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INFLUENCE OF PREMATURITY ON POSTNATAL MATURATION OF HEART RATE AND ARTERIAL PRESSURE RESPONSES TO HYPOXIA IN LAMBS

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ABSTRACT

Background: While hypoxic events with bradycardias are a frequent occurrence during the first weeks of life in preterm infants, the impact of preterm birth on maturation of the cardiovascular response to hypoxia in early postnatal life is unknown.

Objectives: In the present study, we tested the hypothesis that preterm birth influences postnatal maturation of cardiovascular responses to hypoxia.

Methods: Six preterm lambs (term 132 days, birth weight 2.9 kg) and six full-term lambs (term 147 d, birth weight 4.3 kg) were studied without sedation at day 3, 7, 14, 21 and 28 during acute, steady-state hypoxia (FI_{O2} = 0.08, 15 min).

Results: Results show that full-term lambs increased their HR in response to hypoxia from the first day of life, with no postnatal maturation thereafter. In preterm lambs, HR did not change before day 14 and the full-term-like response was not acquired before two weeks after theoretical term. In both groups, MAP tended to decrease below baseline, but the amplitude of variations remained of small magnitude.

Conclusion: the present results bring unique evidence that preterm birth is associated with a blunted heart response to steady-state hypoxia during the first postnatal week and a delay in the maturation of HR response.

INTRODUCTION

In mammals, acute sustained hypoxia triggers complex adaptive cardiorespiratory responses, including hyperventilation, tachycardia, systemic hypertension and blood flow redistribution in an attempt to preserve cellular activity in vital organs. In particular, the cardiovascular response to hypoxia depends on the complex balance of excitatory and inhibitory influences, including those from arterial chemo- and baroreceptors, from bronchopulmonary stretch receptors, and the direct effect of hypoxia on the central nervous system (1). In addition, the responses are dependent on the level of hypoxia and CO_2, the presence of acidemia, and likely on species type and level of maturation. In mature mammals, while the response to *mild* hypoxia most often includes tachycardia, increased cardiac output, variable changes in blood pressure and reduced total peripheral resistance, there is a tendency towards bradycardia and increased peripheral vascular resistance during *severe* hypoxia (1).

In newborn mammals, the overall cardiorespiratory response to hypoxia is characterized by a predominance of inhibitory central mechanisms upon excitatory peripheral chemoreceptor input. Consequently, the ventilatory response is blunted after an initial hyperventilation (2,3). In addition, the cardiovascular response to hypoxia is reportedly variable. Hence, while a bradycardia (at times after an initial tachycardia) and a decrease in MAP have been reported in newborn piglets (4), the response to hypoxia in full-term newborn infants and lambs includes an increase in both HR and cardiac output with small or transient changes in MAP (5,6) and depends, at least partially, on sympathetic activity (7).

While recurrence of hypoxic episodes is especially frequent in preterm infants, the effect of premature birth on the cardiovascular response to acute hypoxia in mammals is largely unknown. The fall in HR and the increase in MAP observed in the fetal lamb during *in utero* hypoxia is clearly different from the response observed in the full-term, newborn lamb (review in 8). The same is true for the absence of significant changes in HR and cardiac output in response to hypoxia at birth in preterm lambs (9).

Previous data on the postnatal maturation of the cardiovascular response to hypoxia have proven inconsistent. While the response does not change in full-term lambs during the first weeks of life (6), both HR and MAP increase in response to hypoxia from 2 days to 2 months of age in full-term piglets (4,10). To our knowledge, despite its obvious clinical relevance, the effect of preterm birth on the postnatal maturation of the cardiovascular response is unknown.

The aim of the present study was to assess the effect of preterm birth on the cardiovascular response to hypoxia during the first month of life in lambs, and to test the hypothesis that preterm birth blunts HR and MAP response to acute, steady-state hypoxia, and influences its postnatal maturation during the first weeks of life.

METHODS

Animals

Six preterm lambs (postconceptional age 132 ± 1 d, range 130 to 132 d) and six full-term lambs (normal term 147 d) were studied. Mean birth weight was 2.9 ± 0.7 kg (range 1.9 to 4.3 kg) for preterm lambs and 4.3 ± 0.9 kg (range 3.1 to 5.8 kg) for full-term lambs, the wide range being due to the presence of multiple gestations (2-3 lambs) in both groups of lambs. The protocol of the study was approved by our institution's Ethics Committee for Animal Care and Experimentation.

