

## Nitric Oxide Response to Acute Exercise in Patients with Coronary Artery Disease

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### Abstract

Nitric oxide (NO) has been identified as a vasodilatory substance released from the endothelium which decreases in the presence of atherosclerosis. This study aimed to evaluate the systemic NO response to acute exercise in untrained diabetic and nondiabetic patients with atherosclerotic coronary artery disease (CAD). This is a prospective, clinical study consisting of three groups. Group A (n=50) consisted of nondiabetic CAD patients, group B (n=20) consisting of diabetic, CAD patients and group C (n=20) of healthy controls. All patients underwent a standard symptom limited treadmill exercise test according to the modified Bruce protocol after 24 hours low nitrite/ nitrate diet. End products of nitric oxide metabolism (NOx) were determined as the half life of NO is very short. Basal serum NOx levels of both diabetics ( $24 \pm 8.4 \mu\text{mol/L}$ ,  $p < 0.0001$ ) and nondiabetics ( $43.5 \pm 13.7 \mu\text{mol/L}$ ,  $p < 0.01$ ) were significantly lower compared to controls ( $66.5 \pm 3.4 \mu\text{mol/L}$ ). Only in the nondiabetic, CAD patient group was an increase in NOx levels observed with exercise. No increase in NOx with exercise was observed for the diabetic, CAD patient group or for the control group. In normal controls exercise induced no significant change in NOx levels. Exercise induced increase in systemic NOx levels in patients with atherosclerotic disease may indicate a compensatory mechanism which is not present or diminished in the diabetic subgroup.

**Keywords:** atherosclerosis, diabetes, exercise, nitric oxide,

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### Introduction

Nitric oxide (NO) has been identified as a vasodilatory substance, continuously released from the endothelium, keeping the vasculature in a state of basal relaxation. Additionally synthesized from its substrate L-arginine by the enzyme nitric oxide synthase (NOS), which exists in both a constitutive (calcium-dependent) and inducible (calcium-independent) form in endothelial cells and platelets (Moncada et al. 1991). Agonists such as acetylcholine and bradykinin as well NO inhibits platelet aggregation and suppresses smooth muscle proliferation (Busse et al. 1996

Sladek et al 1997). It is as mechanical stimulation (eg. increased blood flow during exercise) increases endothelial NO production (Wang et al 1993; Canty and Schwartz 1994 and Awolesi et al. 1995). The earliest detectable manifestation of atherosclerosis is a decrease in the bioavailability of NO. Decreased NO is a common feature of many atherosclerosis risk factors like ageing, hyperlipidaemia, diabetes and hypertension (Stroes et al. 1995; Williams 1996; Panza et al 1995 and Heitzer 1996). The positive effect of exercise training on endothelial NO production was shown in animal models, as well as in healthy subjects

and in patients with hypertension (Arvola et al. 1999; Tanabe et al. 2003; Stefanie et al. 1995; Vassale 2002; Surdacki *et al.*, 1998). Chronic exercise increases NO synthase expression in skeletal muscles which results in enhancing the glucose uptake in skeletal muscle (Roberts et al. 1999). Data on the effect of acute exercise are limited. The aim of this study was to evaluate the systemic NO response to acute exercise in untrained diabetic and nondiabetic patients with atherosclerotic coronary artery disease (CAD).

### Material and Methods

The study group consisted of 50 nondiabetic (Group A) and 20 diabetic (Group B) men with angiographically proven coronary artery disease (>50% stenosis in one or more epicardial arteries) and 20 healthy controls (Group C) without CAD, referred to exercise stress testing. As the study is a nonrandomized study, 70 consecutive CAD patients were included so that there are 50 diabetic and 20 nondiabetic patients. Diabetes was defined as being on antidiabetic medication/ diet control and/ or fasting blood glucose levels >120 mg/dl. Informed consent was obtained from all patients. All patients underwent a standard symptom limited treadmill exercise test according to the modified Bruce protocol (Case 15 Marquette). Subjects were prescribed with low nitrite/ nitrate diets (no spinach, beets or cured meats, the most ample sources of alimentary nitrite and nitrate) for 24 hours before the test day. All medications which can influence the test were discontinued 1-3 days before the test. Each patient underwent antecubital vein cannulation for the collection of plasma samples. Samples for NO determination were collected before (basal) and immediately after cessation of exercise. Blood samples were collected in EDTA tubes and centrifuged immediately at 1000x g (30 min). Plasma were placed in 0.5-1 ml portions into Eppendorf tubes and kept at -20 °C until used. The Griess reaction was used for the measurement of plasma nitrite (NO<sub>2</sub><sup>-</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>) concentrations (NO<sub>x</sub>), two end products of nitric oxide metabolism as the

