Breathing Frequency Changes at the Onset of Stepping in Human Infants

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Short title: Breathing frequency changes with stepping in infants

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ABSTRACT

Breathing frequency increases at the onset of movement in a wide range of mammals including adult humans. Moreover, the magnitude of increase in the rate of breathing appears related to the rate of the rhythmic movement. We determined whether human infants show the same type of response when supported to step on a treadmill. Twenty infants (aged 9.7±1.2 months) participated in trials consisting of sitting, stepping on the treadmill, followed by sitting again. Breathing frequency was recorded with a thermocouple, positioned under one naris and taped to a soother which the infant held in his/her mouth. A video camera, electrogoniometers, and force platforms under the treadmill belts recorded stepping movements. We found that the rate of breathing changed at the beginning of stepping. Most surprisingly, we found that when infants stepped at a frequency slower than their breathing frequency in sitting, the breathing frequency decreased. Average breathing frequency during stepping was positively correlated with stepping frequency. There was no evidence of entrainment between stepping and breathing. In conclusion, the rapid change in breathing frequency at the beginning of movement is functional in infants. The direction and magnitude of change in breathing is associated with the leg movements.

Keywords: respiration, locomotion, exercise hyperpnea, development
INTRODUCTION

Exercise is accompanied by an increase in pulmonary ventilation, so that gas exchange in the lungs matches with the increased demand for oxygen from muscles (reviewed in Mateika & Duffin 1995; Waldrop et al. 1996; Ward 2000; Haouzi 2006; Mitchell & Babb 2006). The initial increase in ventilation from rest to exercise at constant-load is virtually simultaneous with movement (first shown in humans by Krogh & Lindhard 1913, reviewed in Asmussen 1983; Whipp 1983; Mateika & Duffin 1995; Bell 2006). Moreover, the magnitude of this initial increase seems to be related to the rate of the movement (Dejour 1967; Duffin & Bechbache 1983; Casey et al. 1987; Wells et al. 2007). The mechanisms responsible for this initial rise in respiration remain unclear and highly controversial (see Point:Counterpoint, J Appl Physiol 100:1077-1083 and 100:1417-1418).

Maturation of respiratory function is necessarily early in mammals although very little information exists on the relationship between movement and breathing in young humans. School-aged children show adult-like changes in respiratory frequency and ventilation at the onset of rhythmic exercise (Shea et al. 1993; Sato et al. 2000). We do not know when this response matures. One would expect that some controls are in place to coordinate respiration and limb movement early in life, so that the demands of muscles can be met. Electrical stimulation of lumbar dorsal roots in neonatal rats can entrain the breathing rhythm (Morin & Viala 2002). Electrical stimulation of forelimb afferents in a working heart-brainstem preparation of juvenile rats similarly entrains breathing via identified brainstem neurons (Potts et al. 2005). Hence, there are functional pathways for limb movements to influence breathing in at least some young mammals.
We wished to determine whether human infants show an abrupt increase in respiratory frequency at the beginning of stepping on a treadmill, and if it occurs whether it is associated with the rate of stepping. Infants can be induced to step by placing their feet on a moving treadmill belt (Yang et al. 1998). We hypothesized that there would be an immediate increase in the rate of breathing at the onset of stepping.

MATERIALS AND METHODS

Subjects

A total of 52 subjects were recruited for the study through the Public Health Division of Capital Health in Edmonton. Not all infants provided useful data (see Results for numbers), but all were born at term and developing normally. None of the infants were walking independently at the time of data collection. Ethical approval for the study was obtained through the Health Research Ethics Board, University of Alberta and the Capital Health, Edmonton, Alberta. A parent or legal guardian provided informed, written consent for the infant to participate in the study.

Prior to the experiment, the parent/guardian was contacted by phone to determine if the infant showed stepping behaviour when supported on a firm surface, and whether the infant liked using a soother (i.e., pacifier, see Recording Procedures below). Only infants who liked using a soother and showed at least 10 consecutive steps at a time, as reported by the parent/guardian were recruited for the study.

Instrumentation
**Respiration:** We recorded breathing frequency using a miniature thermocouple. The thermocouple was custom made from thermocouple alloy wire (YSI Temperature, Dayton, Ohio). Thermocouples of this sort have been used successfully to measure the rate of breathing in adults (Marks et al. 1995) and children (Marks et al. 1993). The wire was taped to the top portion of an infant’s soother so that the thermocouple was directly below the right naris. Breaths were detected by the change in air temperature: warm (expiration), cool (inspiration). After extensive pilot work with a variety of methods to measure breathing, we found the thermocouple to be the most reliable and unobtrusive. Room temperature was kept just above 20°C, in accordance with recommendations for measuring respiration with a thermocouple (Marks et al. 1995).