Preterm lambs were obtained as described in a previous study (11). Briefly, pregnancies were dated accurately by means of single mating after induction of estrus. Fetal lung maturation was accelerated by administration of betamethasone and thyrotropin-releasing hormone to the ewe within 48 h prior to delivery to prevent respiratory distress syndrome at birth. Vaginal delivery was induced by oxytocin IV infusion, 12 h after intravaginal placement of prostaglandin E_2. Exogenous surfactant replacement (5 ml/kg) (BLES, London, ON) was given to all lambs by direct intratracheal injection immediately after delivery, aiming at giving it before the first breath. After standard initial neonatal care, the preterm newborn lamb was returned to its mother as soon as its general status, rectal temperature, glycemia, respiration and transcutaneous oxygen saturation were normal and stable. In case of hypothermia (< 38.5^{o}C) and/or hypoglycemia (< 2.3 mmol/L) because of insufficient spontaneous feedings, the lamb was warmed in an incubator and bottle-fed with mother's milk. In cases of persistent retractions or grunting and/or tachypnea (> 80/min) or arterial saturation below 95% despite supplemental nasal oxygen, a second dose of exogenous

surfactant was given. This was necessary in 2 lambs. One lamb needed endotracheal ventilatory support (Bourns-BP200, Life System, Riverside, CA) for 2 hours within the first 24 hours of life.

Full-term lambs were born in our local provider's farm and arrived in our laboratory within 8 hours of birth.

Study Design

An arterial catheter was inserted into the brachial artery under local anesthesia (2% lidocaine), one or two hours before the first experiment. The catheter was glued on the skin and covered by gauze dressing secured by medical tape. Daily flush with heparinized saline solution and dressing change with application of antibiotic ointment were systematically performed to help preventing local infection and maintaining catheter patency. A second catheter was placed in the other brachial artery when the first catheter was displaced or no more patent. Antibiotics (25000 U/kg IM long-acting penicillin and 5 mg/kg IM gentamicin) were repeated daily. A second catheter (Insyte, 18GA, Infusion Therapy Systems Inc., Utah, USA) was introduced transcutaneously into the superior vena cava through the superficial jugular vein prior to each experiment. The lamb was then comfortably positioned in a sling with loose restraints. A plaster-molded face-mask with a pressure port was glued on its snout using latex. The face-mask was connected via a T piece to a tubing with constant airflow. The inspiratory gas could be switched from air to various gas mixtures within < 1 s (model 21049 and P314 Collins valves). Gas analyzers (Sable Systems, Henderson, NV) were used to measure the inspired fraction of O_2 (FI_{O2}) (model FC-1B) and to monitor end-tidal CO_2 (model CA-1B) at the mask. Electrocardiogram (subcutaneous needle-electrodes) and arterial blood

pressure (pressure transducer Trantec model 60-800, American Edwards Laboratories, Santa Ana, CA and pressure monitor model 78342A Hewlett Packard, Waltham, MA) were continuously monitored. All variables were recorded on an Apple microcomputer, using data-acquisition software (AcqKnowledge version 3.2, MP100A, Biopac Systems Inc., Santa Barbara, CA) and stored on compact disks for further analysis.

The following experiment was first performed between day 2 and day 4 (named day 3 in the results) then repeated on days 7, 14, 21 and 28. All experiments were performed in non-sedated lambs during quiet wakefulness. Ambient temperature was maintained at 23°C.

Steady-State Hypoxia. All animals underwent one hypocapnic hypoxia run per experimental day, as previously described (12). Baseline ventilation was recorded for 3 min. The inspiratory gas was then abruptly switched to F_{IO2} = 0.08, for a duration of 15 min. After 15 min of hypoxia, the lambs were abruptly switched back to room air for 3 min of recording. The entire test was completed in 21 min.

Data Analysis

Analysis of the cardiovascular response to hypoxia was performed as follows. Both heart rate (HR) and mean arterial pressure (MAP) values were expressed for each hypoxic run as a percentage change from baseline (%HR and %MAP). For example, the percentage change in HR (%HR3min) at 3 min of hypoxia was calculated as [(HR 3min – HR BL) x 100 / HR BL]. MAP was only studied in 4 lambs within each group and only during the first three weeks of life because of technical difficulties in maintaining the patency of arterial catheters on the long-term. Measured values were averaged over 1 min during baseline, room-air breathing, and over 10 seconds at 3, 6, 9 and 15 min of

hypoxia and 2 min after the return to room-air breathing. In the absence of a biphasic response to hypoxia, all HR and MAP values obtained during the hypoxic period were averaged for each lamb and at each experimental day. The average values were then used to describe the changes in HR and MAP within each group of full-term and preterm lambs and to compare the response between the groups in order to assess postnatal maturation of the response within each group and between groups. In addition, the HR and MAP responses to hypoxia were compared at the same postconceptional age, in order to assess whether preterm birth alters normal maturation, which occurs in utero during the late gestation period in term animals.

