Abdominal Muscle Function in Ventilation and Locomotion in New World Opossums and Basal Eutherians: Breathing and Running With and Without Epipubic Bones

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ABSTRACT All tetrapods have the same four basic abdominal hypaxial muscle layers that wrap around the abdomen between the pelvis, ribcage, and spine. However, the marsupials and our immediate mammalian ancestors have epipubic bones extending anteriorly into the ventral hypaxial layers with two additional muscles connecting them to the ventral midline and femur. Studies of two marsupials have shown that all of the abdominal hypaxials play a part bilaterally in resting ventilation and during locomotion there is an asymmetrical pattern of activity as the hypaxial muscles form a cross-couplet linkage that uses the epipubic bone as a lever to provide long-axis support of the body between diagonal limb couplets during each step. The cross-couplet epipubic lever system defines the earliest mammals and is lost in placental mammals. To expand our understanding of the evolution of mammalian abdominal muscle function and loco-ventilatory integration we tested the generality of the cross-couplet system in marsupials and conducted the first formal studies of hypaxial abdominal motor patterns in generalized placental mammals focusing on a representative rodent and insectivore. These new data reveal 1) that continuous abdominal muscle tonus during resting ventilation and a 1:1 breath to step cycle during locomotion appear to be the basal condition for mammals, 2) that the loss of epipubic bones in eutherians is associated with a shift from the cross-couplet dominated motor pattern of marsupials to a shoulder-to-pelvis system with unilateral activation of abdominal muscles during locomotion and 3) that hypaxial function in generalized eutherians is more similar to marsupials than cursorial mammals. J. Morphol. 270:1014–1028, 2009.

KEY WORDS: locomotion; marsupial; mammals; hypaxial; eutherian; breathing; mammal evolution

INTRODUCTION

Tetrapods, including mammals, have the same four basic abdominal hypaxial muscle layers that wrap around the abdomen between the pelvis, ribcage, and spine (external and internal obliques and the rectus and transverse abdomini, Fig. 1A). The marsupials, however, have epipubic bones extending anteriorly from a synovial joint on the pelvis into these abdominal muscle layers with two additional muscles, the pyramidalis and pectineus. The pyramidalis spans the epipubic bones medially and extends obliquely forward to the midline. The pectineus extends from the proximal half of the femur to the base of the epipubic bone just anterior to the pelvic articulation point (Fig. 1B). Recently, we have shown in two South American marsupials (Didelphis virginiana and Monodelphis domestica; Reilly and White, 2003, in press) that 1) all of the hypaxial layers (but not the pectineus) exhibit mild continuous bilateral tonus during resting ventilation and 2) that during locomotion each epipubic bone is retracted like a lever by the pectineus as part of a “cross-couplet” pattern of primary activity in abdominal muscles that provides long-axis support of the body between diagonal limb couplets (Fig. 1B). In addition, both species exhibited coordinated abdominal muscle contractions with exhalation during locomotion (Reilly and White, in press). These results are significant because they reveal that abdominal muscles are mildly active in stiffening the abdominal walls during resting ventilation and that, at least in these marsupials, all of the abdominal hypaxials play a part in ventilation during locomotion (Reilly and White, 2003; in press). From an historical perspective, the cross-
couplet motor pattern appears to be related to the presence of epipubic bone levers, which appear in the fossil record in concert with a large suite of unique characters that define the earliest mammals (Kielan-Jaworowska et al., 2004). Thus, it is likely that locomotion in the earliest mammals would have involved a functional line of tension from the ribcage and shoulder muscles on one side through the epipubic bones to the opposite side femur.

Equally significant is the question of what happened to abdominal hypaxial function when the epipubic bones were lost in the eutherians? All living placental mammals lack epipubic bones, the pectineus has moved onto the pelvis proper as a small femoral adductor, and the pyramidalis is variably vestigial or absent. The function of the four hypaxial abdominal layers in eutherians has seen little formal study. The transversus abdominis has been shown to compress the abdomen during resting exhalation in dogs (De Troyer and Lor- ing, 1986; De Troyer et al., 1989; Ainsworth et al., 1996; Deban and Carrier, 2002) and in resting and active marsupials (Reilly and White, 2003, in press). Because the same pattern is seen in resting salamanders the transversus abdominis has been regarded to be a generalized tetrapod exhalatory muscle (Brainerd and Monroy, 1998; Brainerd, 1999). The few quadrupedal eutherian electromyographical (EMG) studies to date on abdominal muscles during resting ventilation (also on dogs) show that the rectus abdominis and the oblique muscles are used differently among individual. They may be silent, in tonus, or sometimes actively bursting with exhalation (De Troyer et al., 1989; Deban and Carrier, 2002). In addition, abdominal muscle activity in dogs and horses is variably coupled or not coupled to ventilation and footfall patterns during locomotion (Koterba et al., 1988; Fife et al., 2001; Deban and Carrier, 2002).