**Stepping:** Stepping was induced with a split-belt treadmill, custom made at the University of Alberta (model IN-FSBT-FP, designed and built by R. Gramlich and S. Graziano). This treadmill was used because it has separate force plates under each belt, which is optimal for measuring ground contact during walking. The two treadmill belts ran at the same speed, which was estimated by the rotation of the main drive shaft for each belt, and confirmed with video data. A Plexiglass partition was placed between the two belts to ensure that the infant’s legs remained on separate belts.

Stepping movements in the sagittal plane were monitored with electrogoniometers (Penny & Giles; Biometrics, Blackwood, Gwent, UK) placed over the hip joints bilaterally. The two arms of the goniometers were aligned with the mid-axillary line of the trunk and the long axis of the femur, respectively. In addition, a standard miniDV camcorder recorded the motion from the
right side at 30 frames per second. Infants were dressed in black stockings to enhance the contrast with the reflective markers. Markers were placed on the right side over the superior border of the iliac spine, the greater trochanter, the knee joint line, the lateral malleolus and the fifth head of the metatarsal. The audio track on the video recording was also important for monitoring verbalizations from the infant, which would change the rate of breathing.

All electrical signals were digitized on-line using an A/D program (AxoScope; Modular Devices, Foster city, CA) at a rate of 250 Hz, and backed up on VHS tape using a pulse-code-modulated encoder (Vetter, Rebersburg, PA). The force plate and electrogoniometer signals were low pass filtered at 30 Hz prior to digitization. The video and analog data were synchronized using a custom built timer, which generated pulses at 1 Hz and advanced an LED counter in view of the camera.

**Recording Procedures**

Infants were studied in a single experimental session, typically 1 hour in duration. The infant played on a floor mat while we attached the instruments. The experimental trials always started with at least 30 s of quiet sitting on an arm support just above the treadmill. We did not start the infants from a standing position, which would have been more similar to the methods used in adults, because it is very difficult to ensure the babies stand quietly for that length of time. Stepping was initiated by placing the infant’s feet on the treadmill belt, which was already running at the chosen speed (see below for how speeds were selected). The infant was supported under the arms by the experimenter whose arms rested on the arm support. An effort was made not to constrict the infant’s chest wall, but simply provide vertical support. Each stepping trial
lasted for 1-2 minutes, after which the treadmill was stopped and the infant was placed on the arm support to sit quietly for approximately 1 minute. The treadmill belt speed for the first trial was always around 0.4 m/s, to familiarize the infant to the treadmill. In subsequent trials, we included some faster (>0.4 m/s) and slower speeds (0.1 m/s to 0.2 m/s). The speeds were not tested in any specific order. A number of trials were recorded from each infant, typically separated by at least 1 minute. During the data recording, we tried to ensure a quiet environment to prevent an undesired change in breathing frequency.

Control experiments

Two types of control experiments were done. 1) Since the infants were lifted from a sitting position to a walking position on the treadmill, we determined if the act of lifting and standing the infant changed the rate of breathing. Hence, 3 infants were tested while sitting, standing, then sitting again. Many more infants were tested for this sit to stand transition, but the data could not be used because infants at this age do not like to stand still. Hence, the low numbers. 2) We also determined whether the use of a soother altered the breathing rate in infants. Five additional infants (aged 3 to 6 months old) participated in this control experiment. Breathing without a soother was recorded with a variable inductance plethysmograph (Respitrace, Ambulatory Monitoring Inc, Ardsley, NY). Transduction bands from the Respitrace system were placed directly on the rib cage and abdomen to sense the change in size. In order to derive a single kinematic signal representing respiratory behaviour, signals from the rib cage and abdomen were summed (Boliek et al., 1996). The resulting summed signal was used to measure breathing frequency for each condition. Infants sat quietly in an infant seat throughout the
experiment. Breathing was recorded with and without a soother repeatedly. The experiment was also videotaped. A minimum of 15 breaths were averaged for each condition.

**Data Analysis**

The audio-video files were reviewed to select successful trials. A successful trial for walking was one in which the infant was sitting quietly at the beginning of the trial with at least 10 consecutive breaths, followed by consistent stepping on the treadmill with at least 10 consecutive steps and a minimum of 10 analyzable breaths during stepping, followed by another period of quiet sitting with a minimum of 8 breaths. The analog data from successful trials were reviewed together with the video data to exclude periods when the infant vocalized or did things that might alter the breathing, such as large upper arm movements. These events were marked so the breath could be removed from analysis.