Statistical analysis. The Statistica software (Statsoft, Tulsa, OK) was used. Values were expressed as mean ± standard deviation and compared by Student's t test for paired or unpaired observations and Wilcoxon or Mann-Whitney tests as appropriate. Hypoxia response curves were compared by two way repeated measures ANOVA and by post-hoc Student's t-test or Mann-Whitney test as appropriate. In addition, covariance analyses adjusting for postnatal age were performed to test whether overall postnatal maturation was different between preterm and full-term lambs using the Super-ANOVA software (Abacus Concepts, Berkeley, CA). A simple linear regression analysis was used to study the relationship between arterial blood gases and pH during hypoxia and postnatal age. Significance was set at $p < 0.05$.

RESULTS

Baseline room-air breathing

HR and MAP values measured in baseline conditions at all postnatal ages are shown in figure 1 for both full-term and preterm lambs. While HR did not change with postnatal age in full-term lambs, it decreased from D3 to D28 in preterm lambs ($p < 0.01$). More specifically, baseline HR was lower on D7, D21 and D28 than on D3 in preterm lambs ($p < 0.05$). In addition, HR was significantly higher in preterm lambs than in full-term lambs on D3 and D7 ($p < 0.01$). Resting MAP was not significantly modified in either group between D3 and D21, with no significant differences between groups. At the same post-conceptional age, no significant differences in resting HR (figure 2) or MAP were observed between preterm and full-term lambs. No significant differences in arterial pH, PaO_2, $PaCO_2$ were observed in baseline conditions within or between each group (Table 1).

Cardiovascular response to steady-state hypoxia

In full-term lambs, a significant increase in HR (% HR = 50 ± 27 %; range between 29 and 89%) was observed in response to hypoxia at all ages (overall hypoxia effect: $p < 0.05$ *vs.* baseline by two way, repeated measures ANOVA). Post-hoc test did not reveal any significant differences at any age however. Interestingly, the HR response was sustained throughout the 15-min period, with no change with maturation (Figure 2 and 3). In contrast, alterations in MAP during hypoxia were limited, with a tendency towards a decrease below baseline value, which did not exceed 5 % (Figure 4).

In preterm lambs, the HR response was strikingly different from that observed in full-term lambs, with an absence of increase in HR during hypoxia until D14 (figures 2 and 3) and a significant difference in the HR response between groups ($p < 0.001$ at D3 and < 0.01 at D7). From D14 thereafter, the overall increase in HR during hypoxia was significant in preterm lambs, with no difference between preterm and full-term lambs. Overall, postnatal maturation of the HR response to hypoxia during the first 4 postnatal weeks was significantly different between preterm and full-term lambs ($p < 0.01$). Moreover, the HR response to hypoxia was less important at D153-154 of post-conceptional age (term: D147) in preterm than in full-term lambs (Figure 2, $p < 0.01$). The HR response was identical in both groups two weeks after theoretical term.

A mild decrease in MAP was observed in response to hypoxia at all post-natal ages (overall hypoxia effect: $p < 0.01$ to 0.05 *vs.* baseline by two way, repeated measures ANOVA, Figure 4). This decrease in MAP was significantly greater on D3 (- 16 ± 12 %) than later, and significantly more important in preterm than in full-term lambs on D3 ($p < 0.01$). Finally, the overall postnatal as well as postconceptional maturation of the MAP response to hypoxia were not significantly different in preterm and full-term lambs (Figure 4).

Arterial blood gases changes during hypoxia

We did not observe any significant differences between preterm and full-term lambs with regards to arterial blood gases and pH (Table 1). When pooling preterm and full-term lambs altogether, a correlation was found between postnatal age and arterial blood gases during hypoxia, with a decrease in $PaCO_2$ ($r = - 0.85$, $p < 0.01$), an increase in PaO_2 ($r = 0.95$, $p < 0.01$) and an increase in arterial pH ($r = 0.71$, $p < 0.05$) with

postnatal age. In addition, $PaCO_2$ during hypoxia was correlated with PaO_2 ($r = - 0.91$, $p < 0.01$), suggesting that postnatal maturation was responsible for enhancing the hypoxic ventilatory response, which consequently limited hypoxemia. Those correlations between blood gases and postnatal age, and between PaO_2 and $PaCO_2$ were observed within each group, except for pH in full-term and $PaCO_2$ in preterm lambs.

