

## Mechanics of the respiratory system in the newborn tammar wallaby

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### Summary

We investigated whether the mechanical properties of the respiratory system represent a major constraint to spontaneous breathing in the newborn tammar wallaby *Macropus eugenii*, which is born after a very short gestation (approximately 28 days, birth mass approximately 380 mg). The rate of oxygen consumption ( $\dot{V}_{O_2}$ ) through the skin was approximately 33% of the total  $\dot{V}_{O_2}$  at day 1 and approximately 14% at day 6. The mass-specific resting minute ventilation ( $\dot{V}_E$ ) and the ventilatory equivalent ( $\dot{V}_E/\dot{V}_{O_2}$ ) were approximately the same at the two ages, with a breathing pattern significantly deeper and slower at day 1. The mass-specific compliance of the respiratory system ( $C_{RS}$ ) did not differ significantly between the two age groups and was close to the values predicted from measurements in eutherian

newborns. Mass-specific respiratory system resistance ( $R_{RS}$ ) at day 1 was higher than at day 6, and also higher than in eutherian newborns. Chest distortion, quantified as the degree of abdominal motion during spontaneous breathing compared with that required to inflate the lungs passively, at day 1 was very large, whereas it was modest at day 6. We conclude that, in the tammar wallaby at birth, the high resistance of the respiratory system and the distortion of the chest wall greatly reduce the mechanical efficiency of breathing. At this age, gas exchange through the skin is therefore an important complement to pulmonary ventilation.

Key words: chest wall, development, skin, gas exchange, neonatal respiration, newborn marsupial, tammar wallaby, *Macropus eugenii*.

### Introduction

After a short gestation, marsupials are born at a very early stage of development. Their oxygen requirements are low in comparison with those of other newborns, possibly because the maternal pouch eliminates the energy cost of body temperature regulation. Hence, the large body surface area-to-mass ratio determined by the small body size, coupled to the high rate of gas diffusion across the skin, are characteristics sufficient to make the skin the major route for gas exchange, with little need for lung ventilation (Mortola et al., 1999; MacFarlane and Frappell, 2001).

Whether gas exchange across the skin represents an alternative to gas exchange through the lungs or is the only possibility in marsupial neonates, which, because of their altricial characteristics, do not have a mechanically functional respiratory apparatus, is not clear. In the newborn Julia Creek dunnart *Sminthopsis douglasi*, in which the skin is the primary gas exchanger for several days after birth, the compliance of the respiratory system, normalized by body mass, was not much lower than in newborns of other eutherian mammals (Frappell and Mortola, 2000). This suggests that, even in this species, born after approximately 12 days of gestation, the lungs contain type II epithelial cells and have a functional complement of surfactant, as demonstrated in neonates of other marsupials (Krause et al., 1976; Ribbons et al., 1989).

Visual inspection indicated that in these newborns coordinated chest wall expansions are a rare occurrence; rather, wiggling and random movements of the whole body are the common patterns (Mortola et al., 1999; Frappell and Mortola, 2000). In foetal rats and mice, rhythmic bursts of activity of the phrenic nerves occur only from embryonic day 16, or 2–3 days after the phrenic axons have reached the primordial diaphragm (Greer et al., 1999). In contrast, the central nervous system of the neonatal opossum *Monodelphis domestica*, born after approximately 13 days of gestation, already contains neurons with properties that enable rhythmic respiration (Eugenin and Nicholls, 2000). Further, in the pouch young opossum, *Didelphis virginiana*, the firing rates of the medullary respiratory neurons do not represent a limitation to breathing (Farber, 1993). Hence, it is unlikely that pulmonary ventilation in the newborn marsupial is prevented by inadequate neural control. It remains possible that poor muscle coordination and chest wall distortion impede efficient and reliable breathing, despite the fact that, in passive conditions, the mechanical properties of the respiratory system may seem adequate. In such a case, skin gas exchange would be the only option for survival of an organism born at such an early developmental stage.

