decrease (7-12) and others no change in exNO (7, 13) after exercise.

We hypothesized that NO released from nitrates could contribute to endogenous NO production and this might be detected in exhaled air, both at rest and after physical exercise. The aim of the present study was to find out whether the presence of stable coronary artery disease (CAD) (with standard medical treatment) would affect the exNO level. The second objective was to investigate how physical exercise affects the exNO level in healthy subjects and in CAD patients.

MATERIAL AND METHODS

Subjects

The study protocol was approved by a local Ethics Committee and informed consent was obtained from all participating subjects.

exNO levels were examined in 78 patients who were divided into two groups. Group I (n=44) consisted of patients with stable CAD (14) and Group II (n=34) of healthy volunteers. Both groups were further divided into subgroups A (non-smokers, IA – 31 patients, IIA – 21 patients) and B (smokers, IB – 13 patients, IIB – 13 patients). Smoking participants used, on average, 11.5 cigarettes/day, range 2-20 cigarettes/day, which gives 210 packs/year.

Group I patients were taking oral nitrates: 37 were taking isosorbide mononitrate (Mononit®; Sanofi-Synthelabo, France), Effox® (Schwarz Pharma, Germany) or Mono-Mack® (H Mack Nachwf., Germany) and 7 patients were taking isosorbide dinitrate (Sorbonit®; Argon, Poland)).

Doses of these drugs varied from 40 to 60 mg per day. Additional medications in this group consisted of β -adrenoreceptor blockers, calcium channel blocking agents, angiotensin-converting enzyme inhibitors, and statins. Of the 26 patients on β -blockers, 18 used metoprolol (Metocard[®]; Polpharma, Poland) and 8 others used atenolol (Atenolol[®]; Polpharma, Poland) - doses varied from 50 to 100 mg daily. Of the 14 patients on calcium channel blocking agents, 7 used amlodipine (Amlozek[®]; Adamed, Poland) 5 mg daily and 7 others used diltiazem (Dilzem[®]; Godecke/Parke-Davis, New York, NY) 180mg daily. Of the 17 patients on angiotensin-converting enzyme inhibitors, 5 used enalapril (Enap[®]; Krka, Slovenia) 10 mg daily, 3 used quinapril (Accupro[®]; Godecke/Parke-Davis, New York) 10 mg daily, 3 used captopril (Captopril[®]; Biofarm, Poland) 50 mg daily, 3 used perindopril (Prestarium[®]; Servier, France) 4 mg daily, and 3 others used cilazapril (Inhibace[®]; Roche, France) 5 mg daily. Of the 12 patients on statins, 8 used simvastatin (Zocor[®]; Merck Sharp & Dohme, New York, NY), 3 used lovastatin (Lovastin[®]; Polfa Grodzisk, Poland) and one patient used pravastatin (Lipostat[®]; Bristol Myers Squibb, New York, NY), all statins were given in a dose of 20 mg/day.

The exclusion criteria included: age <18, any signs of infection, chronic lung disease, and the use of inhalational medications. Some exclusion criteria were different for each group and included: class III and higher on Canadian Cardiovascular Society scale classification (14), class II and higher on New York Heart Association classification, recent myocardial infarction (<30 days), and use of intravenous nitrates in Group I and any medications in Group II. The health status of the participants in our study was assessed by the questioning of patient as well as by medical history and physical examination.

Study protocol

exNO was assessed with a high resolution chemiluminescence analyzer (280 NOA, Sievers, Boulder, Colorado) adapted for on-line recording of NO concentration. An internal restrictor in the breathing circuit allowed expiration against a resistance of 10 cmH₂O to keep the soft palate closed and to prevent contamination of the exhaled air with nasal NO. A single breath measurement was performed at a constant flow of 200 ml/s. Plateau values were obtained from the exNO single breath curve. The mean value from six consecutive and reproducible measurements was automatically calculated and considered for analysis. The whole procedure of the exNO measurement was performed according to the recommendations of the European Respiratory Society (12).

Smokers were asked to refrain from smoking a minimum of 2 h before the examination. Every one in the study who was in the smoking subgroup was an active smoker. Ex-smokers were classified as non-smokers if they had stopped smoking more than one year earlier or, otherwise, they were excluded from the study.

The exercise test was performed according to the Bruce protocol (15, 16) in 32 patients: 21 patients in Group I and 11 patients in Group II. Patients with CAD underwent the exercise test for medical reasons. The exNO measurement were performed before and 2-5 min after exercise.

