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Effects of short term forced oral breathing: Physiological changes and structural adaptation of diaphragm and orofacial muscles in rats

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ABSTRACT

Objective: We studied adaptation of diaphragm and orofacial muscles as well as hormonal responses to forced oral breathing (lasting for only 4 days) following reversible bilateral nasal obstruction performed on day 8 post-natal male rats.
Design: Muscle myosin heavy chain (MHC) composition and hormone levels were analysed during two periods: 1 and 3 days after obstruction (days 9 and 11 post-natal), and following 3 months recovery with nasal breathing (90 days, adult).
Results: Diaphragm muscle showed significant increases in adult isoforms (MHC 1, 2a) in oral breathing group versus control. We observed increases in MHC neonatal and adult type 1 isoforms in muscles involved with oral breathing, *masseter superficialis* and *anterior digastric*. No changes were observed in the *levator nasolabialis* muscle involved with nasal breathing. Reversible nasal obstruction was associated with reduced growth of the olfactory bulbs lasting into adulthood, and an initial decrease in lung growth followed by recovery at 90 days. Adrenal hypertrophy was observed after 1 day of nasal obstruction and lasted into adulthood. The ''stress'' hormone response was variable, increased (over 1000%) during the obstruction but normal by adulthood. An increase in plasma testosterone was observed during the obstruction, and a decrease in thyroid hormone levels throughout.
Conclusions: Very short term nasal obstruction, i.e. forced oral breathing, leads to long term hormonal changes and respiratory muscle fibre adaptation.

1. Introduction

Evidence is now available showing that muscle contractile properties and myosin heavy chain (MHC) composition are correlated.[1] The four major myosin heavy chain isoforms detectable in adult skeletal muscles are three fast types, MHC 2a, 2x and 2b, and one slow type, MHC 1.[2] During development, two perinatal MHC forms can be found in muscle fibres depending on the stage considered: MHC embryonic and neonatal. These different MHC appear sequentially in fast and slow muscles and the developmental programme (except 'program' in computers) of myosin expression depends greatly on the type of muscle.[3] Thus, at birth, rat muscles are phenotypically immature and during development, or when the working conditions are changed, marked transitions in the myosin content can occur in rat fast and slow-twitch

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muscles.[4,5] These modifications generally adapt the muscle to the new environmental requirements.[6]

Chronic nasal obstruction is a non-specific condition observed in many pathological conditions, e.g. rhinitis. Nevertheless, because this disorder is not life threatening (at least in adults) its importance could be underestimated. 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,[7] and chronic mouth opening.[8] Furthermore, in contrast to oral breathing, nasal breathing allows the optimal conditioning of inhaled air, clearing, moistening and warming the air before gas exchange in the lungs.[9,10] Thus, nasal obstruction could be associated with both social (maternal behaviour, relation with congeners) and physical (environmental privation, respiratory modification) stress. In other words, it is possible that nasal obstruction causes a loss of the sense of smell and this hyposmia could disrupt the orientation of young rats to the mother, with consequent deprivation of food and feeding. It has been shown in rats that deprivation of food for 3 days causes a diminution in thyroid hormones,[11] and other studies have also shown that increased mother licking of the pups is needed for testosterone production necessary for masculinisation of the young male rat.[12]

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 very important in the normal development of vertebrate skeletal muscle, notably in muscle MHC distribution.[16] For example, thyroid hormones are known to stimulate the transition from neonatal type to adult type and to anticipate the expression of MHC 2b during post-natal development.[17] Thus, reduced thyroid hormone levels and increased EMG activity could explain the fast-to-slow transitions found in muscles related to mouth opening in oral breathing animals, but this remains to be confirmed.

In addition, the contribution of orofacial muscles to the variation in bite force magnitude is correlated with craniofacial morphology,[18] and chronic oral breathing is known to be a contributing factor in deviant facial growth patterns in preschool children.[19,20] These patterns are the result of a prolonged presence of unbalanced oro-pharyngeal muscle activity. Through mechanoreceptors, oral breathing stimulates oro-pharyngeal electromyographic activity of the muscles facilitating respiration.[21] These modifications of electric stimulation may have an effect on MHC isoform expression,[22] and MHC isoform composition of orofacial muscle could be involved in the adaptation to oral breathing during nasal obstruction. In a previous study, Gelhaye et al.[23] showed that nasal obstruction induced by external cauterisation of the nostril, in 8 day old rat female pups, produced total nasal obstruction during the next 4 days followed by gradual reopening of the nostrils, complete at 15 days. Furthermore, these authors found that this nasal obstruction was associated with chronic oral breathing. At weaning (day 21), the only period studied, the chronic oral breathing animals presented an atrophy of olfactory bulbs, hypertrophy of the adrenal glands and reduced muscle growth for all muscles studied except for the diaphragm. A decrease of MHC 2b compared to MHC 2a in *levator nasolabialis*, a muscle involved with nasal breathing in the oral breathing group was observed. In *masseter superficialis* and *anterior digastric*, muscles involved with oral breathing, an increase of MHC 2b in *masseter superficialis* and a decrease of MHC 2a in *anterior digastric* to the benefit of MHC 2x were detected. No significant difference was detected at day 21 (D21) in diaphragm MHC expression in oral breathing animals.To our knowledge no work has been published of MHC changes during short term reversible nasal obstruction in male rats. Thus, our hypothesis was that nasal obstruction would have a significant effect of MHC isoform expression of muscles involved in breathing including the diaphragm during the very short period of forced oral breathing. We investigated also if these changes were maintained over the long term, i.e. up to adulthood, day 90 (D90). The effect of early nasal obstruction on various organ weights, on the stress response and on plasma levels of thyroid hormones (T3 and T4) and androgens (testosterone) was also studied.