Fig. 1. Mammalian abdominal anatomy and the cross-couplet linkage used in marsupial locomotion. For simplicity, muscles are labeled only on one side of the body. A: Extant placental mammals have four abdominal hypaxial layers. They have lost the epipubic bones, the pyramidalis muscles are vestigial or absent and the pectineus has retreated to the lateral aspect of the pubis as a femoral adductor. B: Marsupial hypaxial muscles and epipubic lever system; the sequence of muscles figured illustrates the asymmetrical pattern of muscle activity in the cross-couplet linkage shown previously in the South American marsupials Monodelphis and Didelphis (Reilly and White, 2003) that stiffens the body across diagonal couplets during trotting steps.
Thus, abdominal muscle function is highly variable in the few large cursorial mammals studied. It is not well understood in other erect cursorial eutherians, and has not been studied in smaller, generalized noncursorial or semierect (crouched) eutherians (e.g., Rodentia, Insectivora).

In this study, we sought to expand our understanding of mammalian abdominal function and loco-ventilatory integration by addressing two goals. First, to examine the generality of the cross-coupled system in marsupials we quantified abdominal motor patterns in a third South American marsupial, the four-eyed opossum (Philander opossum). Second, we conducted the first formal studies of abdominal motor patterns in generalized placental mammals focusing on a representative rodent (Norway rats, Rattus norvegicus) and insectivore (four-toed hedgehogs, Atelerix albiventris). These new data reveal the general pattern in which basal mammals exhibit continuous abdominal muscle tonus during resting ventilation and share some similarities in hypaxial function during locomotion. However, the loss of epipubic bones in eutherians is associated with a shift from the cross-coupled dominated motor pattern of marsupials to a shoulder-to-pelvis system with unilateral activation of abdominal muscles during locomotion.

MATERIALS AND METHODS

Three generalized terrestrial mammals with crouched limb postures were obtained from commercial sources. Two male four-eyed opossums, Philander opossum (0.836 kg, 0.856 kg) were used as representatives of a third species of South American marsupial to test the generality of the patterns observed recently in two other opossums (Monodelphis domestica and Dasyurus virginiana; Reilly and White, 2005; in press). To sample generalized eutherian mammals we studied five Norway rats (Rattus norvegicus: 2 males, 3 females, 0.397–0.650 kg) as a representative rodent, and 4 four-toed hedgehogs (Atelerix albiventris: 1 male, 3 females, 0.377–0.587 kg) as a representative insectivore (generic names are used herein). All animals were run on a speed-controlled treadmill (70 × 27 cm2) to 1) accustom them to the experimental procedure prior to data collection, and 2) to determine the range of speeds each species would perform.

Stride patterns (gaits) used by these mammals were sampled over a range of treadmill matched speeds from high-speed video (120 Hz) recorded during the EMG experiments. The timing of footfalls was measured for each foot for each stride (for a complete cycle for each of the four feet) from lateral views of numerous strides of the animals moving over the widest range of speeds we could obtain in treadmill locomotion. Gaits were described following Hildebrand (1976) by plotting limb phase (percent of stride duration that the foot follows the ipsilateral hind foot) versus duty factor (percent of stride duration that the hind foot is on the substrate).

To examine the relationship between ventilation and stride timing we used videofluoroscopy to film a subset of the animals (2 philander, 3 rats, 3 hedgehogs) while resting and running on the treadmill over several weeks prior to the EMG experiments. Animals were filmed (60 Hz) at rest and moving at speeds between 0.05 and 0.4 m/s with a Phillips BV-25 C-Arm mobile videofluoroscopy unit. From the videofluoro images the coordination of footfalls (and epipubic bone movements in Philander) and diaphragmatic movements (craniocaudal displacement cycles of the apex of the diaphragm imaged laterally) were noted at different speeds. Prelocomotion and postlocomotion ventilation patterns and loco-ventilatory integration patterns were also noted following videofluoroscopy for subsequent comparison in abdominal muscles. Resting and locomotor breathing patterns were recorded 1) from cycles of diaphragmatic movement on videofluoroscopic images (during rest and in relation to footfall patterns during locomotion), 2) from abdominal/thoracic movements filmed with high-speed video at rest during EMG experiments, and 3) from EMG activity in the transversus abdominis muscles which are known to fire during exhalation in amniotes (Brainerd, 1999) and were correlated with thoracic exhalation in all three species during locomotion (except in “sniffing”, see below). In one of the Philander individuals, ventilation synchrony could be directly observed in movements of nasal secretions at the nares at rest and in locomotion during EMG experiments (this was indicative of “sniffing”).