Statistical analysis

All data are shown as means \pm SD. Data distribution was analyzed using the Kolmogorow-Smirnow test. Depending on the results, data were analyzed either by a *t*-test for repeated measures or Wilcoxon test. Differences between groups were analyzed by a *t*-test for independent samples or Mann-Witney test. Differences in frequency distribution were analyzed by the Fischer exact test. $P < 0.05$ was considered significant.

RESULTS

Comparison of demographic data revealed that the Group I patients were significantly older and had higher body mass index (BMI) values than the Group II healthy subjects. It was impossible to find control subjects who were of CAD patients' age, were healthy, and did not take any medications. The percentages of smokers and non-smokers and of men and women were similar in both groups. Demographic details are shown in *Table 1*.

There were no significant differences in the exNO level between the two whole groups. The lack of differences hold true after the division of each group subjects into smokers and non-smokers, although exNO tended to be lower in the healthy subjects (*Table 2*).

CAD patients in Group I who completed the exercise test were significantly older and had lower values of the metabolic equivalent (MET) of basic oxygen uptake (3.5 ml O₂/kg/min) (*Table 3*). Group I, as a whole, and in both subgroups related to smoking history, had a significantly lower exNO level after exercise (*Fig. 1*). No such decrease in exNO was observed in healthy subjects.

The percentage difference in the exNO values before and after exercise correlated with the MET values in the non-smoking healthy subjects (n=8 subjects; r=0.76, P=0.03). In fact in this subgroup, the values of exNO were higher after than before the exercise test (*Fig. 1*).

Table 1. Demographic data of subjects in the two groups studied.

		Coronary artery disease patients (n=44)	Healthy subjects (n=34)	P
Age (yr)		57.0 ±6.8	35.6 ±9.3	<0.01
Height (cm)		168.0±7.1	172.0±8.2	<0.05
Weight (kg)		81.5 ±14.8	71.8 ±13.9	<0.05
BMI (kg/m ²)		28.8 ±4.7	24.1±3.5	<0.01
Gender	Male	28 (63.6%)	19 (55.9%)	
	Female	16 (36.4%)	15 (44.1%)	
Smoking	No	31 (70.5%)	21 (61.8%)	
	Yes	13 (29.5%)	13 (38.2%)	

Table 2. Mean exhaled NO levels.

exNO level (ppb)	Coronary artery disease patients (n=44)	Healthy subjects (n=34)	P
Total group	6.01 ±4.67	4.91 ±2.85	NS
Non-smokers	7.02 ±5.17	5.89 ±3.15	NS
Smokers	3.62 ±1.59	3.33 ±1.19	NS

Table 3. Demographic data of coronary artery disease patients who completed the exercise test.

Parameter		Group I Coronary artery disease patients (n=21)	Group II Healthy subjects (n=11)	I vs. II P
Gender	Female	6 (28.6%)	3 (27.3%)	
	Male	15 (71.4%)	8 (72.7%)	
Smoking	No	8 (38.1%)	8 (72.7%)	
	Yes	13 (61.9%)	3 (27.3%)	
Age (yr)		55.0 ±6.2	36.3 ±10.8	<0.01
Height (cm)		170.0±6.9	172.0±6.0	NS
Weight (kg)		82.5 ±15.7	77.7 ±15.3	NS
BMI (kg/m ²)		28.2 ±4.7	25.8 ±3.7	NS
MET (3.5 ml O ₂ /kg/min)		8.5 ±1.6	10.6 ±3.1	<0.05

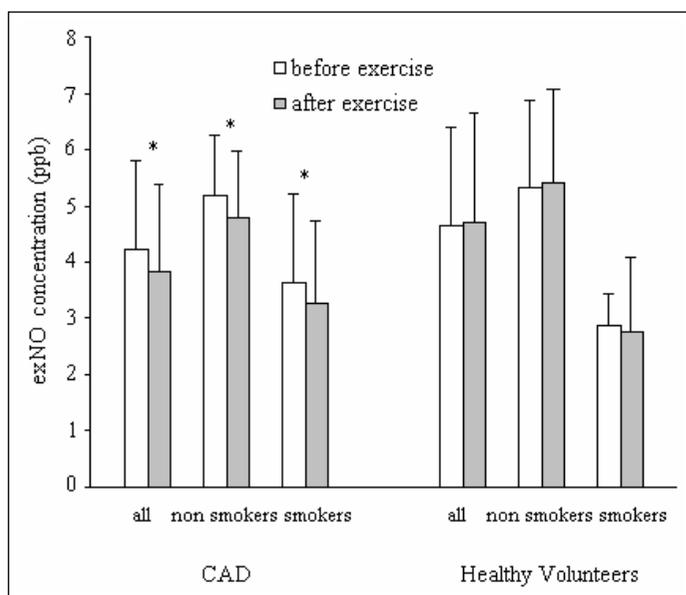


Fig. 1. Comparison of exNO concentrations in coronary artery disease patients who completed the exercise test.