2. Materials and methods

2.1. Animal care

Forty-two male Wistar rats (IFFA-CREDO, France) were used for this experiment. The animals were born in the laboratory from twenty litters, culled to 7 pups per litter to ensure normal body growth. The animals were housed in standard cages under controlled temperature conditions (22 ± 1 °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 Nancy 1. All efforts were made to minimise animal suffering.

At the age of 8 days (D8), the litters were first anesthetised. 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 Meisami,[7] and Waguespack et al.[24] 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

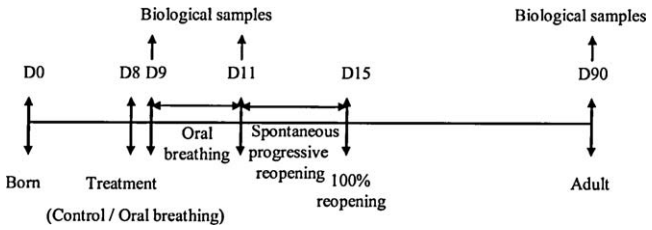

Fig. 1 – Time line of the experimental protocol.

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) and day 11 (D11) and at 90 days after post-reopening of the nostrils, i.e. at the beginning of adulthood (see Fig. 1). The animals started breathing through their mouths immediately after nasal occlusion, as has been reported in infants.[25] 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 °C) for 30mn and returned to their mothers.

Exploratory and feeding behaviours of the pups after weaning were the same for both cauterised and control group rat pups suggestive of no serious long term central effects of the treatment, especially in the forced oral breathing group.[26]

2.3. *Sample collection*

Seven male rats per group (control and oral breathing) and per age (D9, D11 and D90), were removed, immediately humanely killed, weighed and an intracardiac blood sample (500–1000 μl) was taken between 11 h and noon for hormonal measurements. Blood was collected over 1–2 min into sterile heparinised syringes fitted with a 26-G needle. Plasma was immediately separated from cells by centrifugation (4 °C, 15 min at 3000 rpm) and the extracts were stored at −18 °C until assayed.

After blood sampling, olfactory bulbs, lungs, testicles and adrenal glands were removed bilaterally and weighed. Adrenal weight is a direct indicator of chronic stress exposure.[13]

2.4. *Muscle sampling and myosin extraction*

After sample collection, the entire Diaphragm (Dia, respiratory muscle) was dissected, and the following muscles removed unilaterally (right hand side): *Anterior Digastric* (AD, depressor mandibular muscle), *masseter superficialis* (MS, propulsive mandibular muscle) related to mouth movements and oral breathing,[27,28] and *levator nasolabialis* (LN, active sniffing muscle) related to nasal breathing.[29] After dissection, muscles were weighed and myosin was isolated in a high ionic strength buffer, as described by D'Albis et al.[30]

2.5. *Electrophoretic analysis of myosin heavy chain isoforms*

Electrophoresis was performed according to the method of Talmadge and Roy,[31] with little modification. This allowed the separation of the developmental MHC as described by Janmot and D'Albis,[32] and Ohnuki et al.[33] Mini-gels were used in the Bio-Rad Mini-protean II Dual Slab Cell. Electrophoresis took place in a cold room (temperature of 6 °C) for the whole run. To separate all the heavy chains, the duration of the run was 32 h (70 V) for animals aged D9 and D11, and 28 h (70 V) for adults (D90). Three separate loads were made per sample (2.5 μg of protein/well). The MHC isoforms were identified according to migration rates compared with an adult diaphragm containing only adult isoforms 2a, 2x, 2b and 1.[34] The gels were stained with Coomassie blue R-250. The relative amounts of the different myosin heavy chains were measured using an integration densitometer Bio-Rad GS-800 and analysed with the Molecular Analyst Program (except 'program' In Computers) (Quantity One 4.2.1).