DISCUSSION

Results of the present study show the influence of preterm birth on the early postnatal maturation of the cardiovascular responses to steady-state, poikilocapnic hypoxia. It was found that the normal tachycardic response to hypoxia, observed from the first days of life in full-term lambs, was inhibited in preterm lambs until 14 days of life. In addition, while the overall postnatal maturation of HR response was significantly greater in preterm lambs, complete recovery of their HR response was delayed for two weeks after theoretical term. Simultaneously, the effects of preterm birth on the normally weak MAP response to hypoxia were modest and only observed at D3.

Effects of preterm birth on heart rate response to hypoxia

The increase in HR from birth in full-term lambs, with no postnatal maturation, confirms previous results in lambs obtained during the first weeks of life (6). Indeed, hypoxia (FiO_2 = 9%) has been reported to induce an increase in HR, with a tendency for MAP to decrease and an increase in cardiac output in full-term lambs (6). While the reason for the absence of HR response in preterm lambs is not straightforward, there are several potential explanations. Available data suggest that, while an increase in both beta (7) and alpha (14) sympathetic activity is involved in HR response to acute hypoxia in the full-term lamb from the first week of life, this increase is blunted in preterm lambs (15). This blunting has been attributed to reduced sympathetic innervation and adrenomedullary catecholamine release and to a delayed or incomplete development of adrenergic receptor-effector mechanisms in target tissues. It should be cautioned however that antenatal administration of betamethasone in preterm lambs, which was

an absolute necessity to prevent respiratory distress syndrome at birth, may have blunted the differences in HR response to hypoxia between preterm and full-term lambs. Indeed, administration of antenatal glucocorticoids has been shown to improve cardiovascular function and enhance sympathetic responsiveness to hypoxia in preterm lambs (9,16,17). Of note, the absence of increase in HR during hypoxia in newborn rats exposed to nicotine prenatally has also been related to a deficit in adrenomedullary catecholamine release (18), which poses the question as to whether the effects of preterm birth and prenatal cigarette exposure are additive on HR response to hypoxia during the first postnatal days.

Several other mechanisms, aside from enhanced sympathetic activity, may also be involved in explaining the effect of preterm birth on the HR response to hypoxia. First, the increased vagal efferent activity due to peripheral chemoreceptor stimulation by hypoxia may in fact decrease HR through a muscarinic efferent pathway in preterm lambs, as previously reported in term fetal sheep (19). Secondly, while the lung inflation reflex tends to increase HR in adults through stimulation of bronchopulmonary stretch receptors during hypoxic hyperventilation (20), previous results suggest that this reflex is not present in premature newborns (21). And the significantly smaller increase in V_T observed in preterm comparatively to term lambs during hypoxia for the first postnatal week (unpublished personal results) is a weaker stimulus for bronchopulmonary stretch receptors. Accordingly, the association of bradycardia and absence of hypoxic hyperventilation in the compromised newborn has been previously attributed to absence of the lung inflation reflex (22). Thirdly, while previous studies in full-term lambs during the first 2 weeks of life have shown a moderate decrease in metabolism during acute hypoxia (23 and unpublished data), results obtained in newborn mammals from more

altricial species (24) suggest that this hypoxic hypometabolism could be enhanced in the more immature preterm lambs. Although metabolism was not measured in this study, it is conceivable that such a decrease would predominate in the first week of life and would participate, in turn, in the decreased HR response to hypoxia observed initially in preterm lambs. Fourthly, brainstem regions related to autonomic outflow may contribute to the initial absence of HR response to hypoxia in preterm lambs, through either immaturity of neuronal integration or heightened direct hypoxic depression, as reported previously in young piglets (10). Finally, the potentially differential effect of hypocapnia observed during hypoxia is difficult to assess in preterm *vs*. full-term lambs. Indeed, hypocapnia has been reported to modulate the responses to hypoxia both by causing a depression of the central vasomotor neurons and by decreasing the effectiveness of afferent chemoreceptor activity (20), with a consequent increase in HR in adult mammals (25).

