

# Effects on Nasal Nitric Oxide Production of 2 Mechanisms of Vasoconstriction

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## ■ Abstract

**Background:** Vasoconstrictor drugs reduce nitric oxide (NO) production in vitro by inhibiting the enzyme involved in the regulation of inducible and constitutive NO synthases (iNOS and cNOS). Intranasal vasoconstrictors also decrease nasal NO concentration in vivo. It is as yet unclear if this last finding is due to the effects of the drug on the enzyme or on the vessels. Physical exercise also induces nasal vasoconstriction and reduces nasal resistance.

**Objectives:** The aim of this study was to clarify the mechanisms involved in xylometazoline-induced reduction of nasal NO concentration.

**Methods:** We compared 2 randomized groups of patients with moderate-severe persistent allergic rhinitis. The first group (n=24) underwent a physiological nasal vasoconstrictor stimulus (exercise) whereas the second group (n=29) was treated with a nasal vasoconstrictor drug (topical xylometazoline). Nasal volume and NO were determined at baseline and 15 to 20 minutes after the end of each stimulus using acoustic rhinometry and chemiluminescence, respectively.

**Results:** Baseline values of nasal volume and NO did not differ between the 2 groups. Nasal volume increased by 57% ( $P = .0001$ ) after exercise and 71% ( $P = .0001$ ) after xylometazoline. Nasal NO decreased (25%,  $P = .001$ ) after xylometazoline, but not after exercise.

**Conclusion:** Physical exercise and topical xylometazoline cause vasoconstriction and similar effects on nasal volume. In contrast nasal NO decreased with xylometazoline but not after exercise. These findings suggest that vasoconstrictor drugs reduce nasal NO by mechanisms other than vasoconstriction.

**Key words:** Exercise. Nasal nitric oxide. Nasal volume. Rhinitis. Topical nasal vasoconstrictors.

## ■ Resumen

**Antecedentes:** Los fármacos vasoconstrictores reducen la producción de óxido nítrico (NO) in vitro inhibiendo la enzima implicada en la regulación de las sintetasas inducible y constitutiva (iNOS y cNOS) del óxido nítrico. Los vasoconstrictores intranasales también disminuyen la concentración de NO nasal in vivo. Hasta el momento se desconoce si este último hallazgo es debido a un efecto del fármaco sobre la enzima o sobre los vasos sanguíneos. El ejercicio físico también induce la vasoconstricción nasal y reduce resistencia nasal.

**Objetivos:** El objetivo de este estudio fue intentar clarificar los mecanismos implicados en la reducción de la concentración de nasal NO inducida por xilometazolina.

**Métodos:** Diseñamos un estudio comparativo de dos grupos paralelos de pacientes con rinitis alérgica persistente moderada/grave. El primer grupo (n=24) fue sometido a un estímulo vasoconstrictor nasal fisiológico (ejercicio) mientras que el segundo grupo (n=29) fue tratado con un vasoconstrictor nasal (xilometazolina tópica). El volumen y el NO nasales fueron determinados de forma basal y entre 15 a 20 minutos después de cada estímulo usando rinometría acústica y quimioluminiscencia respectivamente.

**Resultados:** Los valores basales del volumen y del NO nasales fueron similares en ambos grupos. El volumen nasal aumentó un 57% ( $P=0,0001$ ) después de ejercicio y un 71% ( $P=0,0001$ ) tras la aplicación de xilometazolina. El NO nasal disminuyó un 25% ( $P=0,001$ ) con respecto a su valor basal después de la xilometazolina, pero no tras el ejercicio.

**Conclusión:** Tanto el ejercicio como la xilometazolina tópica ocasionan vasoconstricción y efectos similares sobre volumen nasal. Sin embargo, el NO nasal se redujo con la xilometazolina pero no tras el ejercicio. Estos resultados sugieren que los fármacos vasoconstrictores reducen el NO nasal por mecanismos diferentes a la vasoconstricción.

**Palabras clave:** Ejercicio. Óxido Nítrico Nasal. Volumen Nasal. Rinitis. Vasoconstrictores Tópicos Nasales.

## Introduction

Nitric oxide (NO) is a mediator of various biological processes such as vasodilation and cellular immune responses. Through these actions NO can contribute to regulate inflammatory responses in the upper airways. NO and its metabolites are found in high concentrations in the nose of both healthy subjects and patients with rhinitis [1-3]. This is attributed to the high levels of production of NO in the paranasal cavities, from which it flows towards the nasal cavity. The synthesis of NO is regulated by the action of the NO synthase (NOS) enzyme on the amino acid L-arginine. There are 3 isoforms of NOS. Two endothelial and neuronal forms, termed constitutive (cNOS), synthesize NO in normal conditions. The third, termed inducible (iNOS), is not expressed (or very weakly so) in normal conditions [2,3]. iNOS is present in the epithelium of the respiratory airway and in various inflammatory cell types (macrophages, neutrophils, mastocells, endothelial cells) and can be induced by various proinflammatory cytokines (tumor necrosis factor  $\alpha$  and  $\beta$ , interferon  $\gamma$ , interleukin-1 $\beta$ ) [3]. On the basis of these observations iNOS has been linked to the regulation of the immune inflammatory response in the airways [2,3]. iNOS induction requires gene transcription activation, thus, any increase in NO production usually occurs several hours after gene induction and may last for several days [4]. All in all, these findings have led to the consideration that NO can be used as an indicator of inflammation [2].