DISCUSSION

In the present study we failed to show any significant differences in exNO level between CAD patients on typical medication including oral nitrates and a group of healthy volunteers. A possible link between CAD and exNO concentration has not been systematically studied in humans, thus a direct comparison of our results with the literature is difficult. In a study of Sumino et al, the amount of NO excreted in the lungs in patients with heart disease was estimated (4). The authors studied 20 patients of whom 14 had CAD. They found that basic NO production was impaired in non-compensated heart failure, but not

in CAD patients (4). It should be pointed out that that study was based on a different methodology - exhaled air was in a Teddlar bag and NO was estimated taking into account respiratory patterns (4). In the present study we used on-line measurement of end-tidal NO concentrations and patients with heart failure were excluded.

exNO levels in patients with heart disease (including patients with CAD on typical nitrate medications) also were studied by Clini et al (3). Again, it was confirmed that exNO levels are significantly lower in decompensated heart failure. The authors mention that exNO levels appear slightly higher in patients on nitrates, but this finding assumed significance only with regard to peak exNO concentrations and not during the plateau of the expiratory phase (3). Different results were reported by Chua et al (17). The authors compared exNO concentrations in 10 patients with severely impaired left ventricular function (mean left ventricular ejection fraction - 20%) with those in healthy volunteers. Surprisingly, no differences were found between these groups. It may be raised that a potential decrease of exNO concentration in patients with heart failure might have to do more with the severity of clinical symptoms than with ventricular function indices.

There is no clear answer as to what the influence of nitrate medications on the exNO level in humans is. Theoretically, this influence should be substantial as nitroglycerin is clearly biochemically transformed to NO (18, 19). Patients in our study were on isosorbide mononitrate and dinitrate in a moderate therapeutic range. There are no data in the literature regarding exNO concentrations in this kind of nitrate class and dosing. The issue seems to be important, because such usage of nitrates is most common among patients with CAD and thus may constitute a potential “confounder” for interpretation of exNO measurements.

Some studies have investigated exNO levels in humans after intravenous administration of “NO donors” such as nitroglycerin and sodium nitroprusside. In a study of Dirnberger et al (20), there were no changes in exNO concentration after infusion of such drugs in healthy subjects, even in doses that could potentially cause a significant drop in systemic blood pressure. In another study, exNO measurements were taken from 7 intubated and ventilated patients treated with high but therapeutic doses of “NO donors” (21). An increase in exNO level was noted, but relevant data were analyzed just in 3 patients, too few for reaching final conclusions on the subject.

In the present study, CAD patients were older than healthy volunteers. Our finding that age did not correlate with the exNO level is in accord with the literature concerning both patients (22) and children (23). We also assessed the exNO level after exercise and found that in CAD patients this level, detected shortly after termination of exercise, was significantly lower than that before exercise. This effect was not seen in the control group of healthy subjects. The result is not readily explainable, but suggestions that exNO level could decrease after physical exercise may be found in the literature.

Physical exercise is capable of changing NO release, distribution, and metabolism. NO production in endothelial cells is likely unchanged during physical exercise, as opposed to that in the lungs, where it increases with a simultaneous decrease in exNO. This apparent discrepancy may be easily explained. A mathematical model of NO metabolism, recently proposed by Hyde et al (10), predicts that if NO production remained steady during exercise, NO concentration in the airways would decrease due to increased ventilation. This, in turn, should decrease the NO gradient between the blood and alveoli, reducing the proportion of NO absorbed by blood and increasing that remaining in the airways. This model explains why physical exercise does not increase NO production by endothelial cells, causing only a larger proportion of NO being eliminated during exhalation. The net effect of all these changes may be recognized as an increase of NO production in the lungs. A significant reduction of exNO levels during exercise may thus be explained by a “dilution” of NO produced in the lungs (17).

During exercise, a large proportion of NO originated from the lungs is transported to capillary vessels in pulmonary circulation and rapidly metabolized by binding to hemoglobin due to a high volume of distribution of NO (10). Rapid inactivation of NO causes its partial pressure to decrease, thereby producing a gradient between cells on the surface of airways and capillary blood – a “driving force” finally pushing NO to pulmonary microcirculation. As a result, 94% of NO produced distally in the lungs is absorbed into the blood and only 6% is exhaled (10).