Muscle activity was recorded using standard fine-wire electromyography (EMG; Reilly et al., 2005). Electromyographical recordings were made from tiny (0.002 in diameter) bipolar stainless steel electrodes implanted percutaneously through the skin or via small incisions directly into each muscle layer while the animals were under anesthesia. The bared metal tips of each electrode were 0.25-mm (hedgehogs, rats) or 0.5-mm long (Philander). The bundle of electrodes was glued together, passed distally around the abdomen to a suturing pattern on the midline dorsal to the pelvis. Animals completely recovered from anesthesia within 2 h and synchronized EMG and kinematic data were sampled for initial resting breathing, during several bouts of locomotion, and then resting breathing after locomotion. Animals were rested (about 5–10 min) between bouts of locomotion (lasting 20 s maximum). EMG signals were amplified 10,000 times using AM Systems model 1700 differential AC amplifiers with a bandpass of 100–3,000 Hz (and a 60-Hz notch filter), and then recorded on a Cambridge Electronics MICRO 1401 analog to digital converter that generated a synchronization pulse simultaneously recorded on the video frames. EMG data were recorded and analyzed using the Cambridge Electronics SPIKE 5.0 software with sample rate for each channel set at 10,000 Hz to obtain reliable recordings of EMG burst patterns (Jayne et al., 1990). EMG profiles were inspected for possible patterns revealing crosstalk. Immediately following data collection, each animal was euthanized by overdose of anesthetic and preserved in 10% formalin. Electrode position was then confirmed by dissection and only individual preparations in which the electrode lay completely within the muscle were used. Samples sizes ranged from 2 to 5 individuals per unilateral muscle site and are indicated in Table 1. All attempts to record bilateral motor patterns in each of the four abdominal hypaxial layers (rectus abdominis, transversus abdominis, external, and internal obliques) and the pyramidalis and pectineus muscles in Philander in each individual. To record muscle activities at different speeds, each individual was subjected to a series of treadmill bouts in which they were carefully brought up to a given speed and a sample of 10–20 s of EMG recording was made as they matched that given speed. Speed effects on loco-ventilatory integration were observed by relating footfalls to diaphragmatic movements over the range of steady state speeds we could obtain from each animal. To examine the relationship between muscle activation bursts and stride timing, footfall timing patterns digitized from videos were aligned with simultaneous EMG recordings for multiple strides from each individual. Unilateral versus bilateral patterns of muscle activity could easily be identified from the presence of either one or two bursts of EMG activity per stride. All procedures were completed under approved animal research protocols.

RESULTS

We attempted to run the animals at set treadmill speeds ranging from 0.050 to 0.55 m/s, how-
ever, all three species matched treadmill speeds over numerous consecutive strides only at speeds between 0.15 and 0.25 (rats and hedgehogs) and 0.15 and 0.5 m/s (Philander). All three species used diagonal couplets gaits in all strides observed in across these speed ranges (see Fig. 2).

Thoracic Ventilatory Movements at Rest and During Locomotion

During resting ventilation and locomotion all three species exhibited observable diaphragmatic movements under videofluoroscopy between 2 and 10 mm (Table 2) with no measurable increase in diaphragmatic excursion during locomotion. Resting diaphragmatic cycle rates were very consistent within species but varied with size from 0.9 Hz to 1.75 Hz across species (Table 2). In all three species, all strides observed in videofluoroscopy (nearly 900 steps) exhibited one cycle of diaphragmatic movement per diagonal couplet step (2 breaths per stride; Table 2). Thus a 1:1 pattern of limb couplet cycling and diaphragmatic ventilation was characteristic of locomotion over preferred speed ranges.

Abdominal Motor Patterns Before, During and After Locomotion in Philander

Hypaxial electromyography revealed that resting Philander exhibited continuous low amplitude activity in all of the hypaxial muscles, including the pyramidalis (Fig. 3A). Philander exhibited resting ventilation rates under videofluoroscopy of between 0.69 and 0.94 Hz (mean, 0.87 Hz; Table 2). The pattern of bilateral, continuous tonus of the abdominal muscles was maintained over many thoracic ventilatory cycles (externally observed as visible movements of the abdomen/thorax) and exhibited the same resting ventilation rate of diaphragm movements (0.91 Hz +0.02, N = 24) observed under videofluoroscopy (Table 2). Thus, at rest, constant low intensity abdominal tonus is maintained in the face of continuous cycles of contraction and relaxation of the diaphragm. The pectineus was silent in Philander at rest except for occasional bursts of activity when the animal moved while changing positions.

During locomotion Philander exhibited the cross-couplet motor pattern where the internal and external oblique muscles of the forelimb side fired in concert with the rectus abdominis, pyramidalis, and pectineus muscles of the hind limb side (Fig. 3B). Videofluoroscopy showed each epipubic bone retracting with its ipsilateral femur and then rapidly protracting in early swing phase. The transversus abdominis was active bilaterally during each couplet step firing in synchrony with the exhalation phase of each ventilatory cycle. As speed increased the couplet rate and ventilatory rate (transversus abdominis burst rate and diaphragm cycle rate) increased in a 1:1 fashion up to a maximum of 6.8 couplets per second at a speed of about 0.5 m/s. Both individuals, however, preferred slower speeds between about 0.15 and 0.4 m/s where couplets steps ranged from 1.3 to 2.9 Hz (mean = 2.19 Hz; Table 2).

Samples of abdominal EMG immediately after locomotion revealed that Philander exhibited large bursts of bilateral motor activity synchronous with the exhalation phases (inferred from thoracic contraction) and no activity during the inhalation phases (posterior diaphragmatic movements inferred from thoracic expansion) in all the abdominal muscles except the pectineus, which was silent (Fig. 3C). After stopping, EMG amplitudes of bilat-
Intermittent Superimposed High-Speed Ventilation

While following the general rest- locomotion-rest pattern outlined above (from Fig. 3A to B to C to A), about half the time Philander would intermittently exhibit a pattern of higher speed low amplitude bilateral EMG pattern on top of the resting tonus pattern (Fig. 3D) and on top of the cross-couplet motor pattern during locomotion (Fig. 3E) but not during post-running active breathing (Fig. 3C). Rapid activity bursts occurred in all of the abdominal muscles but not the pectineus. This high frequency motor pattern occurred consistently at rates between 5 and 6 Hz based on measuring the rate of transversus abdominis bursts (resting: 5.17 ± 0.05, N = 260; locomotion: 5.36 ± 0.02, N = 508). The rapid bilateral pattern would come and go from both the tonus (Fig. 3A vs. D) and pure couplet locomotion (Fig. 3B vs. E). These EMG patterns show that the abdominal muscles are able to add small amplitude high frequency motor pattern to the pure tonus or cross couplet motor pattern. However, when the high-speed bilateral pattern appeared, activity in the transversus abdominis shifted from diaphragmatic synchrony to the new high frequency motor pattern.