2.6. *Hormone assays*

Corticosterone and testosterone concentrations were measured without an extraction procedure, using a commercially available EIA kit and performed according to the manufacturer's guidelines (Assay Designs Inc., USA). The concentration of corticosterone and testosterone in plasma samples was calculated from a standard curve and expressed as ng/ml. The intra- and inter-assay coefficients of variation were under 8.4% and 13.1%, respectively for corticosterone, 10.8% and 14.6% respectively for testosterone.

Thyroxine (T4) and triiodothyronine (T3) were assayed using commercial RIA kits and performed according to the manufacturer's guidelines (Immunotech SA, Marseille, France). The concentrations of T4 and T3 in plasma samples were calculated from standard curves and expressed as pg/ml. The intra- and inter-assay coefficients of variation were respectively under 6.7 and 6.5% for T4 and under 6.4 and 5.5% for T3.

2.7. *Statistical analysis*

The results were expressed as group means ± SEM. Student's *t*-test was used to establish the comparison between control and oral breathing animals since all data were normally distributed. Body weight group differences were determined using a two-way ANOVA (factor treatment × factor age). Specific mean comparisons were then made using *t*-test with the Bonferroni correction. Differences were considered significant at $P < 0.05$.

3. Results

3.1. *Morphometric characteristics*

Before treatment, at 8 days of age, the body weights of control and oral breathing pups were not significantly different: 17.78 ± 0.52 g and 17.95 ± 0.28 g, respectively (t = 2.80,

Table 1 – Effects of temporary forced oral breathing on body weight (g), olfactory bulbs, lungs, adrenal glands and testicles specific weights (mg/g) at age 9, 11, and 90 days in controls and animals exposed to nasal obstruction. Values are means ± SEM (n = 7/group/age). Analysis of two-way ANOVA summary: age effect: F = 105.44–4584.50 at two degrees of freedom $P < 0.0001$; treatment effect: F = 5.17–7.04 at one degrees of freedom P = 0.02 to <0.0001; age × treatment: F = 6.18–11.86 at two degrees of freedom P = 0.005–0.0001. Analysis of t-test with Bonferroni correction: *significantly different from control group at 9, 11 and 90 days at $P < 0.05$.

	9 days	11 days	90 days
Control group			
Body weight (g)	18.60 ± 0.49	23.08 ± 0.59	408.47 ± 9.61
Olfactory bulbs (mg/g)	1.62 ± 0.13	1.21 ± 0.03	0.22 ± 0.01
Lungs (mg/g)	20.60 ± 0.52	18.83 ± 0.54	4.69 ± 0.14
Adrenal glands (mg/g)	0.34 ± 0.03	0.32 ± 0.01	0.19 ± 0.01
Testicles (mg/g)	2.11 ± 0.07	2.66 ± 0.11	7.55 ± 0.22
Oral breathing group			
Body weight (g)	15.51 ± 0.48*	19.75 ± 0.79*	394.18 ± 8.65
Olfactory bulbs (mg/g)	1.12 ± 0.12*	0.88 ± 0.04*	0.13 ± 0.01*
Lungs (mg/g)	20.61 ± 1.12	16.20 ± 0.02*	4.50 ± 0.36
Adrenal glands (mg/g)	0.41 ± 0.01*	0.49 ± 0.03*	0.23 ± 0.01*
Testicles (mg/g)	2.24 ± 0.05	2.65 ± 0.10	7.95 ± 0.24

P = 0.14). Table 1 shows that there was a significant difference in body weight already at D9 (t = 4.45, $P < 0.0001$) which continued on D11 (t = 3.23, P = 0.002) between control and oral breathing rats. No differences in body weights were observed at D90 (t = 1.11, P = 0.28).

Body weight of animals decreases by 14% at D9 in the oral breathing group compared to weights at D8 (respectively, 15.51 g vs 17.95 g) and also by 14% compared to control at D11 (respectively, 19.75 g vs 23.08 g).

Relative organ weights are presented in Table 1. Olfactory bulb weights: a significant reduction in olfactory bulb weight was found for the three ages in the oral breathing group compared to control animals (F = 16.34, $P < 0.0001$). The reduction was 30% during nasal obstruction and 41% at D90 in oral breathing males compared to control animals.

Lung weights: A significant reduction of lung weight was observed only on D11 in the oral breathing group compared to the control group (F = 5.29, P = 0.003). The reduction was 14% after three days of nasal obstruction.