half life of NO is very short. Specifically, NO<sub>3</sub> was reduced to NO<sub>2</sub> by 0.1U nitrate reductase, 5x10<sup>-6</sup> mol/l flavin adenine dinucleotide and 250x10<sup>-6</sup> NADPH. The samples were incubated at 37 °C for 3 h, then 8.8U lactic dehydrogenase and 10<sup>-2</sup> mol/l pyruvate was added, and the samples were incubated for an additional 90 min at 37 °C. Finally the Griess reaction was added to each well, and the samples were read at 540nm. (Verdon et al. 1995) Results are given as mol/L. *Statistical analysis:* Data were given as mean ± standard deviation (SD). Comparisons among the groups were made with one-way ANOVA test and chi square test. A p < 0.05 was considered significant.

### Results

Baseline characteristics of the three groups are given in Table 1. The groups were similar in age. When the presence of risk factors is considered the only statistically significant difference was the higher prevalence of hypertension in Group B. Except for higher LDL-C levels in Group A which is of borderline significance groups were similar in respect of lipid profile and the mean fasting blood glucose level of diabetics (Group B) was significantly higher as expected. Exercise parameters are given in Table 2. Total exercise duration was significantly shorter in the patient groups compared to controls. Although diabetics have the shortest exercise duration the difference was only significant in comparison to the control group. Resting blood pressure values of diabetics were higher compared to nondiabetic patients (p<0.01) and controls (p<0.01). Diabetics have also higher peak diastolic blood pressure values, but only the difference to the control subjects was statistically significant (p=0.006). Mean resting heart rates of the groups were similar. The control group achieved the highest peak heart rate. Mean plasma nitrite/nitrate concentrations (NO<sub>x</sub>) in patients and control groups before and after exercise are presented in Table 3. In-group and between groups comparisons are given in Table 3 as mentioned. Basal serum NO<sub>x</sub> levels

of CAD patients, both diabetics (Group B) and nondiabetics (Group A) were significantly

lower compared to controls. Diabetics have the lowest values. In the patient groups an increase

**Table 1:** Baseline characteristics

	<b>Group A nondiabetic</b>	p A-B	<b>Group B diabetic</b>	p B-C	<b>Group C control</b>	p A-C
Mean Age	57±9	NS	61±10	NS	56±7	NS
Smoking	30%	NS	37%	NS	40%	NS
Hypertension	47%	0.005	87.5%	<0.00	-----	<0.00
Cholesterol mg/dl	185±24	NS	183±33	NS	180±30	NS
HDL-C mg/dl	41±8	0.05	36±4	0.04	43±7	NS
LDL-C mg/dl	140±27	0.055	127±36	NS	126±20	0.04
Triglyceride mg/dl	153±40	NS	172±47	NS	142±86	NS
Glucose mg/dl	88±7.5	<0.01	119±26	<0.01	86±5	NS

NS: nonsignificant

**Table 2:** Exercise parameters

	<b>Group A nondiabetic</b>	p A-B	<b>Group B diabetic</b>	p B-C	<b>Group C control</b>	p A-C
Exercise duration (min)	9.4±2	NS	8.1±2.8	0.003	11.3±1.9	0.001
MET	6.5±1.3	NS	5.9±1.3	0.000	7.5 ±1.2	0.005
SBP* rest (mmHg)	128±11	<0.01	136±11	<0.01	125±9	NS
SBP peak (mmHg)	172±12	NS	176±13	NS	167±12	NS
DBP** rest (mmHg)	8±5	<0.01	85±7	<0.01	80±4	NS
DBP peak (mmHg)	9±12	NS	94±12	0.006	84±7	NS
HR***rest	81±8	NS	84±16	NS	8±10	NS
HR peak	156±20	NS	157±12	<0.01	166±9	<0.01

\*systolic blood pressure, \*\*diastolic blood pressure, \*\*\*heart rate

**Table 3:** Pre- and postexercise NOx levels (mol/L)

	<b>Group A nondiabetic</b>	p A-B	<b>Group B diabetic</b>	p B-C	<b>Group C control</b>	p A-C
Baseline	43.5 ±13.7	0.0001	24 ± 8.4	<0.0001	66.5 ± 23.4	0.01
Postexercise	77.7 ± 29	0.0001	28.8 ± 11.8	0.01	48.8 ± 20.5	0.003
Difference	34 ± 21	0.0001	4.8 ± 4.5	0.01	-17.7 ± 24.7	<0.0001
SBP peak (mmHg)	172 ± 12	NS	176 ± 13	NS	167 ± 12	NS
% Change	83 ± 52.2	0.0001	17.9 ± 14	0.0001	23.8 ± 20.5	<0.0001
P pre-postexercise	<0.0001		0.31		0.08	

NS: non significant

in NO<sub>x</sub> levels with exercise was observed but only in the nondiabetic group was the increase significant. In normal controls exercise induced a slight, insignificant decrease in NO<sub>x</sub> levels. Nondiabetic CAD patients (Group A) had the highest post exercise NO<sub>x</sub> levels. The diabetic group had again significantly lower post exercise NO<sub>x</sub> levels compared to both controls and nondiabetic CAD-patients.