Abdominal Motor Patterns Before, During and After Locomotion in Basal Eutherians

Rats and hedgehogs exhibited the same resting pattern as Philander of maintaining continuous low amplitude activity on all of their four hypaxial muscles over the course of many thoracic ventilatory cycles observed with videofluoroscopy of the diaphragm and visible movements of the thorax externally (see Fig. 4). The eutherians exhibited mean resting ventilation rates of 1.5 Hz (hedgehogs) and 1.75 Hz (rats), about twice that of the larger Philander (Table 2).

The eutherians also exhibited higher rates of limb cycling during locomotion (Table 2). In terms of hypaxial locomotor patterns, the eutherians were similar to Philander in that the external and internal obliques always fired only with the ipsilateral forelimb contact and the transversus abdominis fired bilaterally with each bout of exhalation (see Fig. 5). The rat and hedgehog differed from the marsupials, however, in shifting the primary (greater amplitude) activity of the rectus abdominis from the hind limb side (as in didelphids) to the forelimb side. At slow speeds, the rectus abdominis exhibited large amplitude bursts unilaterally with the other three hypaxial muscles. However, as speed increased we observed the appearance of small amplitude bursts in the opposite side muscle (as illustrated in Fig. 5, right rectus abdominis muscles). Thus, all of the four abdominal muscle layers exhibited large unilateral amplitude bursts with each ipsilateral forelimb during each couplet step. The addition of bilateral rectus abdominis activation with increasing speed may indicate an accessory contribution of this muscle to ventilation or locomotion.

After locomotor bouts, the rats and hedgehogs returned to the tonus resting pattern (see Fig. 4) as ventilation rate returned to resting levels. We did not observe an active pattern of resting bilateral hypaxial bursts (like Fig. 3C) in the eutherians even after high speed running or when coming out of anesthesia.

DISCUSSION

Data from three didelphids and two eutherians give us the first comparative information with which to examine patterns of hypaxial function and ventilatory integration among generalized mammals. These comparisons reveal 1) some vari-

**TABLE 2. Observations of thoracic ventilation patterns in mammals at rest and during locomotion at preferred speeds from videofluoroscopy (means ± SE)**

<table>
<thead>
<tr>
<th>Species (n)</th>
<th>Mean mass (g)</th>
<th>Resting ventilation rate (n)</th>
<th>Couplet step frequency (n) (Speed range)</th>
<th>Ventilation cycles/stride</th>
<th>Diaphragm displacement (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Philander (2)</td>
<td>846</td>
<td>0.87 Hz ± 0.01 (41)</td>
<td>2.19 ± 0.02 (387) (0.15–0.5 m/s)</td>
<td>2</td>
<td>2–10</td>
</tr>
<tr>
<td>Atelerix (3)</td>
<td>500</td>
<td>1.50 Hz ± 0.08 (15)</td>
<td>2.43 ± 0.02 (193) (0.15–0.25 m/s)</td>
<td>2</td>
<td>3–5</td>
</tr>
<tr>
<td>Rattus (3)</td>
<td>431</td>
<td>1.75 Hz ± 0.09 (25)</td>
<td>3.12 ± 0.04 (312) (0.15–0.25 m/s)</td>
<td>2</td>
<td>4–10</td>
</tr>
</tbody>
</table>
**Fig. 3. Abdominal hypaxial motor patterns for *Philander*.**

**A**: Abdominal hypaxial motor patterns during resting breathing with constant low amplitude bilateral tonus maintained in all the hypaxial muscles over several respiratory cycles.

**B**: Diagonal pattern of hypaxial motor activity during normal locomotion illustrating the cross couplet locomotion with bilateral transverses abdominis activity during exhalation.

**C**: Active bilateral hypaxial activation after exercise.

**D**: High frequency low amplitude motor pattern superimposed on the resting tonus.

**E**: High frequency low amplitude motor pattern superimposed on the cross-couplet pattern. Exhalation and inhalation timing and foot fall gait patterns indicated with bars at the top and bottom of each panel.
Similarities and Differences in Abdominal Function Among Didelphids

The generality of the cross-couplet pattern is confirmed by the results from Philander (Fig. 3B). All three didelphids studied so far have exhibited the pure cross-couplet pattern at some time during locomotion. However, the three species differ in the details.

Philander used the pure cross-couplet pattern about half the time in concert with bilateral activity in the transversus abdominis muscle coincident with the exhalation phases of the ventilation cycle (Fig. 3B). However, Philander was the only species with extended periods of high frequency abdominal contraction with concerted ~5 Hz bursts across all the hypaxial muscles both at rest and while running (Fig. 3D,E). We propose that this may be a sniffing olfactory behavior rather than a thoracic ventilatory behavior for several reasons. First, the ~5 Hz pattern is basically constant and occurs intermittently on top of the demonstrated ~1 Hz resting ventilation frequency and the ~2 Hz 1:1 couplet to diaphragm cycle during locomotion. Second, we could observe the ~5 Hz ventilatory rate on one individual Philander in which nasal fluids were pulsating in and out of the nares at rest. This nasal pulsing was in synchrony with simultaneously recorded high frequency hypaxial EMG burst patterns (Fig. 3D) and the burst patterns and nasal movements disappeared when the animal went back to pure resting tonus (Fig. 3A). Third, we could not observe any rapid diaphragmatic cycles in the videofluoroscopy (during nasal pulsing) or in high-speed video of thoracic movements. Thus, the ventilatory effects of this behavior on the diaphragm and thoracic cavity appear to be small—perhaps just enough to move air over the nasal passages, to sniff the surrounding environment.