Nasal vasoconstrictors are sympathetic mimetic amines that activate the  $\alpha$ -adrenergic receptors located in vascular smooth muscle. These drugs effectively and quickly relieve nasal obstruction but are less efficacious over other rhinitis symptoms such as sneezing, pruritus and rhinorrhea according to Westerveld et al [1], who cite recent studies suggesting that vasoconstrictors exert part of their beneficial pharmacological effects by mechanisms other than vasoconstriction. They note that oxymetazoline and xylometazoline inhibit cNOS and iNOS activity in rat macrophages *in vitro* in a dose-dependent manner, and that this effect results from reduced induction of the enzyme rather than a direct effect on the enzyme activity. A decrease in nasal NO after intranasal administration of vasoconstrictors has been previously reported, and although the mechanisms involved in this effect are still unclear, it has been suggested that the constriction of venous sinusoids results in a reduction in blood flow which in turn reduces the diffusion of NO from the tissues to the nasal cavity [5,6]. Another hypothesis the authors have put forward is that the reduction of blood flow could prevent the metabolite substrates from reaching the cells where NO is produced. A toxic effect of the drugs has also been suggested as a potential explanation.

Physical exercise is also associated with an increase of nasal volume that in turn reduces nasal resistance in both healthy subjects and patients with rhinitis, and it has been shown that these changes are largely caused by a vasoconstrictive phenomenon (increase in sympathetic activity) that reduces the volume of the venous sinusoids [7,8].

Acoustic rhinometry is a technique used to assess the geometry of nasal cavities, including both the size of the cross-sectional areas as well as the volume of the nasal cavities at various distances from the nostrils [9].

The aim of this study was to gain insight into the mechanisms responsible for the effect of administering a vasoconstrictor drug on nasal NO. Because the reduction of nasal blood flow has been proposed as one of the possible mechanisms to explain findings of reduced nasal NO, we compared the effects of the drug with those of physical exercise, which is a well-known method of inducing vasoconstriction by the release of endogenous sympathetic hormones.

## Patients and Methods

### *Patients and Design of Study*

A cohort of patients with persistent severe-moderate allergic rhinitis according to classifications in the guidelines of the Allergic Rhinitis and Its Impact on Asthma group [10] was recruited. We designed a prospective study using 2 parallel groups. Subjects were randomized to receive 2 puffs of xylometazoline 1% in each nostril or to perform an exercise challenge for 6 minutes on a bicycle ergometer to reach 80% of maximal heart rate (Cardio O<sub>2</sub> MGC, St Paul, Minnesota, USA) according to methods previously described [11]. The selected patients had not received any treatment with topical or systemic corticosteroids in the last 4 weeks or with antihistamines in the last week.

The study was approved by the ethics committee of our hospital and all patients gave their informed consent to participation.

### *Nasal Volume*

Nasal volume was measured by acoustic rhinometry (acoustic rhinometer SER 2000 RhinoMetrics, Lyngø, Denmark) at baseline and at 15 minutes after the end of either the exercise test or the intranasal instillation of xylometazoline. Because the main changes in nasal geometry occur in the most vascularized segment, which are the turbinates, the nasal volume was evaluated between the second and sixth centimeters (Vol<sub>2-6</sub>) of the nasal cavity using a standardized method [9].

### *Nasal NO*

Nasal NO was measured after acoustic rhinometry at similar time points. Measurement was performed by chemiluminescence (SIR System N6008 NO tracer, SIR, Madrid, Spain), following a standardized method [12]. The system uses the light generated by a reaction between environmental ozone and the NO from the sample. The light is then amplified and analyzed by specific software. To evaluate the nasal NO, the nasal air sample was obtained from one nostril using a negative pressure pump with a flow rate of 0.05 L/s. The patients were instructed to blow out their cheeks to elevate the mouth pressure in order to lift up the palate and isolate the nasal cavity from the rest of the respiratory system. Measurement was made when the level reached a plateau value.

## Characteristics of Patients at Baseline\*

	Exercise Group	Xylometazoline Group
Gender, male/female	12/12	10/19
Age, mean, y	27 ± 6.7	29 ± 7.8
Nasal volume (Vol <sub>2-6</sub> ), cm <sup>3</sup>	14.1 ± 3.0	13.2 ± 7.4
Nasal nitric oxide, ppb	1165 ± 439	1287 ± 432

\*Vol<sub>2-6</sub> indicates the volume evaluated between the second and sixth centimeters. Data are means ± SD.