Adrenal gland weights: To determine if the absence of nasal respiration and the related transition to temporary forced oral breathing were associated with an enhanced level of stress, the weight of the adrenal glands was measured (Table 1.). Animals exposed to nasal obstruction presented a greater adrenal gland specific weight compared to control animals (F = 6.18, P = 0.001). This significant difference was observed within 24 h after the treatment (+20%; t = 2.79, P = 0.008) and became more marked at D11 (+53%; t = 3.14, P = 0.003). Nasal obstruction was thus associated with a significant increase of adrenal gland weight. This significant augmentation was still present at D90 (+21%; t = 2.15, P = 0.05).

Testicle weights: There were no significant differences in testicular weights between oral breathing and control animals. The development of the testicles was not affected by nasal obstruction.

3.2. MHC isoform expression in neonatal rats (D9, D11)

Based on densitometric analysis of the SDS–PAGE, the relative MHC isoform compositions of respiratory and orofacial muscles (LN, MS and AD) were determined. Results are shown in Figs. 2 and 3. The order of increasing electrophoretic mobility of developmental and adult MHC isoforms is as follows: emb, adult fast 2a, adult fast 2x, neo, adult fast 2b, and slow adult 1 type.

In the neonatal diaphragm muscle at D9 and D11, MHC neonatal and adult isoforms were observed in both control and oral breathing animals. In the orofacial muscles (LN, MS and AD) the relative expressions of embryonic and neonatal MHC isoforms were most abundant (>85%). In the diaphragm (Fig. 3A) we found a significant difference in the relative distributions of MHC isoforms between control and oral breathing animals. Oral breathing was associated with an increase of MHC 1 in the diaphragm at D9 (t = 3.24, P = 0.009) and D11 (t = 12.29, $P < 0.0001$). In LN (Fig. 3B), related to nasal breathing and rearing behaviour, we found no significant differences in the relative distribution of MHC isoforms between control and oral breathing animals. In MS (Fig. 3C), related to jaw lift, oral breathing were associated with a related decrease in embryonic MHC isoforms at D9 (t = 2.22, P = 0.005) and D11 (t = 2.22, P = 0.005). In AD (Fig. 3D), related to jaw depression, oral breathing was associated with decreased MHCneo at D9 (t = 3.52, P = 0.005) and D11 (t = 10.37, $P < 0.001$), with an increase of MHC1 at D9 (t = 5.88, P = 0.002) and D11 (t = 5.85, P = 0.002).

3.3. MHC isoform expression in adult rats (D90)

Results are shown in Figs. 2 and 3. In adult diaphragm, four MHC isoforms could be detected, in order of increasing electrophoretic mobility; the four fast types, MHC 2a, 2x, 2b, and the slow type, MHC 1 (Fig. 3). These MHC isoforms were observed in both control and oral breathing animals in the diaphragm muscle. In orofacial muscles (LN, MS and AD), the three adult fast isoforms could be observed.

In the diaphragm (Fig. 3A), we found a significant difference in the relative distribution of MHC isoforms between control and oral breathing animals. Oral breathing was associated with an increase in MHC 1 (t = 3.83, P = 0.002), 23.7% and 27.6%, respectively, in control and oral breathing rats. In LN (Fig. 3B),

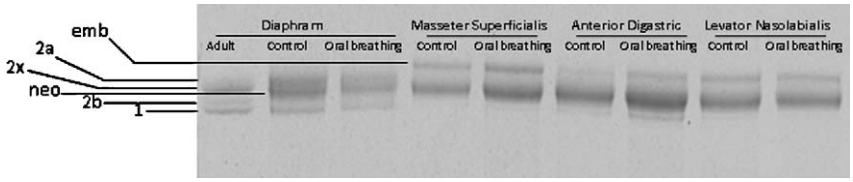

Fig. 2 – Example of effects of temporary forced oral breathing on myosin heavy chain expression in four skeletal muscles: embryonic (emb), neonatal (neo), adult fast 2a, adult fast 2x, adult fast 2b, and slow adult 1 type.

related to nasal breathing and rearing behaviour, oral breathing was associated with an increase in MHC 2a ($t = 26.03$, $P < 0.0001$) and a decrease in MHC 2x and 2b ($t = 8.20$, $P < 0.0001$ and $t = 19.33$, $P < 0.0001$, respectively). In MS (Fig. 3C), related to jaw lift, oral breathing was related to an increase in MHC 2b ($t = 14.62$, $P < 0.0001$) to the detriment of MHC 2x ($t = 6.56$, $P < 0.0001$). In AD (Fig. 3D), related to jaw depression, oral breathing was associated with an increase in MHC 2x ($t = 6.10$, $P < 0.0001$) and a decrease in MHC 2a ($t = 8.80$, $P < 0.0001$).