Monodelphis and Didelphis use the “pure” cross-couplet pattern only at very high speeds (Reilly and White, 2003). However, at preferred speeds Didelphis exhibits a bilateral pattern in all the hypaxial muscles that would appear to aid in hypaxial-assisted exhalation by bilaterally compressing the trunk. This couplet dominated but bilateral abdominal activity maintains a 1:1 couplet/ventilation pattern up to its maximum pre-
ferred speed. The added small amplitude contra-couplet hypaxial contraction in Didelphis could be related to the fact that it is the largest didelphid and has a series of peculiar axial skeletal traits that give it a neck like a burrower and the most rigid vertebral column known among living marsupials (Argot, 2003). Argot (2003) argued that the extreme rigidity of its axial skeleton limits its

Fig. 5. Locomotor hypaxial patterns in basal eutherian mammals. Exhalation and inhalation timing and foot fall gait patterns indicated with bars at the top and bottom of each panel, respectively. Note the chain of unilateral muscle contraction from the forelimb side shoulder/ribcage to the pelvis.

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Fig. 6. Evolutionary patterns of locoventilatory integration and hypaxial function in marsupials and basal eutherians. See text for discussion.
axial bending to ventral flexion and may be that is why it is one of few mammals that exhibit only symmetrical gaits (White, 1990). In addition, Didelphis is the only marsupial with nonconjoined rectus abdominis muscles (White, 1990). The separated rectus abdominis muscles and the decreased ability to bend the spine laterally during couplet steps may limit the cross-couplet’s effect on exhalation. Thus, bilateral hypaxial contraction is invoked to more effectively assist exhalation with each step. In a sense Didelphis is more like cursorial mammals (dogs) with more ventral flexion, separated rectus abdominis muscles and bilateral motor patterns during locomotion (Deban and Carrier, 2002).

Monodelphis always uses the cross-couplet system in sub-maximal locomotion but differs from other marsupials in sustaining ventilation rates of 10 Hz at rest and during a narrow range of preferred locomotor speeds (Reilly and White, in press). The novel 10 Hz pattern in Monodelphis may be related to constraints of high natural frequencies in these very small mammals (Reilly and White, in press). Briefly, larger mammals having resting ventilation rates below their natural frequencies are able to increase breathing rates from their lower resting rates up to their natural frequencies during locomotion or thermal stress. However, in the smallest mammals (body mass < 150 g) the resting rate rapidly converges on their natural frequency and thus, they must breathe at their natural frequency at rest and during locomotion, as in Monodelphis. In normal locomotion, Monodelphis would only run at a narrow range of speeds where it could match its couplet step rate to 5 Hz which resulted in a novel 2 breaths per couplet ventilation rate. Thus, it was entraining a 5 Hz couplet rate on its 10 Hz natural frequency. However, Monodelphis could be forced to exhibit short bursts of high speed locomotion where it shifted to the same 1:1 pattern seen in Didelphis (1 ventilation per step with bilateral couplet-dominated hypaxial activity). Reilly and White (in press) could not ascertain if this 1:1 ventilatory pattern resulted in larger movements in the diaphragm that would be indicative of greater tidal volume, however, increased tidal volume is likely a result of bilateral hypaxial exhalation. To summarize, small size appears to introduce some constraints on Monodelphis, however, it still retains the basic cross-couplet system.

Given the presence of the cross-couplet system in a generalized didelphid (Philander), the largest and morphologically derived didelphid (Didelphis), and one of the smallest and most generalized didelphids (Monodelphis), we propose that the cross-couplet pattern is a shared primitive trait for the didelphid radiation in South America. Furthermore, given the presence of the epipubic lever system anatomy in the Australian marsupials we predict that same cross-couplet pattern should be present in the Australian radiation of marsupials. Studies are underway to test this hypothesis.

Patterns of Abdominal Hypaxial Function in Basal Eutherians

The rat and hedgehog had the same motor patterns at rest and during locomotion (Figs. 4 and 5). Both species exhibited tonus in all the hypaxial muscles at rest (see Fig. 4). With slower locomotion, the eutherians maintained a 1:1 synchrony of couplet steps and the ventilation cycle with the hypaxial muscles firing unilaterally during the forelimb stance phase (see Fig. 5). This pattern was maintained as these species increased speed up to their maximum running speeds. Unlike the didelphid marsupials, we did not observe an active bilateral resting burst pattern of EMG in these eutherian species, even after high speed running. The unilateral chain of muscle contraction in eutherians reveals a line of tension that would be produced by the external and internal obliques, rectus abdominis and transversus abdominis muscles extending from the posterior aspects of the shoulder muscles and rib cage directly to the midline and pelvis (see Fig. 1). This “shoulder to pelvis linkage” is well positioned to support body weight between the couplet forelimb and pelvis during each step. As the rat (Rodentia) and the hedgehog (Insectivora) had the same patterns during locomotion, the shoulder to pelvis linkage appears to represent the basal condition for eutherians (see Fig. 6).