## Statistical Analysis

Age and values of Vol<sub>2-6</sub> and nasal NO at baseline and at 15 minutes are presented as means ± SD. Differences within groups and between groups were estimated using Wilcoxon and Mann–Whitney analysis, respectively. The software used was SPSS Version 14.0 (Chicago, Illinois, USA).

## Results

Fifty-three patients were studied; 29 received xylometazoline and 24 performed the exercise test. Demographic characteristics and baseline acoustic rhinometry and nasal NO values were similar in both groups (table). All patients were sensitized to house dust mites.

At 15 minutes, the Vol<sub>2-6</sub> increased by 57% ± 29% over baseline in the exercise group ( $P < .0001$ ). In the xylometazoline group, the Vol<sub>2-6</sub> increased by 71% ± 32%

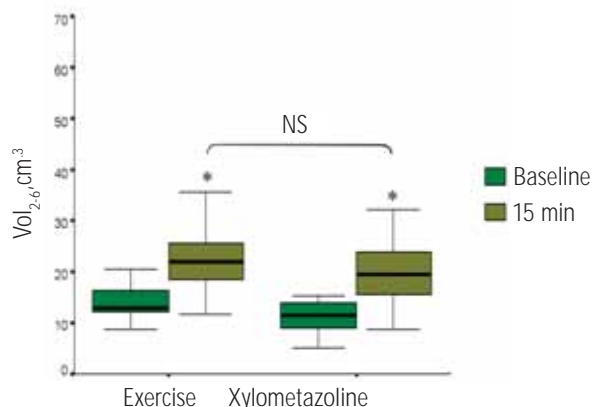


Figure 1. Nasal dilation after exercise and xylometazoline. Ppb indicates parts per billion; NS, not significant. \* $P < .0001$ .

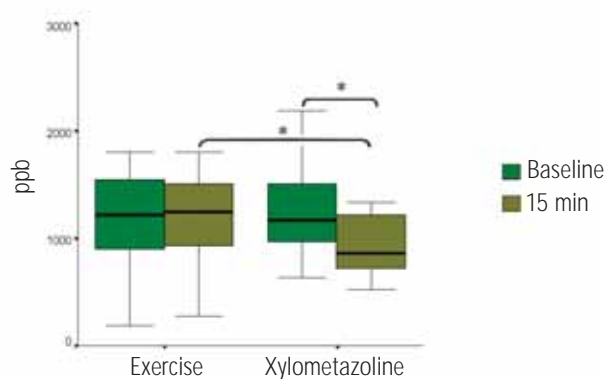


Figure 2. Nasal nitric oxide at baseline and 15 minutes after stimuli. The value is expressed in parts per billion (ppb). \* $P < .05$ .

over baseline at 15 minutes ( $P < .0001$ ). The difference in the magnitude of change in Vol<sub>2-6</sub> between the 2 groups was not statistically significant (Figure 1).

Nasal NO did not change significantly in the exercise group from the mean value at baseline of 1165 ± 439 parts per billion (ppb) to the mean value of 1185 ± 413 ppb at 15 minutes after the end of exercise ( $P = .4$ ). In contrast, in the xylometazoline group, the mean value at baseline of 1287 ± 432 ppb decreased significantly to 969 ± 337 ppb at 15 minutes after the intranasal administration of the drug ( $P < .05$ ). The nasal NO concentration at 15 minutes was significantly lower in the xylometazoline group with respect to the exercise group ( $P = .046$ ) (Figure 2).

## Discussion

Vasodilator agents such as papaverine are associated with increases in nasal NO whereas vasoconstrictor drugs cause the opposite effect [13]. These findings suggest that nasal blood flow is the mechanism involved in the regulation of nasal NO output.

Our study shows that both exercise and vasoconstrictor drugs (xylometazoline) caused a similar increase in the size of nasal cavities. In both cases, the mechanism involved in this action appears to be the reduction in the volume of venous sinusoids resulting from the vasoconstrictor effect of sympathetic substances. In one case the substance was exogenous and artificially administered intranasally and in the other case the sympathetic substances were supposedly produced by the suprarenal glands upon the physiological stimulation of physical exercise and released in the nose. However, even though the effect on vessels was similar, the nasal NO concentration significantly decreased after xylometazoline instillation but not after exercise. This observation suggests that vasoconstriction per se is not the mechanism responsible for the xylometazoline-induced nasal NO reduction. Westerveld et al [1], reported that vasoconstrictor drugs reduce the inducible form of NO synthase activity in vitro. Whether xylometazoline reduces

nasal NO in vivo in the nose by a direct effect on the enzymes involved in the production of NO is a possibility that remains to be demonstrated.

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