In this study, we have measured the mechanical properties

(compliance and resistance) of the respiratory system of the newborn tamar wallaby *Macropus eugenii* and the movement of the chest wall in active conditions, i.e. during spontaneous breathing. The results suggest that chest distortion is a major constraint to efficient breathing in the neonate of this species.

### Materials and methods

The rates of ventilation ( $\dot{V}_E$ ) and oxygen consumption ( $\dot{V}_{O_2}$ ), respiratory system compliance ( $C_{RS}$ ) and resistance ( $R_{RS}$ ) and chest wall distortion were measured in 13 awake and unanaesthetized neonatal tamar wallabies *Macropus eugenii* (Desmarest) at 1 and 6 days after birth (day 1, mass  $0.47 \pm 0.03$  g,  $N=7$ ; day 6, mass  $1.26 \pm 0.20$  g,  $N=6$ , means  $\pm$  S.E.M.). Ages were determined from the day of birth (day 0), and experiments were conducted the following day; hence, animals were aged day 1. The pouch young were removed from the teat of the female at the required age and immediately prepared for the experiment. Experiments were conducted following approval from the Animal Ethics Committee of LaTrobe University.

The apparatus used to measure rates of metabolism and ventilation in this experiment was similar to that described previously (Frappell and Mortola, 2000; MacFarlane and Frappell, 2001). In brief, a small mask constructed from a short length of polyethylene tube was placed over the mouth and nostrils of an animal, and the open part of the mask was inserted through a hole in a flexible rubber stopper. A non-toxic dental compound (Impregum F, Polyether Impression Material, ESPE) sealed the mask to the face of the animal. The stopper holding the mask was placed medially inside a moist, water-jacketed chamber (which maintained temperature,  $36.5^\circ\text{C}$ , and humidity, 100% relative humidity) in a way that divided the chamber into two completely separate compartments. One compartment communicated with the airways and the other enclosed the body; the volume of each

compartment was approximately 10 ml. Each compartment was sealed by a rubber stopper placed at either end of the chamber; injection of a known volume of air and the resultant stability of the pressure change, measured by a sensitive pressure transducer ( $\pm 490$  Pa; Spirometer, PowerLab) ensured that the compartment was sealed.

### Gaseous metabolism and ventilation

The animal was given 10 min to equilibrate to temperature and humidity while each compartment was flushed with room air to prevent the conditions from becoming asphyxic. Airflow through each compartment was maintained at  $60 \text{ ml min}^{-1}$  by a roller pump (Masterflex easy-load, model 7518-10) before each compartment was sealed for 6–8 min depending on the age and size of the animal. Ventilation was measured while the compartments were sealed. The pressure transducer was connected to the compartment that communicated with the airways, and the pressure oscillations associated with breathing (calibrated for volume by injecting and withdrawing  $10 \mu\text{l}$  of air) were digitally converted and recorded for later analysis (PowerLab/800, ADInstruments). The breathing trace was analysed for tidal volume ( $V_T$ ), respiratory frequency ( $f$ ) and ventilation ( $\dot{V}_E = V_T f$ ). At the end of this period, both compartments were again flushed with air, and the gas from each compartment was passed separately through a drying column (Drierite, Hammond Drierite Co.) before being analysed for fractional concentrations of  $\text{O}_2$  and  $\text{CO}_2$  by appropriate gas analysers (PowerLab Gas Analyser, model ML205;  $\text{O}_2$  and  $\text{CO}_2$  accuracy to 0.01%). The outputs of the analysers were digitally converted and recorded (PowerLab/800, ADInstruments, 200 Hz). The rates of oxygen consumption ( $\dot{V}_{O_2}$ ) and carbon dioxide production ( $\dot{V}_{\text{CO}_2}$ ) were calculated from the time integral of the gas concentration curves multiplied by the flow and the reciprocal of the time for which each compartment was sealed (Frappell and Mortola, 2000).