Breath cycles were defined by peaks in the thermocouple signal, which corresponded to the onset of inspiration (i.e., the beginning of a decrease in temperature associated with inspiring room air). Custom written software in MatLab (Mathworks, Natick, MA) filtered the thermocouple signal (4 Hz low-pass), then selected the breaths automatically by searching for local maximums, which were then reviewed by the user to correct any inaccuracies related to baseline shifts or vocalization by the infant. The instantaneous breathing frequency was taken as the inverse of each breath duration. Step cycles were determined from the force plate signal if the infant stepped on the 2 separate force plates. Some infants have considerable varus (bow-legged) making it difficult for them to step with the plastic partition between the legs. In those infants, stepping was induced on a single treadmill belt. In these cases, the electrogoniometer signal
from the two hips provided timing information on the step cycles. Custom written software in
MatLab was used to determine the step cycle duration based on crossing of a threshold (mean) in
the force or goniometer signal. Selection of steps was confirmed with visual inspection of the
video tape.

We wished to determine whether the rapid change in breathing frequency was consistent with the
Phase I response reported in adults, i.e., within the first 10-15 s of exercise (Whipp 1983).
Hence, the breaths prior to and at the beginning of stepping were examined in more detail in a
subset of the infants, who showed an uninterrupted transition from rest to stepping (i.e., no more
than 2 breaths deleted because of an interruption). The instantaneous breathing frequency
calculated from the last 12 breaths prior to stepping were averaged in groups of 3 for each trial
(i.e., breaths 1-3, 4-6 .... etc.), as were the first 12 breaths at the beginning of stepping. Across
subject averages were then estimated from these 8 time windows. The same analysis was done
with data at the end of stepping. The 12 breaths at the end of stepping and the first 12 breaths
immediately after stepping were averaged in groups of 3, generating values for 8 time windows.

**Statistical Analysis**

A 2-way ANOVA with a mixed-design (SPSS, SPSS Inc, Chicago, IL) was used to compare the
average breathing frequency during quiet sitting, stepping and sitting again. The two factors
were movement state (sitting, stepping, sitting), and movement speed (fast and slow stepping).
Each subject provided repeated-measures over movement state (i.e., within-subject factor), but
not across movement speeds (i.e., between-subject factor). Each infant contributed one set of
values (averaged across comparable trials) to the ANOVA for a particular comparison. Tukey’s
test was used for all post hoc analyses. Since the rate of breathing was clearly different during sitting compared to stepping, we wished to determine the time course of this change in breathing frequency both at the beginning and the end of stepping. Two separate 2-way ANOVAs were used, one to compare the transition from sitting to stepping (i.e., onset), and the other for the transition from stepping to sitting (i.e., offset). The two factors in the ANOVA were: time and movement speed (fast and slow stepping). Time was represented by averages obtained from the 8 time points surrounding the transition period (see Data Analysis above), a within-subject factor. Differences were considered to be significant at p<0.05. Tukey’s test was used for post-hoc analysis of significant interactions. Test of within-subject contrasts was used for comparing adjacent means (i.e., breath groups -4 vs -3, -3 vs -2 …etc). Since there was a transition period spanning breath groups -1 and 0 as a result of lifting the infant, the average of the first 3 breath groups (-4, -3, -2) was compared with the last 3 breath groups (1, 2, 3) to determine if the breathing frequency was different in the 2 states.

Pearson’s product-moment correlation was used to determine if there was a relationship between the rates of stepping and breathing. Each trial of walking contributed a data point, which consisted of the average breathing and average stepping frequency during walking in that trial. If the rate of stepping influenced the rate of breathing, then there should be a positive relationship between these variables.

Another factor that could affect the change in breathing at the onset of stepping is the breathing frequency at rest. There was considerable variability in the breathing frequency during sitting both within and between infants. Hence, a difference was calculated between the average rate of
breathing in sitting, and the average rate of stepping that was adopted once stepping started. We would expect this difference to be narrowed if the frequency of stepping is driving the frequency of breathing. In other words, a change in the breathing frequency during stepping that would bring it closer to the stepping frequency. So the difference between the breathing frequency at rest and the stepping frequency was plotted against the change in breathing frequency from sitting to stepping. We predicted that these two factors would vary together (i.e., a positive correlation). A correlation was considered significant at p<0.05.

The presence of entrainment of breathing with stepping was determined with circular statistics, because there was not sufficient data to run cross-correlations. The occurrence of inspiration onset was plotted as a function of the step cycle, with the step cycle represented as a circle. A mean vector length, representing dispersion of the data, was estimated from the circular plot (Batschelet 1981). A Rayleigh Test was used to determine if the dispersion was significantly different from zero (i.e., a score of zero indicates a random distribution).