3.4. *Hormone assays*

As shown in Fig. 4A, plasma corticosterone levels were significantly different between the experimental groups at D9 and D11. Twenty-four hours after treatment, nasal obstruction was associated with a significant augmentation in corticosterone ($F = 4.15$, $P < 0.0001$). At D11 plasma corticosterone levels were significantly increased in oral breathing rats ($F = 16.20$, $P < 0.0001$). At D90, plasma corticosterone levels were no longer significantly different between the 4 days oral breathing and control animals ($P = 0.61$).

As shown in Fig. 4B, plasma testosterone levels were significantly different between the experimental groups at D9 and D11. Twenty-four hours (D9) after treatment, nasal obstruction was associated with a significant augmentation of testosterone ($F = 15.20$, $P < 0.0001$). At D11, plasma testosterone levels were significantly increased in oral breathing rats ($F = 12.15$, $P < 0.0001$). At D90, plasma testosterone levels were significantly higher in both the oral breathing group and the control group compared to D11.

Fig. 5A shows that 24 h after the treatment (D9), thyroxin (T4) concentration was significantly reduced in nasal obstruction compared to control animals ($F = 11.92$, $P < 0.0001$): At D9,

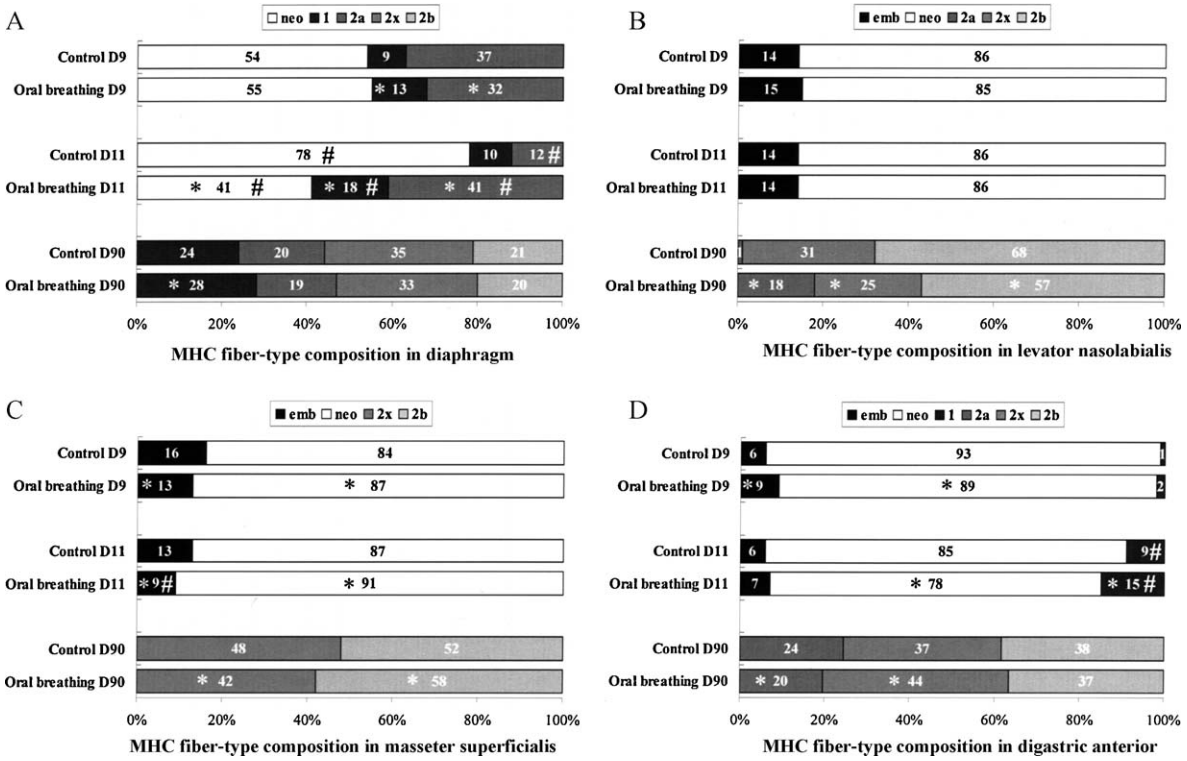

Fig. 3 – Myosin heavy chain distribution in diaphragm (A), *levator nasolabialis* (B), *masseter superficialis* (C) and *anterior digastric* (D) muscles at 9, 11 and 90 days of age in control and rats exposed to temporary forced oral breathing. Values are percentages of total MHC ($n = 7$ per group), SEM values are for all groups included between 0.1 and 0.77. #Significant difference from the previous day at $P < 0.05$ in young rats (D9–11). *Significant differences from control muscles at $t = -10.37$ to 26.03, $P < 0.03$ to < 0.0001.