Basal Eutherian Patterns in Relation to Patterns of Exhalation and Locomotion in Cursorial Mammals

Patterns of resting ventilation. The continuous mild activity to maintain abdominal tone during resting breathing appears to be a general condition for basal mammals. All but the smallest species (Monodelphis) always used mild tonus while at rest. This pattern of tonic activity appears to increase intra-abdominal pressure and rigidity, aiding in expanding the ribcage during inhalation and in the initial passive recoil of the diaphragm during exhalation. In humans, abdominal tone reduces the compliance of the abdominal compartment such that the region in contact with the ribcage (the area of apposition) acts as a fulcrum for the expansion of the lower ribcage during inspiration (Goldman et al., 1987; Iscoe, 1998). What is novel in our data is that all of the abdominal muscles in these basal mammals appear to be serving this purpose in unison. In erect-limbed cursorial mammals, tonic activity in the abdominal hypaxial layers is observed in resting breathing but it is highly variable both within and among...
species. The external oblique has been shown to exhibit resting tonus sometimes in humans (De Troyer et al., 1990) and dogs (Ainsworth et al., 1989; De Troyer et al., 1989; Farkas et al., 1989; Deban and Carrier, 2002). Dogs also exhibit tonus in the transversus abdominis at rest but activity ceases during each inhalation (De Troyer et al., 1989). This suggests that in dogs and humans resting tonus is observed in some of the abdominal muscles but there is a very low threshold for the active recruitment of focused expiratory bursts in the transversus abdominis, rectus abdominis, and the external oblique at rest (De Troyer et al., 1990). Thus, generalized marsupials and eutherians show a consistent pattern of tonus in all of the hypaxial musculature during resting ventilation. However, humans and dogs rely more heavily on active recruitment of hypaxial musculature to aid in ventilation at rest.

There was evidence of phasic rather than tonic bilateral resting hypaxial activity in some of our basal mammals, Monodelphis, in a manner similar to dogs and humans, has adopted active hypaxially-assisted exhalation during resting breathing. This has been argued to be necessary to generate rapid abdominal recoil against the diaphragm to facilitate its extremely high (10 Hz) resting ventilation rate for such a small animal (Reilly and White, in press). Philander also briefly exhibited bilateral hypaxial exhalation under high oxygen demand (post anesthesia and after running) before returning to resting tonus. Therefore, it exhibits a relatively high threshold for using active bilateral hypaxial ventilation. Thus, under high-demand conditions some marsupials exhibit active exhalation in a similar manner to that seen in dogs and humans. The lower threshold for hypaxial recruitment in cursorial mammals may be related to their larger size or possibly because eutherians have higher metabolic rates than marsupials of similar body mass (Dawson and Hurlbert, 1969). Higher metabolic rates may necessitate a lower threshold for the onset of active exhalation with hypaxial musculature to support the need for greater ventilation.

High frequency ventilation patterns—“sniffing” and “panting”. The pattern of intermittent high frequency patterns of ventilation superimposed on slower diaphragmatic breathing in Philander does not appear to be unique, nearly the exact same pattern has been observed in dogs. Ainsworth et al. (1996) found that dogs exhibited either “normal” moderate amplitude ventilations 1:1 with step rate increasing from 2 to 6 Hz as speed increased, or a “mixed” pattern with 4–6 Hz low amplitude ventilation pattern superimposed on top of a 0.5–1.0 resting breathing rate or on top some portions of exercise. They found that the diaphragm only fired during inhalation in both patterns during locomotion, which matches our observations that Philander maintained the normal step/exhalation synchrony when the sniffing pattern appeared. In addition, as in Philander, the transversus abdominis in dogs switched from step/diaphragm synchrony in the normal breathing pattern to a high frequency motor pattern correlated with low pressure ventilation cycles superimposed on the larger diaphragmatic cycles in the mixed pattern. Ainsworth et al. (1996) concluded that the high frequency pattern corresponds to a panting behavior. As in Philander, the high frequency pattern in dogs was intermittently superimposed on top of normal pre-exercise and locomotor diaphragmatic cycling and did not change with speed, thus, we suggest that the dogs may also be sniffing in the mixed pattern. Whether this is sniffing or panting, the shift in transverses abdominis function from lung ventilation/locomotion frequency to a higher constant frequency, illustrates yet another layer of complexity to our understanding of thoracic function in mammals, which extends back as far as the marsupials. The advent of high speed ventilation for either olfactory or thermoregulatory function can be added to the suite of characteristics related to the evolution of homeothermy and higher metabolic rate and activity levels in mammals.