While postnatal maturation allowed preterm lambs to regain responses similar to full-term lambs, this was accomplished after a certain delay, which suggests that *ex utero* maturation of the response is slow compared to late prenatal maturation, which occurs during normal pregnancy. This alteration in maturation of heart rate response to hypoxia during extrauterine life in preterm lambs is in agreement with the observation that, in human preterm infants, short-term reflex cardiovascular control in response to hypercapnia remains altered after term age (26). These alterations in maturation of cardio-vascular responses induced by preterm birth could have short term consequences during the high risk neonatal period as well as long term consequences on cardiovascular function.

Effects of preterm birth on arterial pressure response to hypoxia

In the present study, a small decrease in MAP (mean = - 11%) was observed during hypoxia in the first 2 weeks of life in full-term lambs. Previous results had also shown a slight decrease in MAP during hypoxia (FiO2 = 9%) in full-term lambs in the first week of life (-6%) (6), as well as a tendency towards an increase in MAP from birth to 2 months of age in piglets (4). Overall, the changes in MAP during hypoxia are of small amplitude.

Interestingly, the decrease in MAP was prolonged throughout the 3 week-study period in preterm lambs and significantly more pronounced (significant on day 3), as compared to full-term lambs. Potential explanations for the differences between preterm and full-term lambs may be similar to those discussed at length for HR. Also, the antenatal administration of betamethasone in preterm lambs, with or without TRH (27), as discussed above for the HR response (9,17), may have blunted the differences in MAP response to hypoxia between preterm and full-term lambs. Finally, previous results question whether the simultaneous use of betamethasone, TRH, PGE_2 and oxytocin, all known to increase systemic arterial pressure, has contributed to enhancing cardiovascular adaptation in preterm lambs and blunting the negative effect of preterm birth on MAP response to hypoxia (28-29).

In conclusion,

The present study provides unique evidence that preterm birth inhibits the cardiovascular response to hypoxia during the first postnatal week. This could increase the vulnerability of preterm infants to hypoxic stress. In addition, recovery of the hypoxic response to a level identical to that observed in full-term lambs through postnatal maturation is delayed beyond term age. In addition to the valuable physiological

knowledge gained, clinical importance of the present observations stems from the frequent observation of hypoxic and bradycardic events in the first weeks of life in preterm infants. Further studies will have to delineate the potential mechanisms involved in the effects of preterm birth on the cardiovascular response to hypoxia and its postnatal maturation. For example, differences in the autonomic control of heart rate variability, limitation of myocardial work, as well as differences in maturation of the renin angiotensin system will be especially important to consider.

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Table 1 : Arterial blood gases at baseline and after 5 min of hypoxia (n = 4 in each group). Values are means (SD). D: day

	Baseline					
	pH_a		**P_aCO_2 (mmHg)**		**P_aO_2 (mmHg)**	
Age	*Preterm*	*Full-Term*	*Preterm*	*Full-Term*	*Preterm*	*Full-Term*
D3	7.31 (0.03)	7.34 (0.04)	46 (4.5)	42 (3.5)	76 (15.5)	80.5 (10)
D7	7.35 (0.05)	7.36 (0.01)	39 (5)	39 (3.5)	90 (13.5)	94 (5)
D14	7.30 (0.09)	7.35 (0.02)	40.5 (3)	36.5 (1)	99 (6.5)	98 (5)
D21	7.35 (0.06)	7.35 (0.02)	40 (3)	37.5 (3.5)	109 (26)	111 (22.5)
D28	7.38 (0.05)	7.35 (0.04)	38 (7)	35.5 (10)	102 (13.5)	95 (6.5)
	After 5 min of Hypoxia					
D3	7.39 (0.03)	7.40 (0.05)	38 (5)	36.5 (5)	20.5 (3)	21.5 (3.5)
D7	7.44 (0.06)	7.46 (0.01)	33 (2.5)	33 (2.5)	26 (3.5)	25.5 (2)
D14	7.42 (0.04)	7.44 (0.03)	33 (5)	31.5 (3)	31.5 (4)	31.5 (4)
D21	7.47 (0.03)	7.46 (0.04)	31 (3)	30 (3)	33 (5)	31.5 (2.5)
D28	7.49 (0.04)	7.44 (0.05)	31.5 (1.5)	29 (8)	35.5 (4)	37.5 (17.5)