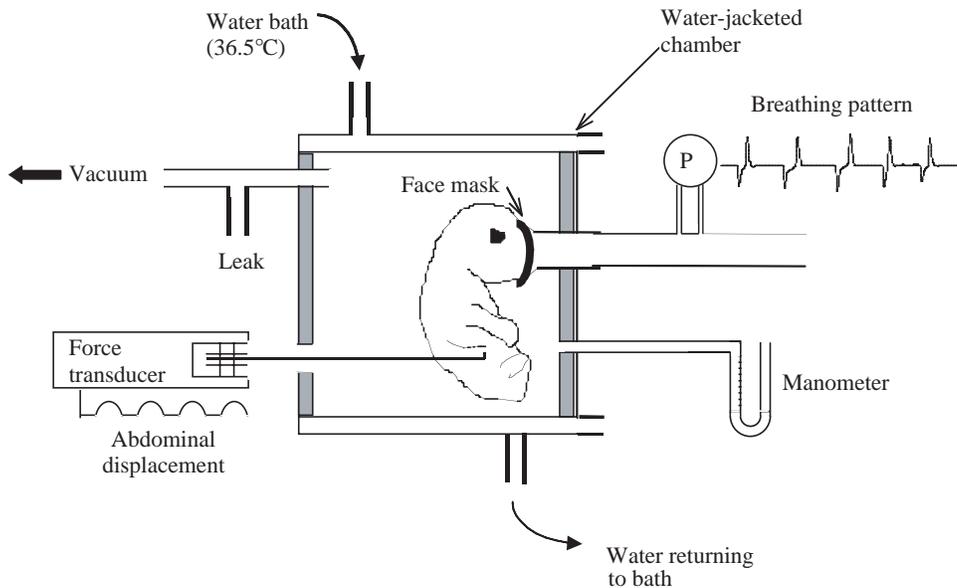


Fig. 1. Schematic diagram of the apparatus used to measure the mechanics of the respiratory system. The joey fitted with a facemask was placed in a water-jacketed chamber to maintain temperature and humidity; the facemask opened to room air. A pneumotachograph (P) was inserted directly into the open end of the facemask and the corresponding flow trace electronically integrated for volume. A vacuum was applied to the compartment enclosing the body to produce negative pressures of 981 Pa, adjusted by altering the size of a leak and monitored by a water-filled manometer. Abdominal displacement was measured using a force transducer with a wire arm resting laterally on the abdominal surface just below the final rib. A moist swab maintained humidity in the chamber.

*Mechanics of the respiratory system*

The mechanical characteristics of the respiratory system were measured following slight modification to each of the compartments in the system described above (Fig. 1). The rubber stopper at the end of the compartment that was in communication with the airways was removed. A pneumotachograph, constructed according to the method of Mortola and Noworaj (1983), was inserted directly into the open part of the mask. The two side arms of the pneumotachograph were connected to the pressure transducer, and the corresponding flow signal ( $\dot{V}$ ) associated with breathing was electronically integrated for volume ( $V$ ). Breathing was calibrated for volume by injecting and withdrawing 30  $\mu\text{l}$  of air through the pneumotachograph before it was connected to the mask.

*Respiratory system compliance and resistance*

A vacuum was connected to a side port in the rubber stopper that sealed the body compartment. Negative pressures of 981 Pa were applied to the body surface 5–6 times at 1 min intervals; the pressure was monitored with the aid of a manometer and adjusted accordingly by varying the size of a leak that had been incorporated into the chamber. The passive compliance of the respiratory system ( $C_{rs}$ ) was then calculated from the change in lung volume ( $\Delta V$ ) associated with the corresponding change in pressure ( $\Delta P$ ) ( $C_{rs} = \Delta V / \Delta P$ ; Fig. 2).