Locomotion and ventilation. Functional locomo-ventilatory integration has appeared independently in two groups of endothermic animals, both of which commonly use long bouts of rapid locomotion (mammal and birds, Carrier, 1987; Boggs, 2002). The physiological significance of coupling limb cycling to ventilation cycling is not well understood (Lee and Banzett, 1997) but it is thought to enhance sustained aerobic locomotor capacity by minimizing the conflicts between the locomotor and ventilatory functions of the trunk (Bramble and Carrier, 1983; Young et al., 1992; Nassar et al., 2002) and limbs (Carrier et al., 2008). At slower speeds, cursorial mammals usually exhibit one breath per step associated with diagonal couplet dominated symmetrical gaits. As velocity increases, cursorial mammals transition to a one breath per stride associated with asymmetrical gaits (e.g. bounds, gallops) dominated by sagittal bending of the trunk and paired fore and hind limb supports of the body. Our data provide some of the first data on loco-ventilatory integration in small noncursorial mammals and confirm that the slow speed pattern of one breath per step with symmetrical gaits appears to be the primitive condition for mammals. This does not imply that small mammals cannot employ asymmetrical gaits because Monodelphis has been observed to use asymmetrical gaits at speeds exceeding 1 m/s (Pridmore, 1992; Fisher et al., 2002; Schilling and Hackert, 2006). However, none of our study species exhibited asymmetrical gaits at any speed on the treadmill or trackway at the speeds we obtained.
Thus, the generality and ecological relevance of asymmetrical gaits in small generalized mammals remains to be demonstrated.

During locomotion, cursorial mammals are widely known to bilaterally recruit hypaxial muscles to facilitate ventilation. Increases in ventilatory rates and tidal volumes by recruitment of accessory muscles of the thorax and abdomen are well established in mammals (De Troyer and Loring, 1986, Deban and Carrier, 2002). The accessory ventilatory muscles of mammals appear to be derived from the primary ventilatory muscles of generalized amniotes that lack diaphragms (Liem, 1985, Carrier, 1987). Other retained primitive traits in mammals include the transversus abdominis as the primary expiratory muscle as in amphibians (Brainerd, 1999). Active exhalation with hypaxial muscles functions not only to increase the rate of exhalation but to increase tidal volume beyond the passive end-expiratory volume (Ainsworth et al., 1989; DeTroyer et al., 1989). Subsequent relaxation of the abdomen during inhalation decreases resistance of diaphragmatic contraction and posterior displacement facilitating inhalation (Iscoe, 1998). At least some hypaxial muscles involved in ventilatory function during locomotion have been studied in humans, dogs, and horses. The most striking observation in hypaxial muscle function among these species is the great variation in motor patterns necessary to serve the many functions of this system (locomotion, ventilation, coughing, sneezing, vomiting, straining, vocalization, and independent limb movements). Although variable, dogs, humans and horses all use one or more of the abdominal hypaxial muscles bilaterally during exhalation (Koterba et al., 1988; DeTroyer et al., 1989; Ainsworth et al., 1996; Deban and Carrier, 2002).

Variability in motor control was observed in our generalized mammals as well. The marsupials can, but usually do not, locomote using only the pure cross-couplet motor pattern. Philander added bilateral activity in the transversus abdominis (coordinated with diaphragmatic cycles) to the pure couplet about half the time during locomotion, similar to the transversus abdominis pattern observed in many tetrapods (Brainerd, 1999). The eutherians (rats and hedgehogs) were similar to Philander except in adding bilateral contraction in the rectus abdominis at high speeds. The other two opossums employed variations on bilateral hypaxial ventilation in all the hypaxials more reminiscent of some muscles in dogs. However, Didelphis integrates bilateral activity with the couplet step while Monodelphis integrates bilateral activity with the resting ventilation rate. Thus, bilateral variable hypaxial contraction in some muscles during ventilation appears to be a general feature of mammals, but the generalized mammals use all of the hypaxial muscles when active ventilation occurs.

Functional Changes in the Abdominal External Obliques in Cursorial Mammals

One of the major differences in our results for basal mammals and data available for dogs is in the motor patterns for the abdominal oblique muscles during locomotion. In marsupials and basal mammals both of the oblique muscles contract unilaterally in synchrony with the ipsilateral forefoot stance phase. Given their attachment to the midline (which is shortening with the rectus abdominis) and the pelvis (and opposite hind limb in marsupials), they must primarily act in lateral bending and counteracting the dorsoflexion of the trunk imposed by diagonal footfalls. Studies of dogs have shown that each of the internal oblique muscles function differently than this (Fife et al., 2001; Deban and Carrier, 2002). The abdominal external oblique in dogs is only active with the contralateral forelimb. The internal oblique has a bilateral burst pattern - firing with each couplet step but having the larger amplitude burst with the contralateral forefoot stance phase. Thus, the primary activity in the dog obliques is exactly opposite of the pattern observed in basal mammals. However, the external oblique retains a small amplitude burst with the contralateral forefoot. Therefore, the change from the crouched limb postures of basal mammals to the erect postures of cursorial mammal is associated with a significant change in the function of the oblique muscles. Some insight into this change comes from evidence that the two obliques function differently when dogs go uphill vs. downhill indicating a shift to more of a forelimb controlling function. The obliques in dogs are thought to function to stabilize the trunk to control the shearing forces placed on the trunk by the extrinsic appendicular muscles (Deban and Carrier, 2002) and to control the strut-like behavior of the limb during the step (Carrier et al., 2008).

The Loss of Epipubic Levers and the Basal Condition in Therians

Comparing the basic cross-couplet system of the didelphids to the shoulder-to-pelvis system of the two eutherians reveals several apparent similarities for all therians and a rather simple pattern of transitional change associated with losing the epipubic bone lever system. Several similarities appear to be shared characteristics of the basic ancestral condition for marsupials and placental mammals (see Fig. 6). The first similarity is the presence of bilateral abdominal tonus during resting breathing. Second, a 1:1 couplet to ventilation synchrony is maintained at all speeds in the larger species (but only at forced high speeds in Monodelphis). Third, activity in the oblique muscles is always associated with the ipsilateral forefoot stance phase. Finally, as expected from its wide-
spread prevalence in tetrapods, the transverses abdominis are bilaterally active during expiration during locomotion.