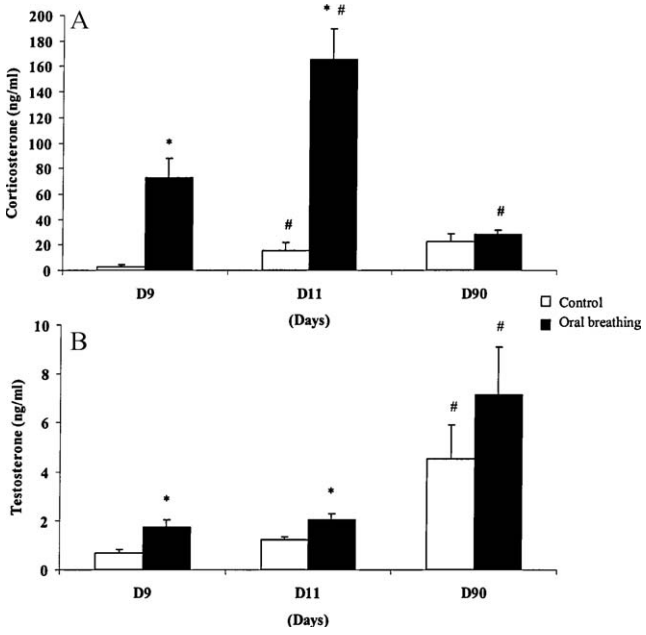

Fig. 4 – Impact of early nasal obstruction on plasma corticosterone (A) and testosterone (B) levels at 9, 11 and 90 days of age in control and temporary forced oral breathing animals. Values are means ± SEM (*n* = 7 rats/group/age). Analysis of t-test: *significant difference from control group at 9 and 11 days at *P* < 0.05. #Significant difference from the previous day at *P* < 0.05.

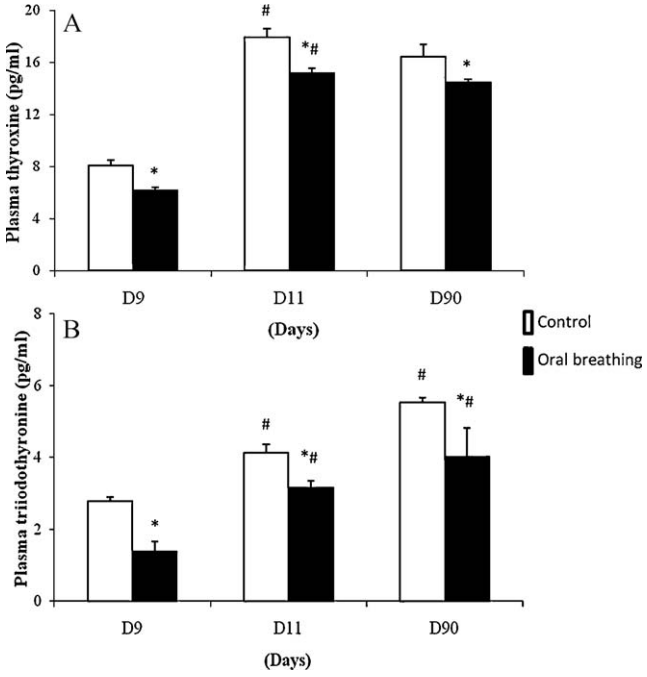

Fig. 5 – Impact of early nasal obstruction on plasma thyroxine (A) and triiodothyroxine (B) levels at 9, 11 and 90 days of age in control and temporary forced oral breathing animals. Values are means ± SEM (*n* = 7 rats/group/age). Analysis of t-test: *significant difference from control group at 9, 11 and 90 days at *P* < 0.05. #Significant difference from the previous day at *P* < 0.05.

plasma T4 levels were significantly reduced by nasal obstruction (−24%; $t = 3.69$; $P = 0.0009$). This difference in plasma T4 levels was maintained at D11 (−15%; $t = 4.20$, $P = 0.0002$), and at D90 (−12%, $t = 3.05$, $P = 0.04$). Fig. 5B shows that plasma triiodothyronin (T3) levels were significantly different between the experimental groups at all ages tested ($F = 14.51$, $P < 0.0001$). Indeed animals with nasal obstruction had lower levels of T3 throughout the period studied. The reduction was 49% at D9, 23% at D11 and 27% at D90.