The resistance of the respiratory system ( $R_{rs}$ ) was measured from the passive decay in lung volume during expiration

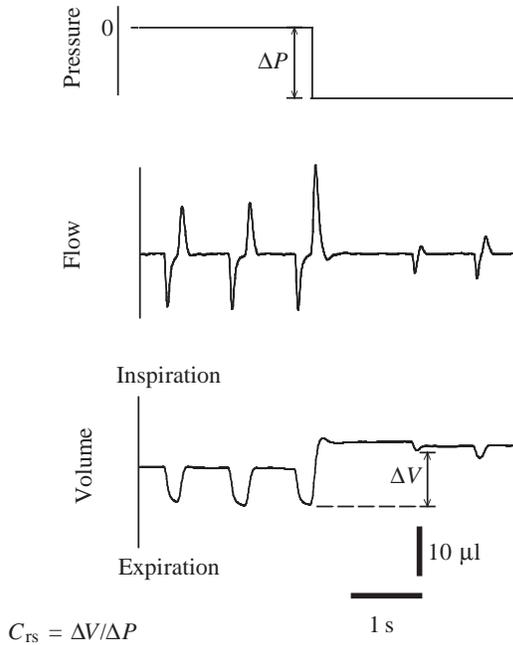


Fig. 2. Determination of respiratory system compliance ( $C_{rs}$ ) in the newborn tamar wallaby.  $C_{rs}$  was determined from the change in volume ( $\Delta V$ ) associated with the change in pressure ( $\Delta P$ ) induced by applying a negative pressure to the body compartment. Note that the breathing pattern is characterized by an end-inspiratory pause, probably due to laryngeal closure (Farber, 1978). Inspiration is upwards.

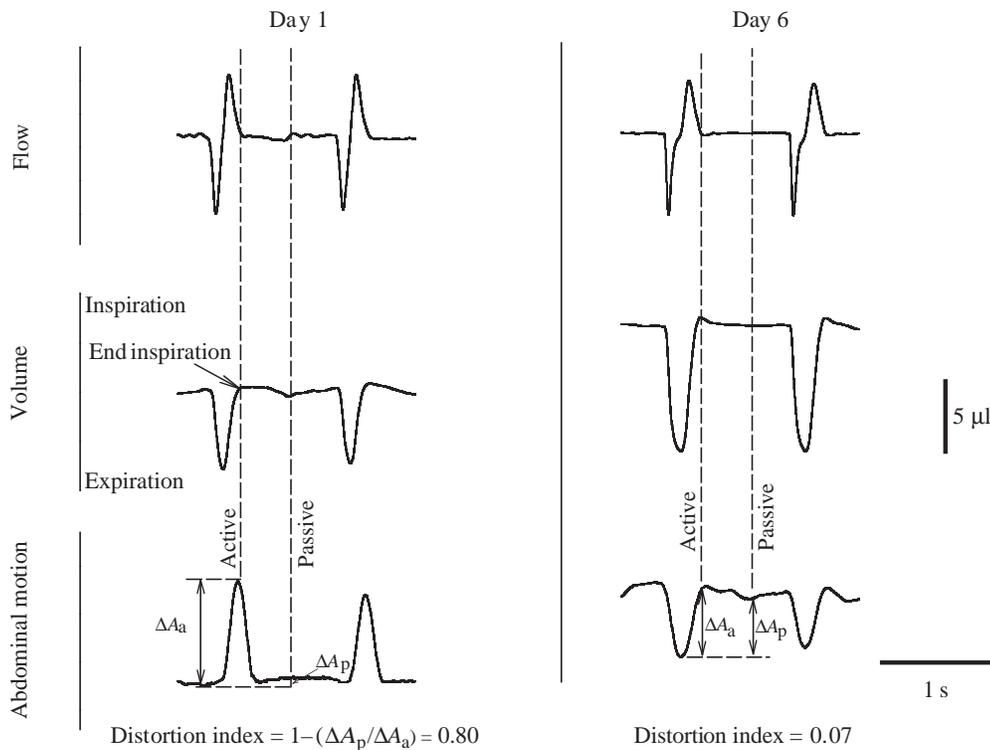


Fig. 3. Breathing pattern (flow and volume) and abdominal motion in newborn tamar wallabies at 1 and 6 days of age. At the end of inspiration, the respiratory muscles are contracting (active), whereas they are relaxed (passive) during the end-inspiratory pause. The distortion index is calculated from the degree of abdominal displacement ( $\Delta A$ ) during active and passive conditions,  $1 - (\Delta A_p / \Delta A_a)$ , with a value of 1 implying maximum distortion and a value of 0 no distortion.