The loss of the epipubic bones in eutherians was accompanied by a shift of the pectineus muscle onto the lateral aspect of the pubis where it takes on an adductor function. In addition, pyramidalis becomes a small or vestigial muscle that, when present, spans across the front of the pubis, where it essentially has no function. Thus, the forces transmitted from the four remaining hypaxial muscles are no longer transmitted to the contralateral femur but are instead transmitted to the midline and pelvis proper in eutherians. The most obvious functional impact of this to eutherians is to decouple the movement of the hind limb from coordinated synchrony with the contralateral forelimb.

Assuming that the ancestral therians (which had epipubic levers) had the same cross couplet patterns as the didelphids, the shift to the eutherian pattern would have required a change in the function of only one muscle (the rectus abdominis). The loss of the epipubic lever system in basal eutherians appears to have had no effect on the function of the obliques or the transversus abdominis. The external and internal obliques in the basal eutherians retain unilateral activity in synchrony with the ipsilateral epaxial region. The transversus abdominis retains its patterns of tonus at rest and active bursts with exhalation during locomotion. The rectus abdominis, however, changes from contralateral activity with the cross-couplet to ipsilateral activity in basal eutherians (see Fig. 6). This fundamental shift in motor pattern means that the shoulder-to-pelvis linkage works in unison (unilaterally except for the transversus abdominis which maintains its bilateral function in assisting with expiration), to generate muscular forces between the stance forelimb side of the ribcage to the midline and the pelvis during each step. At higher speeds the hind limb side rectus abdominis is recruited to develop a bilateral but asymmetrical pattern of activity in the rectus abdominis. An interesting additional morphological transition is the shift from conjoined (marsupials, except Didelphis) to separated rectus abdominis muscles in eutherians (White, 1990).

The second question is why lose the epipubic levers? To address this question we have to probe the differences between marsupials and basal eutherians. In terms of limb kinematics, marsupials have the most laterally displaced femoral movements and footfall positions relative to the midline and the pelvis during each step. At higher speeds the hind limb side rectus abdominis is recruited to develop a bilateral but asymmetrical pattern of activity in the rectus abdominis. An interesting additional morphological transition is the shift from conjoined (marsupials, except Didelphis) to separated rectus abdominis muscles in eutherians (White, 1990).

Two general questions remain in regards to the presence and loss of epipubic levers in early mammals. First, what was their original function? We have argued that through the cross-couplet linkage they provide a system of trunk support across diagonal feet during trotting locomotion (Reilly and White, 2003). Based on the appearance of epipubic bones in the fossil record, the lever system appeared in concert with the appearance of mammalian grade pelvis and the shift to an erect limb posture from the semi-upright posture of advanced cynodont ancestors (Jenkins, 1970; Pridmore, 1985). This also occurred in concert with the first evidence of a diaphragm (Jenkins, 1970; Crompton and Jenkins 1979) at a time when mammals are also thought to have shifted to expanded locomotor needs associated with higher metabolic rates (Crompton et al., 1978; Bennett and Ruben, 1979; Ruben et al., 1987). The shift to endothermy is considered to be related to increased stamina (Bennett and Ruben, 1986). But the primary functional shifts in the lumbar/epaxial region appear to indicate that increased stamina in early mammals was a consequence of being able to breath during locomotion by shifting from lateral to more sagittal bending (Bramble and Carrier, 1983; Carrier, 1987). The shift to the epipubic lever system appears to be a parallel transition in the hypaxial region. Here, however, the epipubic lever system directly spans the diagonal limbs to support the trunk ventrally. This would both facilitate trotting gaits and running mechanics (Parchman et al., 2003) and reduce locomotor loading of lateral body wall muscles to facilitate breathing during locomotion (Carrier, 1987). Thus, epipubic lever system appears to be another innovation in the initial mammalian transition to integrated locomotion and ventilation. However, it is hypothesized that the initial shift to a more adducted and crouched limbs in small mammals was not so much an adaptation for stamina as it was an adaptation to facilitate intermittent locomotion over irregular terrestrial and off-ground substrates (Fischer, 1999; Fischer et al., 2002; Witte et al., 2002). This is evident in the fact that small size, crouched limbs, and noncursorial locomotion remain far and away the most prevalent mammalian strategy today (Witte et al., 2000).

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marsupials the long axis of the pelvis stays much closer to the horizontal plane as it moves during locomotion than in other small eutherian mammals (Fischer et al., 2002; Schilling and Hackert, 2006). Thus, basal eutherians appear to exhibit an evolutionary transition to more vertically directed limb postures and a more vertically positioned and highly pivotal pelvis which may have been related to a release from the need to pull the epipubic bones in the horizontal plane. The change to a more vertical alignment of the pelvis in basal eutherians appears to be related to a significant increase in the degree of arching of the vertebral column. The rather striking difference in the nearly horizontal stance phase vertebral column of marsupials and the highly arched situation in basal eutherians (Fig. 1: Schilling and Hackert, 2006) demands further quantification because it appears to be a major difference between small mammals with and without epipubic bones.

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