



## Review Article

# Temporary forced oral breathing affects neonates oxygen consumption, carbon dioxide elimination, diaphragm muscles structure and physiological parameters



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## ABSTRACT

**Objectives:** We studied adaptation of diaphragm, oxygen consumption and carbon dioxide elimination to forced oral breathing (lasting for only 4 days) following reversible bilateral nasal obstruction performed on day 8 post-natal male rats.

**Methods:** Diaphragm myosin heavy chain (MHC) composition, oxygen consumption, carbon dioxide elimination and hormones level were analysed during nasal obstruction period.

**Results:** Diaphragm muscle showed significant increases in adult isoforms (MHC 1, 2a) in oral breathing group versus control. Reversible nasal obstruction was associated with a decrease of oxygen consumption and carbon dioxide elimination. Nasal obstruction period was associated with reduced growth of the olfactory bulbs and an initial decrease in lung growth. One day after implementing nasal obstruction, basal corticosterone levels had increased (by over 1000). Oral breathing was also associated with a lower level of thyroid hormone.

**Conclusions:** We conclude that a 4 day nasal obstruction period in young rats leads to hormonal changes and to Diaphragm myosin heavy chain structural adaptation.

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## 1. Introduction

Nasal obstruction is considered a risk factor in sleep-disordered breathing [1–3], which has a very negative impact on quality of life in children and adults with increased daytime sleepiness [4]. This symptom resembles that of obstructive sleep apnea (OSA) caused by episodes of upper airway obstruction leading to episodic hypercapnic hypoxia which alters upper airway muscle structure and fibre type expression [5]. The most common clinical manifestations of OSA are nocturnal snoring, respiratory pauses, restless sleep and oral breathing [6]. This disturbed breathing is known to produce lethargy, cognitive and sleep impairment, especially in children [7,8]. Chronic nasal obstruction is a non-specific condition observed in many pathophysiological conditions e.g. allergic rhinitis, rhinosinusitis, adenoid hypertrophy and nasal polyps. Impaired nasal breathing results in obligatory oral breathing, which can be divided into two components: chronic absence of active nasal respiration that results in an olfactory deprivation [9] and chronic mouth opening [10]. Furthermore, in contrast to oral breathing, nasal breathing allows the optimal conditioning of inhaled air, clearing, moistening and warming the air before the gas exchange in the lungs [11,12]. Obligatory oral breathing has been observed in human babies and has been associated with a number of conditions that could have both short and long term effects on the physiology and thus behaviour of these infants later on in adolescence.

Stressful situations correspond to particular changes in environmental conditions that induce modifications in different physiological parameters like plasma hormonal levels. For example, stressful situations produce an adrenal hypertrophy and an increase of plasma glucocorticoid levels [13,14], which are known to induce alterations in MHC isoforms expression [15]. Plasma levels of thyroid hormones can be reduced in stressful situations and these hormones are affected by severity airway obstruction [16,17].

To our knowledge no work has been published on oxygen consumption and carbon dioxide elimination changes during total closure of the nostril in neonates animals before any normal ageing processes could intervene.

Thus, our hypothesis was that early oral breathing (from postnatal day 9 to day 11) would be associated thus having a negative impact on oxygen consumption and carbon dioxide elimination associated diaphragm structure and physiological parameters.

## 2. Materials and methods

### 2.1. Animal care

Twenty eight males Wistar rats (IFFA-CREDO, France) were used for this experiment. The animals were born in the laboratory from 201, culled to 7 pups per litter to ensure normal body growth. The animals were housed in standard cages under controlled temperature conditions ( $22 \pm 1^\circ\text{C}$ ). Food and water were available ad libitum throughout the experiment. From birth, the rats were kept on a reversed 12:12 light–dark cycle (dark period 08:00–20:00).

### 2.2. Nasal obstruction procedure

All experiments conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (no. 85-23, revised 1996), the recommendations of the European Community Council for the Ethical Treatment of Animals (no. 86/609/EEC)—and the regulations of the University of Lorraine. All efforts were made to minimise animal suffering. At the age of 8 days (D8), the litters were first anaesthetised. Animals were weighed and they were then divided randomly into one control group and one experimental group (oral breathing). Bilateral nasal obstruction, resulting in forced oral breathing, was performed in experimental animals (7 per age) as described previously by [18]. The selected method consisted in the cauterisation of the external nostrils, which is the most common and simple procedure allowing spontaneous reopening of nostrils after 4 days. The tissue surrounding the external nostrils was burned by placing a surgical cauterising instrument (1 mm in diameter) on the nostrils, consequently occluding the orifice of the nostrils without mechanical or chemical damage to the olfactory mucosa. This procedure induced complete nasal obstruction between D8 and day 11 (D11) with 100% of the nostrils spontaneously reopened by day 15 (D15). The sampling experiments were conducted during complete nasal obstruction day 9 (D9) (Fig. 1).

The animals started breathing through their mouths immediately after nasal occlusion, as has been reported in infants [19]. Nostril cauterisation earlier in life resulted in rapid death of the pups. In the control group (7 per age), the nostrils were not sealed but the cauterising instrument was placed about 1–2 mm above each nostril to burn the skin. After cauterisation, the nostrils were washed with chlortetracycline (Aureomycine Evans 3%) to prevent infection. The pups were warmed ( $37^\circ\text{C}$ ) for 30 min and returned to their mothers.

### 2.3. Olfactory bulbs and lungs

Seven pups male rats per group (control and oral breathing) and per age (D9, D11), were removed, immediately humanely killed and weighed. Olfactory bulbs and lungs were removed and weighed.

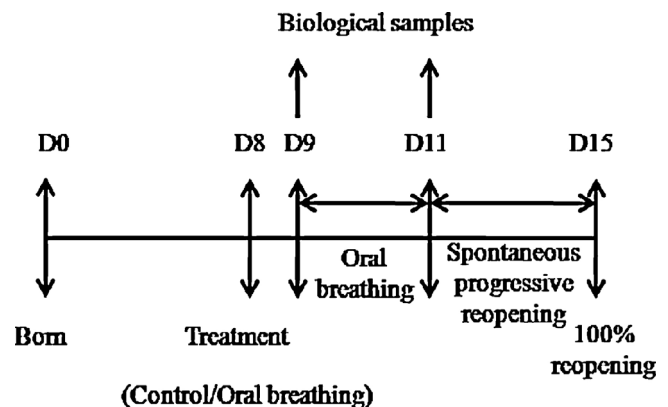


Fig. 1. Time line of the experimental protocol.

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