Metabolic equivalent of basic oxygen uptake calculated after the exercise test was significantly higher in healthy subjects than in CAD patients. This equivalent is calculated according to the duration and grade of exercise. The reason for the difference in the metabolic equivalent could be due likely to different physical fitness and age of the persons in both groups; the healthy subjects were younger and better fit. The exercise test also requires the achievement of submaximal effort, and this is lower in older people, which may be a second possible cause of differences (15, 16). Chirpaz-Oddou et al (7) did not find differences in exNO level in patients with various levels of physical fitness. Our CAD patients presented lower metabolic equivalent values and the exNO level was lower when measured after exercise. This fact was not noticed in healthy subjects in whom exNO tended even to increase. Correlation between the percentage difference in exNO values before and after exercise and the metabolic equivalent of basic oxygen uptake may suggest that the physical fitness and metabolic cost of exercise influence the post-exercise exNO level.

Clini et al (8) is one of a few authors who studied exNO levels after physical exercise using a method similar to that of ours. The author investigated patients with stable chronic obstructive airway disease in comparison with healthy subjects. There was a similar decrease in the exNO level in both groups during exercise and it rapidly came back to the baseline value after exercise (8). Reversal of the exNO decrease after exercise is apparently not dependent on the level of

physical fitness (7, 8). exNO level has also been found to be lower after a marathon race; the decrease being sustained even 10 min after termination of such excessive physical activity (12, 24).

De Gouw et al (11) studied exNO changes during exercise in patients with asthma and, again, they observed that exNO decreased immediately post-exercise. An additional finding was that exNO returned back to normal and even exceeded the baseline value 20 min after exercise in healthy subjects, but not those suffering from asthma. Similar results were reported by Terada et al (3). Such a delayed increase of exNO in healthy subjects once exercise terminated is not adequately explained by the authors of both studies. The lack of exNO increase after exercise in asthmatic patients is more understandable, as it might be a result of bronchial spasm induced by exercise (25).

Neither healthy subjects nor CAD patients of our study presented any signs of heart failure, so that changes in exNO level could not be linked to the mechanisms described for heart failure patients by Clini et al (3) and Sumino et al (3, 4). There might, however, be another possible explanation. Excessive physical exercise and significant heart failure might both create similar changes in NO production. Achievement of the upper limit of heart rate might not be problematic for healthy subjects, but might correspond to excessive physical exercise in CAD patients, thereby generating a decrease of exNO only in this group.

In conclusion, the exNO level is not influenced by the presence of stable coronary artery disease and oral usage of nitrates within the therapeutic dosing range. Physical exercise may lower the exNO level in patients with coronary heart disease and the explanation of this finding warrants further investigation.

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Stable coronary artery disease is generally characterized by episodes of reversible myocardial demand/supply mismatch, related to ischaemia or hypoxia, which are usually inducible by exercise, emotion or other stress and reproducible—but, which may also be occurring spontaneously. Such episodes of ischaemia/hypoxia are commonly associated with transient chest discomfort (angina pectoris).
Table 7 Blood tests for routine re-assessment in patients with chronic stable coronary artery disease. Recommendations. Annual control of lipids, glucose metabolism (see recommendation 3 in Table 6) and creatinine is recommended in all patients with known SCAD. Stable coronary artery disease refers to a reversible supply/demand mismatch related to ischemia, a history of myocardial infarction, or the presence of plaque documented by catheterization or computed tomography angiography. Patients are considered stable if they are asymptomatic or their symptoms are controlled by medications or revascularization. Decrease mortality rates Use caution in pregnant women and in patients with angioedema, renovascular disease, or hyperkalemia. Angiotensin receptor blockers^{1,2,26}.
Algorithm for antiplatelet therapy in patients with stable coronary artery disease. Information from references 1, 2, 49, and 52.
Exercise as a therapeutic intervention in patients with stable ischemic heart disease: an underfilled prescription. In patients with coronary atherosclerosis, exercise training partially improves the endothelial function of large coronary conduit and resistance vessels. This finding provides a pathophysiologic framework for the elucidation of the positive effects of exercise on myocardial perfusion and emphasizes the therapeutic potential of endurance training for patients with stable coronary artery disease. The catheters used in this study were provided by A.D. Krauth Cardio-vascular (Hamburg, Germany).