4. Discussion

These results have shown that in animals with short term nasal obstruction-induced oral breathing there are increases in MHC neonatal and adult type 1 isoforms in two muscles involved with oral breathing, *masseter superficialis* (MS) and *anterior digastric* (AD). During this oral breathing period no changes were observed in the *levator nasolabialis* (LN) muscle involved with nasal breathing. Reversible nasal obstruction was associated with reduced growth of the rat pups during oral breathing, decreased growth of the olfactory bulbs lasting into adulthood, and an initial decrease in lung growth which had recovered at 90 days. After only 1 day of nasal obstruction adrenal hypertrophy was observed and this lasted into adulthood. The consequent plasma levels of ''stress'' hormones were increased during the obstruction but normal by adulthood. An increase in plasma testosterone was observed during the obstruction (but not in adulthood), and a decrease in thyroid hormone levels observed throughout. Thus we have shown clearly that very short term nasal obstruction, i.e. oral breathing, leads to long term respiratory muscle adaptation and significant hormonal changes.

Our results show that nasal obstruction causes early changes in structural development of the respiratory muscles, which begins within 24 h after obstruction and is maintained at least until adulthood. Indeed, in oral breathing animals we have shown an acceleration of structural development of the respiratory muscles during the period of nasal obstruction. The period of nasal obstruction was associated with modifications in MHC isoform expression. During the nasal obstruction period, our results showed a decrease in MHCneo (the predominant neonatal isoform) to the benefit of MHC 1, 2a (the mature isoform) in the diaphragm. In addition, in oral breathing animals the muscles related to jaw movement presented a relative increase in expression of MHCneo to the detriment of MHCemb (the embryonic isoform) in MS, and an increase in MHC 1 (the mature isoform) to the benefit of MHCemb and MHCneo isoforms in AD. These results showed that nasal obstruction induced accelerated structural development of the breathing muscles. During development, muscles usually change directly from embryonic to neonatal to fast, or from embryonic to neonatal to slow isoforms.[3] Geiger et al.[5] have shown that MHCneo increases between D0 to D14 and there after decreases to disappear at the age of 28 days. Our results showed that between D9 and D11 MHCneo

increased in control and decreased in oral breathing animals, which is in accordance with the results of Geiger et al.[5] for control animals. This leads apparently to an accelerated maturation for the forced oral breathing animals because the decrease of MHCneo was to the benefit of MHC adult isoforms. In the adult a similar profile has been found. Indeed, in male rats aged 90 days we observed an increase in the MHC 1 isoform in the diaphragm. At adulthood the LN showed an increase in the 2a isoform at the expense of 2x and 2b isoforms. MS and AD muscles showed antagonist profiles with a decrease in the MHC 2x isoform in MS and an increase in AD muscles.

Thus, oral breathing rats presented a profile in MHC adapted to the transition from nasal to oral breathing, in other words a change facilitating respiration. This is in agreement with results in the literature,[35] that show that environmental conditions (such as hypergravity) could induce structural changes in the development of the muscles.

MHC isoform expression may have a profound effect on muscle fibre contractile and energetic properties.[36,37] Indeed, fibres expressing MHC 1 generate less maximum specific force, slower shortening velocity and greater resistance to fatigue than fibres expressing fast MHC isoforms. Amongst fast fibres, those expressing MHC 2x and 2b generate greater maximum specific force, faster shortening velocity and lower resistance to fatigue than fibres expressing MHC 2a. In addition, in oral breathing animals, the muscles related to mouth opening, AD, will be more susceptible to resist fatigue than MS. This result could be explained by a different control of muscle activity between MS and AD. Indeed, Van Wessel et al.[28] have recently shown that MS and AD presented differences in electromyographic (EMG) activity (in terms of bursts number) during daily activity in the rabbit. In contrast to AD, MS showed a bimodal burst distribution. The authors interpreted this result as the consequence of an additional postural activity for MS only, consisting of many short low-amplitude bursts. In addition, temporary forced oral breathing could produce some behavioural modifications in both nursing and breathing behaviours (mouth opening and rearing behaviour) associated with alterations in specific electromyographic activity of respiratory muscles. Thus, behaviour and related electromyographic activity are probably not the only factors acting upon MHC distribution.

Furthermore, according to these observations, the present investigation has shown that in the long term diaphragm and LN become more resistant to fatigue following temporary forced nasal obstruction. The changes in profile of the MHC in the LN of oral breathing rats could be explained by a decreased solicitation, in fact flaring appears modified in these rats, and they are less able to recognise and respond to receptive females (unpublished results).

Our study has shown that nasal obstruction caused early structural development changes of the respiratory muscles, starting within twenty-four hours of obstruction and remaining throughout the long term. Gelhaye et al.[23] showed that early nasal obstruction caused structural modification of respiratory muscles at D21, and now we have shown that these changes start very early during the period of nasal obstruction (D9–D11) and that they are maintained. Rodent studies have shown the influence of testosterone on the expression and maintenance of MHC fibres especially type IIb.[38,39] This would help to explain the increase in type IIb fibres in the MS muscle. However, the different muscles appeared to react differently to the increased plasma testosterone levels, with reductions in type IIb seen in LN and DA and no change seen in the diaphragm. Further, more detailed, analysis of the effect of testosterone on muscle MHC fibre type expression appears necessary.