RESULTS

Effect of using a soother on the rate of breathing

Comparison of the breathing frequency with and without a soother for the group showed no significant differences between the 2 conditions based on a paired t-test (mean±SD: with soother=1.32±0.38 Hz; without soother=1.07±0.28 Hz). Three out of the 5 infants showed a slight increase in the breathing frequency with the soother compared to without.

Transitions between sitting and stepping
A total of 20 infants (aged 6.9–11.5 months, mean±SD=9.7±1.5 months) contributed useful data to the trials addressing the transition between sitting and stepping. Note that not all infants were tested at both fast and slow speeds, because most infants have a limited tolerance for stepping on the treadmill. Experiments were terminated if the infant became uncooperative. Data were obtained from 12 infants (34 trials) for fast stepping, and 9 infants (16 trials) for slow stepping. Data for slow stepping were more difficult to obtain because some infants do not step consistently at a very slow treadmill speed. There was no relationship between the age of the infant and the rate of stepping in this group of subjects.

**Stepping at different speeds**

The treadmill speeds for fast stepping ranged from 0.4 m/s to 1.2 m/s (mean±SD=0.67±0.19 m/s). Figure 1 shows raw data from a single subject, during sitting (initial 40 s), stepping (~ 50 s) and sitting again (~35 s). The right hip goniometer indicates the steps (top trace), while the thermocouple indicates the breaths (inhalation negative slope, exhalation positive slope). The bottom graph shows the instantaneous breathing frequency (vertical bars), with a running average (calculated with 7 data points) shown in the solid line superimposed on the bars. At the beginning of stepping and sometimes prior to stepping, the breathing frequency increased abruptly as shown in the example in Figure 1 (vertical dashed line indicates beginning of lift). The faster breathing rate persisted throughout the period of stepping (shaded area in Figure 1). After stepping ended, the breathing rate slowly returned towards the resting level with a slower time course compared to the transition from rest to stepping. The average breathing frequency for the subject shown in Figure 1 during each of the 3 periods (sitting, stepping, and sitting
again) is shown in Figure 2A. There was a clear increase in the frequency of breathing during stepping, and a return towards the resting rate after stepping.

(Figure 1 & 2 near here)

Data from all 12 infants together with the group means are shown in Figure 2B. The data from each infant is connected by a line, with each point representing the average from the trials (~3) obtained from that infant. The average stepping frequency at these moderate to fast speeds of walking was 1.04±0.20 Hz. While there was considerable variation in breathing frequency across infants, the majority showed faster breathing frequencies during stepping, and a trend towards a return to resting rates after stepping.

The treadmill speed for slow stepping was set at 0.2 m/s for all subjects. The transition from sitting to slow walking is shown for a single trial in Figure 3, from the same infant as shown in Figure 1. The convention of the graph is the same as Figure 1. Surprisingly, the breathing frequency slowed down when stepping at this slow speed (<0.2 m/s) and rate (average stepping frequency ±SD=0.47±0.08 Hz). This occurred in spite of the presumed increase in metabolic demand of stepping compared to sitting. The average breathing frequency for this infant for each of the 3 phases (sitting, stepping and sitting again) is shown in Figure 2C. Data from all 9 infants is shown in Figure 2D together with the group means.

(Figure 3 near here)

The 2-way ANOVA on data from Figure 2B&D showed a significant interaction between movement speed and movement state. Post-hoc analysis compared the breathing frequency for initial sitting, stepping, and final sitting, at each of the 2 walking speeds separately (see “*” in Figure 2B&D). In addition, we compared breathing frequency between the two walking speeds
for each of the movement states. Significant differences were only found during stepping, so the breathing frequency was the same before and after stepping for the two speeds of stepping.