### Discussion

Reduced NO levels and circulating NO<sub>x</sub>, which can be used as an index of endogenous formation of NO, is the first manifestation of atherosclerosis provided that exogenous intake is restricted and other potential confounders are taken into account (Jurgensten *et al.* 1996). Our study demonstrates that basal systemic NO<sub>x</sub> levels are lower in patients with atherosclerotic heart disease compared to control subjects. The diabetic group has the lowest basal NO<sub>x</sub> levels. This is a finding consistent with the previous data, which indicate that diabetes induced endothelial dysfunction include the production of prostanoid vasoconstrictors and the increased oxidative degradation of NO, as well as reduced NO synthesis caused by asymmetric dimethylarginine (Tsefamariam and Cohen, 1992; Tsefamariam *et al.* 1995 and Valkonen *et al.* 2001). NO<sub>x</sub> levels can also be influenced by age and the presence of other factors which may cause endothelial dysfunction such as smoking and hypertension (Vassale *et al.* 2002). There is no significant difference among groups where age and smoking is concerned but the patient groups include also hypertensive and the rate of hypertensive was significantly higher in the diabetic group. Reduced levels of NO<sub>x</sub> and impaired endothelium dependent vasodilatation, which is mainly mediated by NO, have been reported in essential hypertension, and whether NO<sub>x</sub> levels in essential hypertension is a primary or secondary phenomenon is not completely clear (Node *et al.* 1997; Forte *et al.* 1997; Liyama *et al.* 1996; Cordillo, 1998; Taddei *et al.* 1998 and Paniagua, 2000). The presence of hypertension probably has

contributed to the difference in basal NO<sub>x</sub> levels of our population. Another point to be emphasized is the effect of ongoing chronic medication, which can be considered as a limitation of the study. This has been shown especially relating to two groups of drugs, statins and ACE-inhibitors, used to improve endothelial dysfunction may have (positively) influenced the results of the patient groups (Lipid study group 1998; Yusuf *et al.* 2000). Such a positive effect cannot be detected from our results. The limited number of the study population and the differences in treatment durations and drug doses made further / detailed analysis, which is beyond the scope of this study, impossible. The second finding of the study is the different systemic NO<sub>x</sub> responses of the groups to acute exercise. No significant change (a statistically insignificant decrease) in NO<sub>x</sub> levels was observed in the control group, whereas an increase was observed in the CAD groups. Only in the nondiabetic CAD group was the increase statistically significant, so that the nondiabetic patients with atherosclerosis have the highest post exercise NO<sub>x</sub> levels. The effect of chronic exercise training on systemic NO<sub>x</sub> levels is extensively evaluated but the data on the effect of acute exercise on systemic NO production is limited and controversial. Most of the studies are confined to healthy subjects. Stefanie *et al.*, assessed the systemic NO formation by measuring the urinary excretion rates of NO<sup>-3</sup> and of cyclic GMP in male subjects and reported that acute sub maximal exercise increases the formation of NO in men, which may contribute to the vasodilatation during exercise. Another study performed in sedentary male subjects reported acute elevation of plasma NO<sub>x</sub> content following acute exercise (Tozzi-Ciancarelli *et al.* 2002) Consistent with our findings Akinola *et al.* 1999 reported unchanged NO<sub>x</sub> levels with exercise in healthy subjects and Gordon *et al.*, reported that NO is not a major regulator of peak limb blood flow measured immediately after cessation of dynamic exercise and the contribution of NO to exercise hyperemia is limited to the recovery

period (Akinola et al. 1999; Gordon et al. 2002).

Our findings suggest that systemic NO<sub>x</sub> does not play a major role or is not necessary for the exercise induced vasodilatation in healthy controls. We observed an exercise induced increase in patients with atherosclerosis which is more prominent in the nondiabetic group. This exercise induced increase in systemic NO<sub>x</sub> levels in patients with atherosclerotic disease may indicate a compensatory mechanism which is not present or diminished in the diabetic subgroup due to more prominent endothelial dysfunction. As mentioned above diabetics have reduced NO synthesis as well as increased degradation. This can partly explain the reduced exercise tolerance of these patients. Our findings may also indicate different biochemical or molecular mechanisms underlying the impaired endothelial NO actions in different diseases.

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