Our results showed that animals with nasal obstruction presented an impaired olfactory bulb development during the period of nasal obstruction (D9, D11) as well as in the long-term (D90). Several studies have shown the impact of early nasal obstruction in rats. However, no study has shown that its impact is rapid and lasts through the long term. Gelhaye et al.[23] have shown the impact of early nasal obstruction (D8) on the development of the olfactory bulbs at weaning (D21). Loranca et al.[40] have also shown that early nasal obstruction (D3) causes atrophy of the olfactory bulbs for up to 70 days. Our results show that atrophy of the olfactory bulbs begins within 24 h after nasal obstruction (D9) and remains for the long term (D90). Furthermore, spontaneous reopening of the nasal passage is not complete therefore the olfactory bulb may not receive sufficient stimulation for normal responsiveness, as already mention above with respect to flaring.

Nasal obstruction is associated with the establishment of oral breathing, which causes short-term stress and disruption in development of the male rat. Indeed, our results have shown atrophy of the lungs during the period of nasal obstruction. To our knowledge, no studies have shown an impact of nasal obstruction on lung development. Nasal obstruction and the associated switch to temporary forced oral breathing were correlated with adrenal hypertrophy (72 h after treatment) and increased corticosterone plasma levels (24 h after treatment), but that this result was not maintained in the long term (D90). It is well known that there is a direct relation between stress exposure and increased adrenal gland weight.[13] Accordingly, we suggest that nasal obstruction and its consequences, i.e. low of body weight (perhaps via nutritional depletion), possible hypoxia, as well as olfactory deprivation related to social deprivation represent a multifactorial stressful situation, which enhances global activity of the hypothalamo–pituitary–adrenal axis. This result could be correlated directly with atrophy of the olfactory bulbs. Even if the mechanism is still poorly understood today, several studies have shown links between the olfactory systems and gonadotropin. Pieper[41] showed that bulbectomy in hamsters resulted in an increase in serum gonadotropin approximately one-half of the increase seen after castration. This suggests that the olfactory bulb has an influence on gonadotropin secretion which might be mediated by altering gonadal steroid feedback. Neurons throughout the olfactory and vomeronasal pathways in the limbic system have receptors for androgens and estrogens. In rats, olfactory bulb removal has been reported to decrease androgen receptor binding in both the amygdala and the hypothalamus.[42] If a similar reduction occurred in hamsters, this could explain why there was a decrease in the responsiveness to testosterone feedback on gonadotropin secretion.

Animals exposed to nasal obstruction showed a decrease in plasma T4 and T3 levels up to 90 days after the nostril

reopening. Different factors could be involved in these modifications amongst which are nutritional depletion and the associated secretion of glucocorticoids. A suppressive impact of nutritional deprivation on T3 and T4 levels has been shown,[43] and these effects could be mediated by activation of the hypothalamo–pituitary–adrenal axis.[44] Hypothyroidism observed in animals exposed to nasal obstruction could produce several deleterious effects such as a maturation defect of the central nervous system,[45] or a decrease of basic metabolism and thermogenesis.[46]

In conclusion, the present study has shown that the structure of respiratory muscles and the levels of plasma hormones can be altered by temporary forced oral breathing. The observed changes began very early during the period of nasal obstruction and were maintained over the long term. Indeed, in animals exposed to temporary forced oral breathing, muscles involved in respiratory activity presented an increased relative expression of fatigable MHC isoforms. These modifications could contribute in some part to various human pathologies, and it would be interesting to specify the factors that act to produce these changes in muscular structures and what other morphological changes could be involved. It will also be necessary subsequently to measure the degree of hypoxia and hydration in pups exposed to temporary forced oral breathing and to study the relation between chronic nasal obstruction, nutrition,[47] and craniofacial growth. Brozanski et al.[48] have shown that undernutrition per se delays the postnatal replacement of neonatal myosin by adult forms in the rat diaphragm. Our study showed that forced oral breathing appeared to have had such a large contrary effect with an increase in adult isoforms despite the malnutrition. Furthermore, nasal obstruction is considered a risk factor in sleep-disordered breathing,[49–51] which in children and adults has a very negative impact on quality of life with increased daytime sleepiness.[52] This symptom resembles that of obstructive sleep apnea caused by episodes of upper airway obstruction leading to episodic hypercapnic hypoxia which alters upper airway muscle structure and fibre type expression in ways somewhat similar to those reported here.[53] This could indicate that our model of temporary nasal obstruction could be an appropriate model for looking at potential changes in hormones and other physiological parameters of rhinitis or other temporary obstructive nasal breathing pathology.

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