**Time course of change in respiration at the beginning of stepping**

To obtain a more detailed understanding of the time course of change in breathing, the breaths surrounding the beginning of stepping were analyzed. The 12 breaths just prior to stepping and the first 12 breaths during stepping were averaged in groups of 3, resulting in 8 consecutive averages spanning the transition from sitting to stepping. These consecutive averages provide an estimate of the time course of change in respiration. Transition to fast stepping is shown in Figure 4A, and slow stepping in Figure 4B. Eleven and 6 infants contributed data to the fast and slow stepping, respectively. Not all infants contributed data because some did not have smooth transitions between sitting and stepping (see Methods). A 2-way ANOVA showed a significant interaction for time and speed of stepping (Figure 4A & B). Post-hoc analysis comparing adjacent means at each stepping speed indicated that for fast stepping, breath group -1 was different from group 0. None of the adjacent pairs were different for slow stepping. The mean of the first 3 breath groups (i.e., -4, -3, -2) was compared with the mean of the last 3 breath groups (i.e., 1, 2, 3) using a paired t-test. These means were significantly different for fast stepping only. Hence, the rate of breathing changed well within the first 3 breaths of fast stepping, consistent with the time course for a Phase I response (Whipp 1983). The time course of change for slow stepping was longer. Additional pairwise comparisons for slow stepping were used to determine if breath group 3 was different from any of the breath groups prior to stepping. Breath group 3 was different from breath groups -4, and -1, so there was a change by the 12th breath during stepping.
Averages for the 12 breaths just before and after standing are shown in Figure 4C for the control experiments (n=3). These data illustrate that lifting the infant to a standing position affected the breathing rate during the lift (i.e., Breath Group -1 in Figure 4C). A 1-way repeated-measures ANOVA showed a significant main effect. Post-hoc comparison between adjacent means showed breath group -1 to be the different from breath group -2.

(Figure 4 near here)

**Time course of change at the end of stepping**

The breathing frequency at the end of stepping was analyzed in detail just as for the transition at the beginning of stepping. The 2-way ANOVA showed no significant main effect or interaction. Hence, the changes in breathing frequency after stepping must have a longer time course than the beginning of stepping.

**Relationship between rate of breathing and stepping**

The relationship between the rate of breathing and stepping was estimated by plotting the average frequency of stepping in a trial against the average frequency of breathing while stepping in the same trial (Figure 5A). Each trial from each subject contributed a data point to Figure 5A. A linear regression analysis indicates a significant (p<0.0005) relationship between the two.

(Figure 5 near here)

The rate of breathing while seated was somewhat variable between infants and between trials within an infant. This variability could obscure the changes in breathing frequency when
different infants and trials were averaged together (as was done in Figure 2B&D and Figure 4A&B). For example, an infant with a fast breathing frequency at rest might slow their breathing rate when stepping slowly, but an infant with a very slow breathing frequency at rest might do the opposite when stepping slowly. Hence, data from individual trials were plotted as follows. The change in average breathing frequency from resting to stepping was plotted against the difference between the average breathing frequency at rest and the stepping frequency (Figure 5B). If the stepping frequency is important in driving the breathing frequency, then the change in breathing frequency with stepping should bring the frequency of stepping and breathing closer (i.e., a positive slope in the relationship). This relationship is indeed positive and statistically significant (p<0.0001).

In two infants, the rate of stepping was altered during a trial by changing the treadmill belt speed. Examples from one infant are shown in Figure 6, in which there was a transition between fast and slow stepping rates in both directions as a result of a change in the belt speed (A: fast to slow, B: slow to fast). Note that the rate of respiration changed with the rate of stepping. The same occurred in the other infant.

(Figure 6 near here)

Finally, although it did not appear that there was a 1:1 relationship between the rates of breathing and stepping (see Figure 5A), we determined whether infants tend to take breaths at particular times in the step cycle at submultiples of the stepping rate. Circular histograms (see Methods) were constructed for each infant to indicate the frequency with which inspiration began at different parts of the step cycle. Separate histograms were constructed for fast and slow rates of
stepping. In both cases, there was no particular pattern to the histograms, and the Rayleigh tests showed no trials were different from chance.

**DISCUSSION**

We show for the first time that young infants change their rate of breathing within the first 3 breaths taken during stepping. This indicates the mechanisms underlying the rapid change in breathing at the beginning of rhythmic movement are functional at this age. Also, the rate of breathing is strongly influenced by the rate of stepping. Interestingly, when the rate of stepping is slower than the resting rate of breathing, the breathing rate often decreases from rest to exercise. To our knowledge, this has never been reported in adult humans or other vertebrate species. These results suggest the rhythm generator for stepping and/or its afferent feedback have strong influences on the rhythm generator for breathing in human infants.

**Methodological Considerations**

It was difficult to measure breathing during movement in infants. A long series of pilot work preceded this study to determine a feasible method for recording the rate of breathing. Ideally, it would have been preferable to measure ventilatory volume by using a mouth piece or a mask. However, these methods were not feasible with infants this age, because they dislike anything attached to their faces. Measures of change in the circumference of the chest wall using a variable inductance plethysmograph was tried, but found to contain far too much movement artifact during stepping to be useful, although it was useful for the studies to determine the effects of a soother done in a sitting. The thermocouple method has been used in the past for children 12 months and older (Marks et al. 1993). In that study, the thermistor was taped to the
face near the naris. We modified the approach to avoid having to apply tape to the infant’s face, because infants this age pull off anything attached to their faces. We found that taping the thermistor to a soother worked well.