(Bennett and Tenney, 1982; Mortola, 1983). A semi-logarithmic arithmetic representation of this produces an approximately linear function, and its slope represents the passive time constant of the respiratory system ( $\tau_{rs}$ ). Respiratory system resistance was then calculated as  $R_{rs} = \tau_{rs} / C_{rs}$ . Five breaths were analysed for which the volume trace was clear and free of drift (linear fit,  $r^2 > 0.8$ ).

#### Chest wall distortion

Abdominal displacement was measured using a short length of wire attached to a force-displacement transducer (Grass, model FTO3). The opposite end of the wire was bent perpendicular to its length and then inserted into the body compartment (rubber stopper removed) so that the bent tip was resting gently against the lateral surface of the abdomen just below the final rib (Fig. 1). The force-displacement transducer, clamped to a retort stand, was then positioned at a height that made it easy to manipulate the wire onto the specific position of the animal's abdomen. The displacement of the abdomen associated with each inspiration was detected by the transducer and subsequently recorded (PowerLab/800, ADInstruments, 200 Hz). The pouch young generally remained resting, but on occasion the bent tip slipped from the abdomen and was simply repositioned.

For the quantification of chest distortion, we compared the abdominal motion ( $\Delta A$ ) during spontaneous inspiration (when the inspiratory muscles are active) with  $\Delta A$  during passive lung inflation (when the inspiratory muscles are relaxed) (Mortola et al., 1985). For any given change in lung volume and in the absence of chest distortion,  $\Delta A$  in active conditions ( $\Delta A_a$ ) corresponds with  $\Delta A$  during passive inflation ( $\Delta A_p$ ). In contrast, if chest distortion is present,  $\Delta A_a$  exceeds  $\Delta A_p$ . In fact, in this latter case, some additional diaphragm contraction (and outward motion of the abdominal wall) is required to compensate for the distortion occurring within the chest. The breathing pattern of the pouch young, like that of many other newborn mammals, is characterized by inspirations followed by end-inspiratory pauses, during which the upper airways are occluded and the lungs are maintained inflated without muscle activity (Farber, 1978; Mortola, 2001). Therefore, active and passive conditions occur within a single breath, at the end of inspiration and during the end-inspiratory pause, respectively. Hence, chest distortion can be quantified by comparing  $\Delta A_a$  and  $\Delta A_p$  for the same inspired volume (Fig. 3). In each animal, 10 breaths

were analysed. The distortion index was defined as  $1 - (\Delta A_p / \Delta A_a)$ , an index of 0 implying no chest distortion and an index of 1 implying maximum distortion (see Fig. 3).

#### Statistical analyses

Data are presented as means  $\pm 1$  S.E.M. Comparisons between the two age groups (day 1 and 6) were conducted using two-tailed *t*-tests. The significance criterion was  $P < 0.05$ .

#### Results

The mass-specific compliances of the respiratory system ( $C_{rs}$ ) of the newborn tammars at 1 and 6 days did not differ