FIGURE LEGENDS

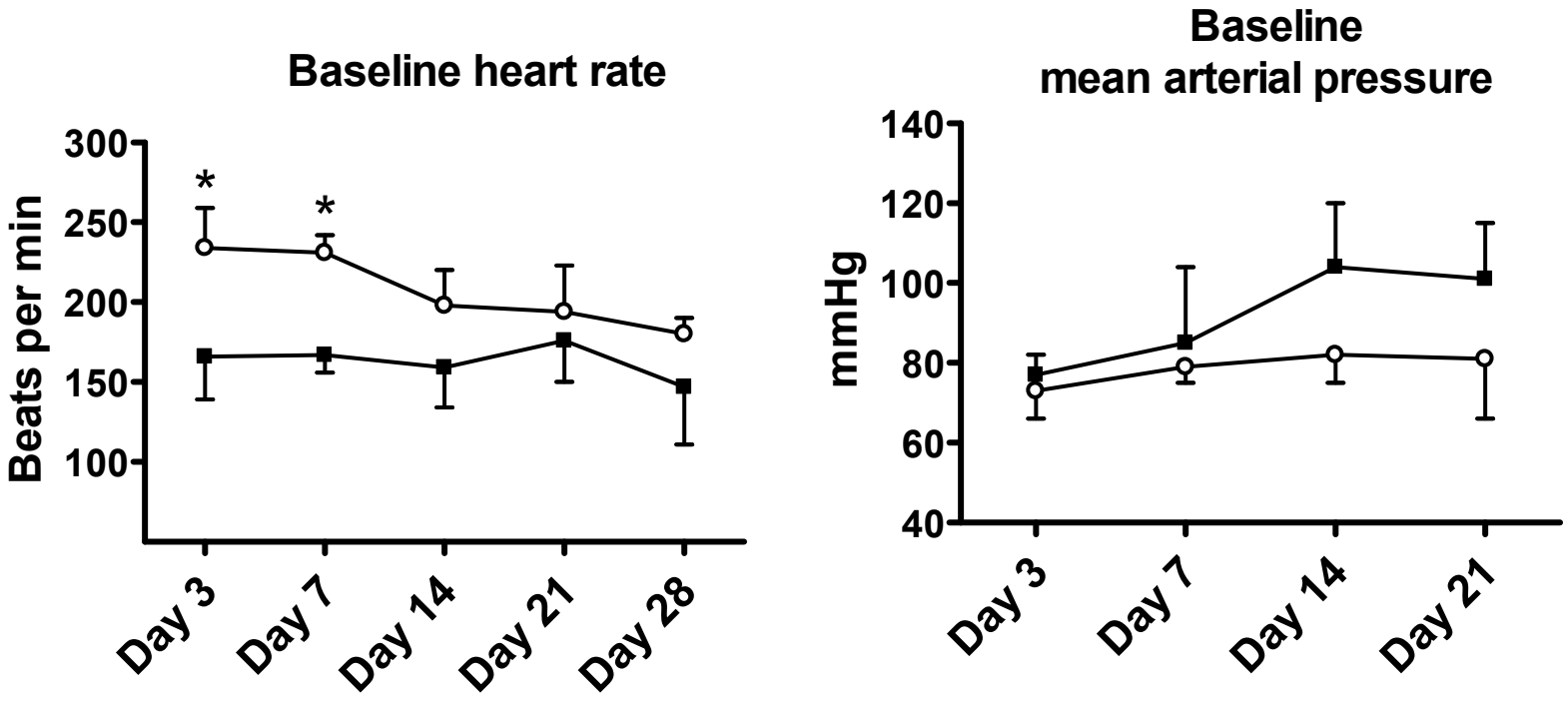
Figure 1: Postnatal maturation of heart rate (beats.min^{-1}) and mean arterial pressure (mmHg) in baseline conditions in preterm (open circles) and full-term (solid squares) lambs. Values are presented as mean ± SD. *, $p < 0.01$ preterm *vs.* full-term; &, $p < 0.05$ *vs.* D3 in preterm lambs

Figure 2: Time course of heart rate response to a 15-min steady-state hypoxic period in preterm (dark grey area) and full-term (light gray area) lambs, presented as a function of post-conceptional age. The mean individual heart rate values obtained at baseline, at each time of the 15 min hypoxia period and after return to room air were used to display changes in heart rate for both preterm and full-term lambs at each post-conceptional day. Heart rate response during hypoxia was significant ($p < 0.05$) at all ages in both groups, but in preterm lambs before D146. *, $p < 0.01$ preterm *vs.* full-term; & $p < 0.01$ preterm *vs.* preterm on postnatal day 3 (day 135 of post-conceptional age).