By using the soother for attaching the thermocouple, we could only study infants who liked using a soother and were willing to use it while stepping. Control experiments indicated that the rate of breathing is not influenced by the use of a soother, although there was a tendency for the rate of breathing to increase with soother use (3 out of 5 control infants). Even if the rate of breathing tended to be faster with the soother, this influence would have been present during both sitting and stepping, so it should not have affected the results. The use of a thermocouple at the naris limited the fastest rate at which we could record stepping, because at higher speeds of stepping, the infant tended to breathe through the mouth, which degrades the signal recorded by the thermocouple.

A possible confound in these experiments is the arousal level of the infant. Putting an infant in an unfamiliar environment (i.e., the lab), and inducing stepping in an unfamiliar way (i.e., the treadmill) could have inadvertently raised the arousal level of the infant, and hastened the rate of respiration. The breathing frequency reported here for quiet sitting is at the upper end of the normal range for awake infants of comparable age (Hoppenbrouwers et al. 1978; Asmussen et al. 1981; Morley et al. 1990; Rusconi et al. 1994). Hence, we cannot discount the possibility that these higher resting rates of breathing influenced what we found during stepping. For example, there may have been less reserve to increase breathing frequency further during movement, resulting in a greater tendency to increase tidal volume rather than increase breathing frequency.
(i.e., as seen in slow stepping). Nevertheless, the rate of breathing changed in both directions depending on the rate of stepping (i.e., increased when stepping was >0.4 m/s and decreased when stepping <0.2 m/s), so there was sufficient reserve for breathing rate to change in either direction. Moreover, in some trials, we were able to vary the treadmill speed as the infant was walking or the infant would spontaneously alter his/her stepping rate. In these situations, the rate of breathing varied with the rate of stepping (Figure 6), again in either direction.

Finally, lifting and placing the infant on the treadmill could change the breathing frequency. Control experiments in which the infant was lifted onto the stationary treadmill indicated that lifting the infant altered the breathing frequency. The acute change in breathing during the lift was complete within approximately 4 seconds, and usually preceded the period of stepping (Breath group -1 in Figure 4C). Since the changes we saw were persistent over at least the duration of stepping (~30s to 60s), we are confident that the changes seen at the beginning of stepping are associated with stepping. It is unclear why lifting the infant did not affect the breathing frequency in the trials with slow stepping (Figure 4B). Inspection of individual trials, however, show that some infants showed the increase in breathing frequency with the lift (e.g., Figure 3) and others did not. Hence, the effect of lifting the infant on breathing frequency was variable between subjects. The important point for this study was that the effects of lifting were brief and disappeared prior to the second group of three breaths analyzed during stepping.

**Change in frequency of breathing occurs at the beginning of movement**

The change in breathing frequency was seen within a few seconds from the beginning of fast stepping in infants, and was sustained typically to the end of the trial (normally less than 60 s).
This time course is consistent with the very early changes in respiration seen at the beginning of exercise in other mammals & adult humans (reviewed in Whipp 1983; Mateika & Duffin 1995). The decrease in breathing frequency when infants stepped at slow speeds had a slightly longer time scale than fast stepping (compare Figure 4A & B), but still occurred within the first 10 s of stepping.

Breathing frequency showed a trend towards a faster rate in standing compared with sitting although the comparison was not significantly different (Figure 4C). Only 3 infants provided data for this comparison, so it is possible that there are real differences in breathing frequency between sitting and standing. Thus, part of the change in breathing frequency with stepping may have been associated with the standing. Nevertheless, the opposite directions of change in breathing frequency for fast and slow stepping indicate the effects of standing are likely small.

There is very limited information on the Phase 1 response to exercise in children. School aged children 7 years old and older show a rapid change in breathing frequency (Shea et al. 1993; Sato et al. 2000) and oxygen consumption (Cooper et al. 1985; Hebestreit et al. 1998) analogous to that seen in adults. Moreover, passive movement of the lower or upper limbs also induces changes in respiratory rate in children just as in adults (Sato et al. 2000). Our results suggest that the changes in respiration at the beginning of stepping are seen in infants as young as 7 months of age. Hence, control of breathing frequency in the early phase of physical activity is functional early in life.