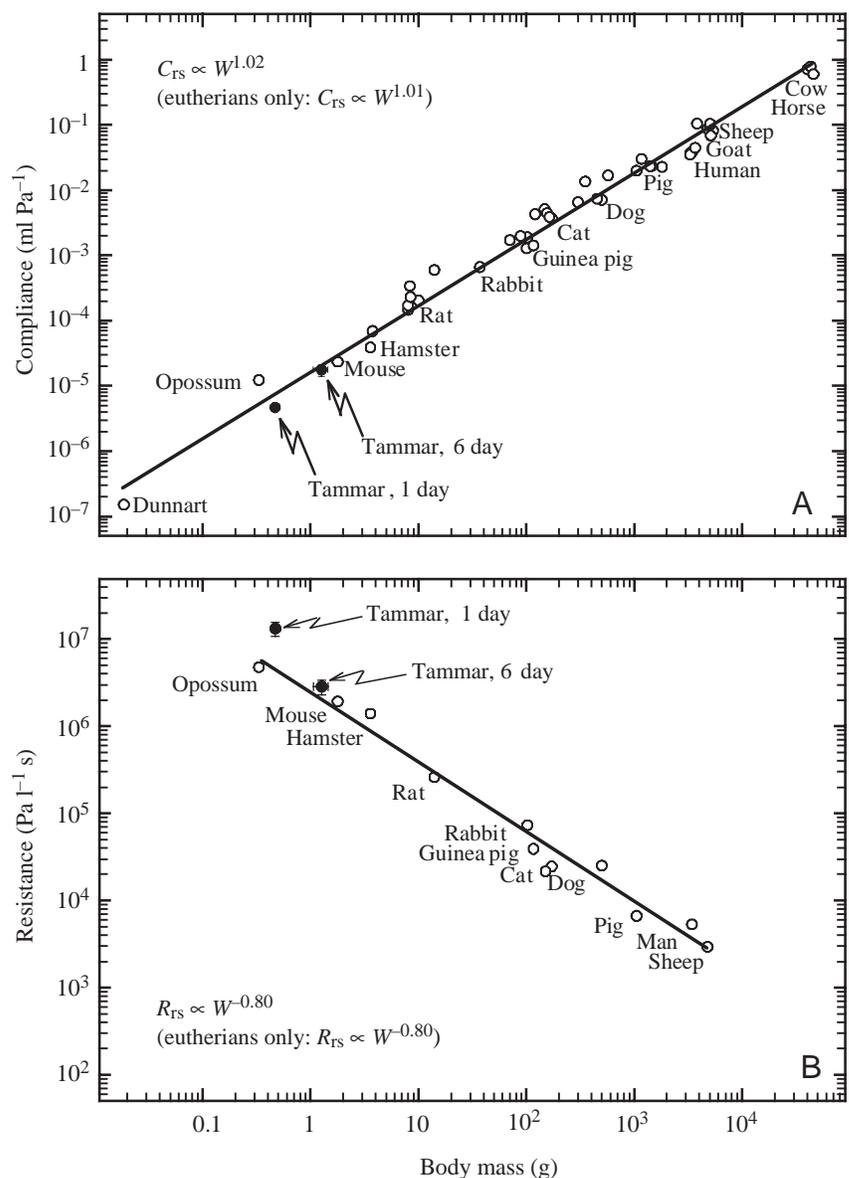


Fig. 4. Respiratory system compliance ( $C_{rs}$ ) (A) and resistance ( $R_{rs}$ ) (B) as a function of body mass ( $W$ ) in newborn mammals [open symbols (Mortola, 2001); solid symbols, this study]. The exponents of the allometric equations for all newborns and for eutherians only are indicated. Values for tammar wallabies are means  $\pm 1$  S.E.M. (day 1,  $N=7$ ; day 6,  $N=6$ ).