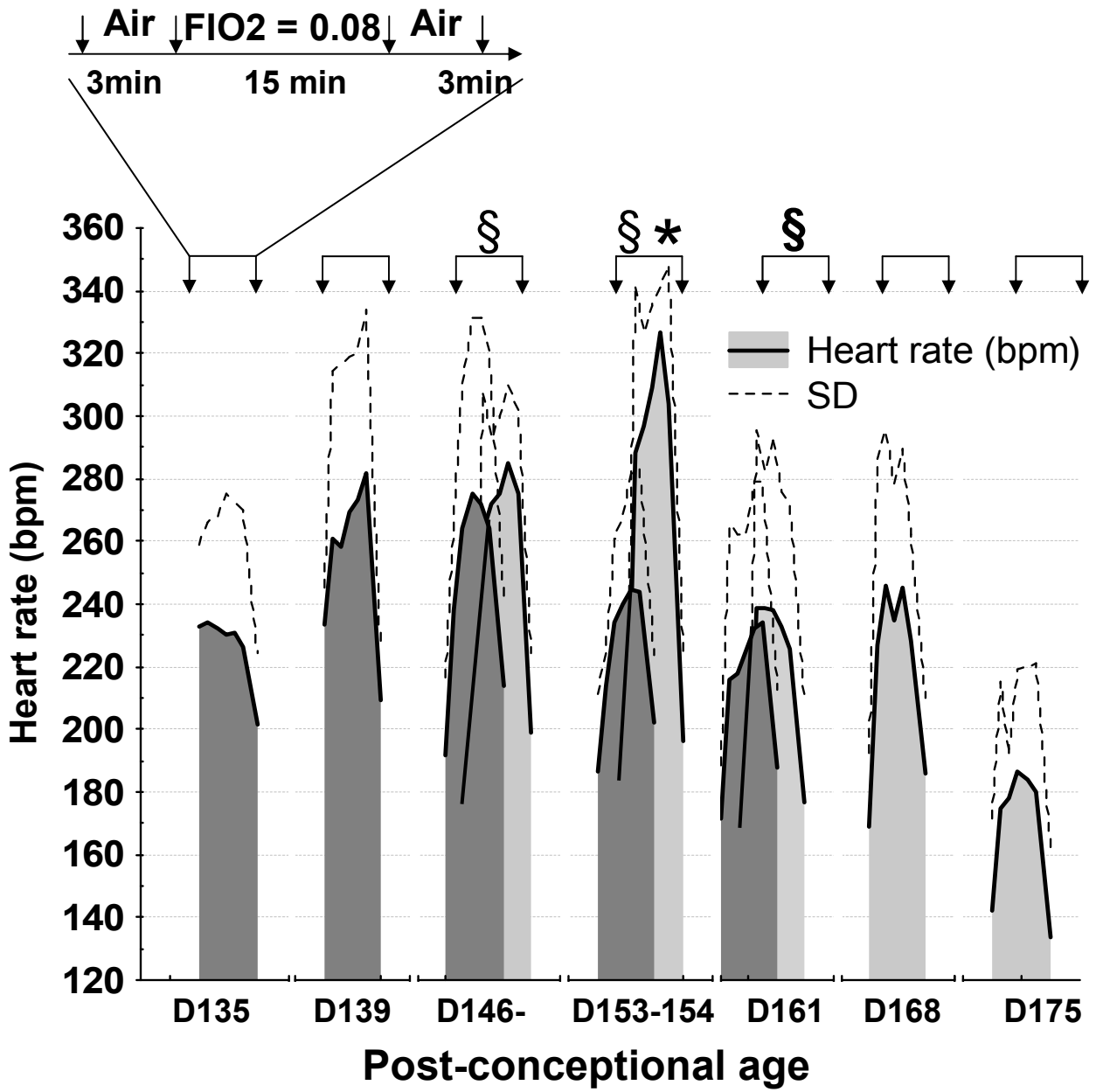
Figure 3: Postnatal maturation of heart rate (HR) response during steady-state hypoxia in preterm (open circles) and full-term (solid squares) lambs. The mean individual heart rate values obtained at baseline, at each time of the 15 min hypoxia period and after return to room air are plotted against postnatal age for both preterm and full-term lambs. The overall heart rate response during hypoxia was significant ($p < 0.05$) at all ages in full-term lambs, and from D14 thereafter in preterm lambs. The results are presented as percentage of change from baseline HR (mean ± SD). *, $p < 0.01$ full-term *vs.* preterm lambs.