Change in the breathing frequency at the end of stepping
Breathing frequency did not change abruptly at the end of stepping. Rather, there was a slow return to the baseline level. This is in contrast to adults (Beaver & Wasserman 1968, 1970; Jeyaranjan et al. 1988; Mateika & Duffin 1992) and older children (Shea et al. 1993) who show a rapid drop in breathing frequency at the end of exercise, followed by a slower exponential return to resting levels. The possible reasons for this difference are many. First, the drop in respiratory frequency at the end of exercise was smaller than the rise in frequency at the beginning of exercise in adults, and required averaging to reveal (Beaver & Wasserman 1968, 1970). We did not have enough trials to perform averaging in every infant, and when we were able to average, the number of trials was far less than that used in the adults. Hence, it is possible there was a drop in respiratory frequency at the end of walking, but we could not detect it. Second, the exercises used in the experiments on adults were of moderate to heavy intensity. We did not have a measure of exercise intensity, but generally, the trials were short (1 to 2 minutes) and the infants showed no signs of exertion. Thus, it is possible that a drop in respiratory frequency at the end of exercise can only be revealed by more intense activity. Finally, it is also possible that the mechanisms responsible for the drop in respiratory frequency at the end of exercise are different from those driving the rise in frequency at the beginning of exercise. In this case, it is possible the mechanisms controlling the rapid drop in frequency at the end of exercise are not yet mature in these infants.

**Rate of breathing is a function of the rate of stepping**

There is a moderate relationship between the frequency of breathing and stepping (Figure 5A). Considerable spread in the data did not justify examining the relationship beyond a simple linear model. The range of treadmill speeds we used were relatively limited, because it was impossible
to record a thermistor signal at fast speeds (i.e., infants start to breath through their mouth). So, the relationship between breathing and stepping frequency may be non-linear, and a larger range of walking speeds would be necessary to explore the relationship.

The slowing of breathing at the beginning of stepping was most surprising, and perhaps the strongest evidence that the breathing frequency is a function of the rate of stepping. All other reports of changes in breathing at the beginning of exercise indicate increases in breathing frequency (hyperpnea). Nevertheless, the magnitude of the change during slow walking was relatively small (equivalent to 0.10±0.13 Hz) and the amount of data we have limited, so further confirmation of these results will be important in the future.

Slowing of the respiratory rate has never been reported at the beginning of exercise in humans, perhaps because the rate of our rhythmic activity is typically faster than our rate of breathing. Adult humans, for example, breathe at a much slower rate (e.g., ~0.2 Hz) than most rhythmic limb movements (e.g., walking ~1 Hz, running ~2 Hz, bicycling ~1 Hz). Studies using electrical stimulation of limb nerves to induce entrainment in reduced mammalian preparations have also not attempted to induce entrainment at rates lower than the spontaneous respiratory frequency (e.g., Kawahara et al. 1988; Morin & Viala 2002). Electrical stimulation of afferents can reset the breath cycle in anesthetized cats, and the resetting can either shorten or prolong the breath cycle depending on when the stimulus arrives during the cycle (Iscoe & Polosa 1976). Hence, it may be possible to slow breathing frequency in other mammals, but this remains to be determined.
The slowing of breathing with slow stepping is counter-intuitive, because the demand on ventilation should be higher during stepping than sitting. We suggest that this higher demand may be accommodated by taking deeper breaths. Independent variation of the respiratory rate and volume (e.g., change in one without the other) during exercise has certainly been reported (e.g., Dejour 1967; Beaver & Wasserman 1970). We suggest here there may be an opposite change in respiratory rate and volume at the beginning of slow stepping, which could be explored in the future with measures of ventilation.

Support for the idea that the rate of movement determines the rate of breathing also comes from previous studies in which the rate of the movement has been changed while keeping the load of the movement constant. For example, in treadmill walking, the incline of the treadmill can be paired with different treadmill speeds so that subjects walk with the same oxygen consumption at very different rates of limb-movement. In all cases, the magnitude of the initial increase in ventilation (i.e., breath-by-breath measures of l/min) was associated with the rate of the movement rather than the load (Duffin & Bechbache 1983; Casey et al. 1987; Kelsey & Duffin 1992; Wells et al. 2007). Other studies in which the load of the movement (bicycling) is changed without a change in the rate of the movement also indicate that there is no rapid change in the minute ventilation when the load is altered (e.g., Whipp et al. 1982).