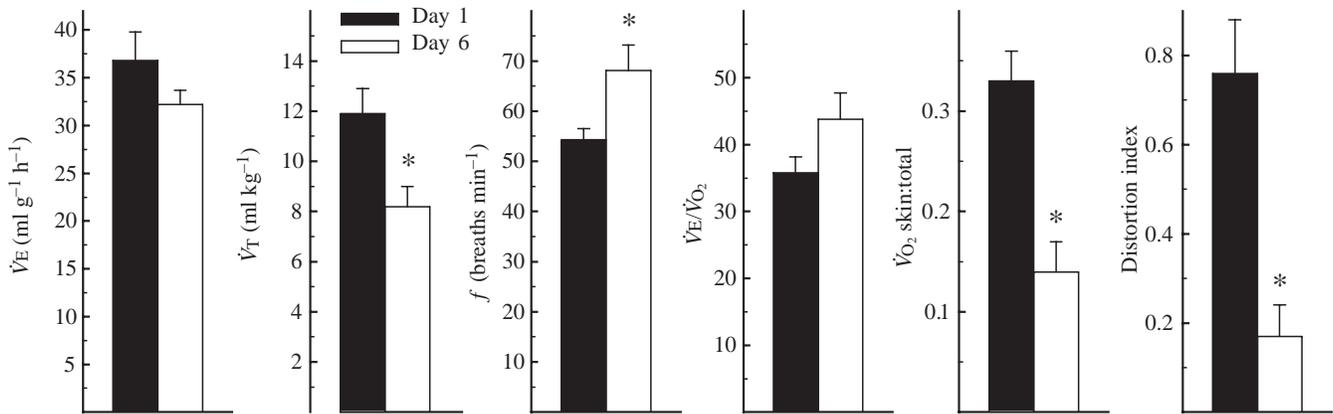


Fig. 5. Mass-specific ventilation ( $\dot{V}_E$ ), tidal volume ( $\dot{V}_T$ ), breathing frequency ( $f$ ), convection requirement ( $\dot{V}_E/\dot{V}_{O_2}$ ), relative importance of skin  $\dot{V}_{O_2}$  ( $\dot{V}_{O_2}$  skin:total) and chest wall distortion index in newborn tammar wallabies at 1 (open columns) and 6 (filled columns) days. Values are means  $\pm$  1 S.E.M. (day 1,  $N=7$ ; day 6,  $N=6$ ); an asterisk indicates a statistically significant difference ( $P<0.05$ ) between the two age groups.

( $t=-1.21$ ;  $P=0.25$ ) and averaged  $0.0103\pm 0.0013$  and  $0.0129\pm 0.0017$  ml kg<sup>-1</sup> Pa<sup>-1</sup>, respectively. Both values are close to that expected from the allometric relationship of  $C_{RS}$  in newborn mammals, whether or not marsupials are included in the relationship (Fig. 4A).

The mass-specific passive resistance of the respiratory system ( $R_{RS}$ ) at day 6 averaged  $3159.9\pm 300.0$  Pa l<sup>-1</sup> kg<sup>-1</sup> s, a value close to that expected from the allometric relationship of  $R_{RS}$  in newborns (Fig. 4B). In the younger tammar,  $R_{RS}$  averaged  $5963.6\pm 823.2$  Pa l<sup>-1</sup> kg<sup>-1</sup> s, a value significantly higher than at day 6 ( $t=3.2$ ;  $P=0.009$ ) and substantially higher than predicted from the allometric curve (Fig. 4B).

The mean values for mass-specific ventilation ( $\dot{V}_E$ ), tidal volume ( $\dot{V}_T$ ), breathing frequency ( $f$ ), convection requirement ( $\dot{V}_E/\dot{V}_{O_2}$ ), skin:total  $\dot{V}_{O_2}$  and the chest wall distortion index in the 1- and 6-day-old tammar are presented in Fig. 5. The breathing pattern of the tammar neonate is deeper and slower at day 1 than at day 6, with no difference in mass-specific  $\dot{V}_E$  or  $\dot{V}_E/\dot{V}_{O_2}$ . Approximately 33% of the metabolic rate of the tammar wallaby at day 1 was supported by the exchange of oxygen *via* the skin, a value substantially greater than at day 6 (14%). The difference in  $\Delta A$  between passive and active conditions was much more marked at day 1 than at day 6 (Fig. 3). Hence, the chest wall distortion index decreased significantly from 0.76 at day 1 to 0.17 by day 6 ( $t=4.3$ ;  $P=0.001$ ) (Fig. 5).