Figure 4: Postnatal maturation of mean arterial pressure (MAP) response during steady-state hypoxia in preterm (open circles) and full-term (solid squares) lambs. Values were averaged in each lamb during the 15-min hypoxic period, then averaged for preterm and for full-term lambs at each postnatal day. The results are presented as percentage of change from baseline MAP (mean ± SD). *, $p < 0.01$ full-term *vs.* preterm lambs.

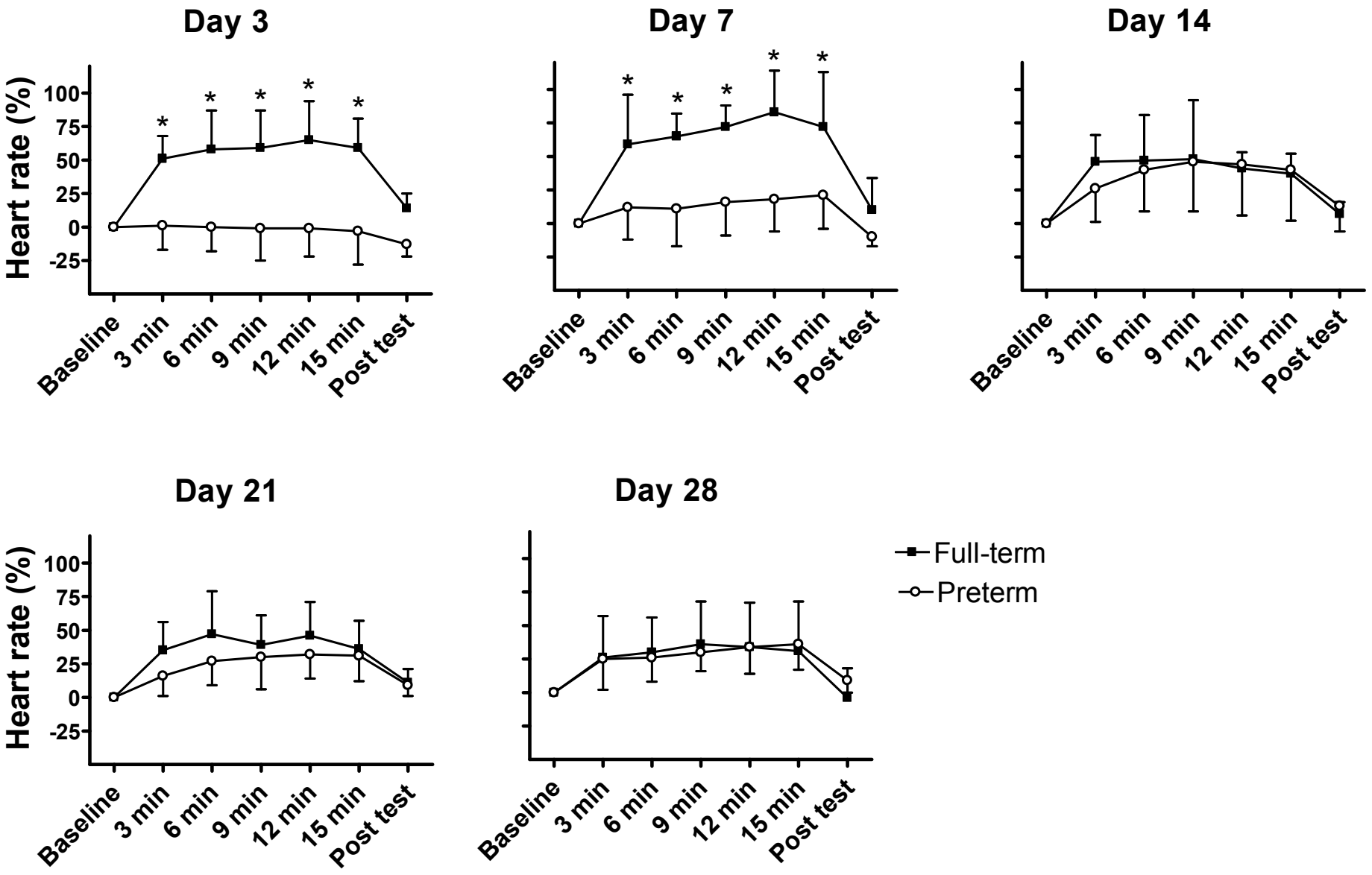
Patrick Pladys, Figure #1



Patrick Pladys, Figure #2



Patrick Pladys, Figure #3



Patrick Pladys, Figure #4