### Discussion

Several recent experiments on newborn marsupials have indicated that the skin can be a major contributor to the animal's total gas exchange (Mortola et al., 1999; Frappell and Mortola, 2000; MacFarlane and Frappell, 2001). In the neonate of the Julia Creek dunnart, which weighs approximately 15 mg, up to 95% of the animal's oxygen requirements are met through the skin (Mortola et al., 1999). The present measurements indicate that in the tammar wallaby, which at birth is one order of magnitude bigger than the dunnart, skin

gas exchange is approximately 33% of the total at day 1 and approximately 14% at day 6. These data agree with values obtained recently in this species (MacFarlane and Frappell, 2001). Although the contribution of the skin to total gas exchange differed between the two age groups, the values of  $\dot{V}_E/\dot{V}_{O_2}$  were similar. This suggests that total gas exchange, and not the fraction exchanged through the lungs, could be the relevant variable in setting  $\dot{V}_E$ , a conclusion also reached from serial measurements in tammar during the first postnatal days (MacFarlane and Frappell, 2001).

Previous measurements in the dunnart indicated that mass-specific  $C_{RS}$  did not differ from that of other neonatal species (Frappell and Mortola, 2000). The present values of  $C_{RS}$  in the tammar also are close to that expected from the allometric curve. In newborn mammals, especially of the smallest species,  $C_{RS}$  is determined largely by the compliance of the lungs ( $C_L$ ) (Mortola, 2001), so these data would indicate that, even in marsupials born after a gestation of less than 2 weeks, the lungs are sufficiently mature to function. Indeed, functional pulmonary surfactant has been demonstrated in neonates of other marsupials (Krause et al., 1976; Ribbons et al., 1989). However, the present results also indicate that  $R_{RS}$  is high in the 1-day-old tammar. Hence, the pressure required for inspiration and the work of breathing may be considerable because of the airflow-resistive component. The deeper and slower breathing of the 1-day-old tammar is therefore appropriate to reduce the energetic losses of the high airflow resistance.

Generally, in newborn mammals, including neonatal marsupials (Frappell and Mortola, 1989), the chest wall is much more compliant than the lungs. One functional consequence of the high compliance of the chest wall is its distortion during inspiration and the associated increased respiratory work (Mortola, 2001). In fact, the reduction in pleural pressure during inspiration tends to collapse the compliant rib cage, and a greater diaphragm contraction must compensate for the loss in volume. Hence, for any given increase in lung volume, the abdominal expansion during spontaneous breathing exceeds that during passive lung

inflation (i.e. without distortion). The greater the degree of chest distortion during spontaneous inspiration, the larger the difference becomes in abdominal expansion between spontaneous and passive lung inflation (Mortola et al., 1985).

The distortion of the chest wall during spontaneous breathing is aggravated by the decreased activity of the intercostal muscles; such a situation can also occur during rapid-eye-movement sleep (Gaultier et al., 1987; Stark et al., 1987) and/or when respiratory resistance is increased (Schulze et al., 1998). In the neonatal marsupial, not only is  $R_{rs}$  high, but the intercostal muscles may also be totally inadequate to stabilize the rib cage and minimize the distortion. In the newborn dunnart, lack of muscle coordination was obvious to an observer, and inflation of the lungs was almost a casual event during the animal's wiggling (Mortola et al., 1999; Frappell and Mortola, 2000). In the tammar, a definite rhythmic pattern with well-defined end-inspiratory pauses is present; nevertheless, at 1 day,  $R_{rs}$  was high and so was the amount of distortion. At 6 days, the distortion was reduced; this may have been the result of a combination of the lower  $R_{rs}$ , a lower chest wall/lung compliance ratio and better function of the intercostal muscles in stabilizing the rib cage.

In conclusion, at birth, the newborn tammar wallaby utilizes the skin to complement the lungs in gas exchange. While the respiratory system has a compliance appropriate for the size of the neonate, a higher than expected respiratory resistance probably accounts for the high degree of chest wall distortion observed during spontaneous breathing. The mechanical inefficiency of the respiratory system in newborn marsupials could explain their reliance to varying degrees on cutaneous exchange.

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