Multiple neurogenic mechanisms, classified as either feedforward or feedback, are proposed to act in concert to produce this initial increase in ventilation with exercise. Neurogenic mechanisms are favored to account for the initial increase in ventilation because the time course is too fast to be humoral (Matell 1963). Feedforward mechanisms associated with the command
to start walking can alter respiration (reviewed in Waldrop et al. 1996), as shown by electrical or chemical stimulation of locomotor regions in the midbrain in anaesthetized intact, or unanaesthetized decerebrate cats, including those in which afferent feedback was eliminated by paralysis of the muscles (Eldridge et al. 1981; DiMarco et al. 1983; Eldridge et al. 1985). Neuroimaging in intact humans further suggest participation of the motor cortex in exercise-related hyperpnea (Fink et al. 1995) even when there is no overt movement (Thornton et al. 2001). Our results cannot directly address the importance of central feedforward mechanisms in these infants. While the motor cortex and its descending input is still immature at this age both histologically (Yakovlev & Lecours 1967; Huttenlocher 1979; Brody et al. 1987) and functionally (Eyre et al. 1991, 2000; Muller et al. 1991; Szelenyi et al. 2003), it remains possible that a feedforward command either from the cerebral cortex or the midbrain could be involved.

There is considerable evidence that sensory afferents from the limbs are involved in generating the initial hyperpnea with exercise. Rhythmic limb movements that have been confirmed to be largely passive in humans (Bell et al. 2003) are associated with a rapid increase in the minute ventilation (Dejour et al. 1959; Ishida et al. 1994; Sato et al. 2000) and frequency of breathing (Ishida et al. 1994; Miyamura et al. 1997, Bell et al. 2003). Interestingly, subjects with clinically complete spinal cord injuries did not show the increase in minute ventilation with passive movements (Morikawa et al. 1989; Jaeger-Denavit et al. 1973) and those with incomplete lesions showed ventilatory changes that were intermediate between healthy controls and those with clinically complete spinal cord injuries (Jaeger-Denavit et al. 1973). Rhythmic electrical stimulation of sensory nerves innervating skin or muscle with no muscle contraction (i.e., cut nerve, paralyzed muscles or in vitro preparation) also leads to a rapid hyperpnea in reduced
preparations of cats and dogs (Koizumi et al. 1961, Senapati et al. 1964, Iscoe & Polosa 1976). In some cases, the stimulation entrained the respiration in cats and rats (Iscoe & Polosa 1976; Morin & Viala 2002; Potts et al. 2005). Many classes of afferents could be involved, including recently favoured group III & IV afferents from muscle (reviewed in Haouzi et al. 2004).

There is no evidence for entrainment between stepping and breathing

Although the rate of breathing changed as a function of the rate of stepping, there was no evidence of entrainment in the infants. Since entrainment is seen in quadrupeds and adult humans when the movement is more rapid, such as cantering and galloping in quadrupeds and running in humans (Bramble & Carrier 1983), it is possible that we did not test the stepping at sufficiently high speeds in the infants. We were unable to test the higher speeds, because infants tended to breath through their mouth when speeds increased, causing a degradation of the signal from the thermocouple. It is also possible that the duration of the exercise we studied was not sufficient for entrainment to develop, since inexperienced adult runners need more running time than trained runners for entrainment to develop (Bramble & Carrier 1983). Finally, entrainment may require experience, since adults who are more highly trained in rhythmic tasks tend to show a higher incidence of entrainment (Bramble & Carrier 1983; Mahler et al. 1991; McDermott et al. 2003).

Summary

We show that infants change their rate of breathing near the beginning of stepping. The magnitude and direction of change is a function of the rate of stepping. Compared to the rate of breathing during sitting, fast stepping induces a faster rate of breathing and slow stepping
induces a slower rate of breathing. The return of breathing frequency to resting levels after stepping has a slower time-course than the change at the beginning of stepping. Moreover, there is no evidence of entrainment between stepping and breathing. Hence, the mechanisms responsible for the rapid adjustment of breathing frequency at the beginning of movement are functional very early in life.
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Figure 1. Example of a trial from a single subject (APG2), walking at a moderate speed of 0.6 m/s. The hip angle (top trace) and thermocouple signal (middle trace) are the analog signals. The bottom trace is the breath-by-breath calculation of the instantaneous breathing frequency, with a running average (7 points) superimposed. The trial starts with the infant sitting. After 40 seconds, the infant is lifted (arrow in top trace and dashed vertical line) onto the treadmill. Stepping continues for approximately 50 s, after which the infant is placed in sitting again. When the infant is verbalizing (arrow in middle trace), the breathing frequency is not calculated. Note that the breathing frequency increases during stepping.

Figure 2. A & C: Average breathing frequency from a single infant during 3 time periods: sitting, stepping and sitting again, from the trials shown in Figure 1 and Figure 3, respectively. The number of breaths in each average for Sit, Step and Sit are: 37, 74 and 41 for A, and 71, 80 and 60 for C. B & D: Data from all infants contributing data to stepping at fast speed (>0.4 m/s) and slow speeds (<0.2 m/s), respectively. Each thin line represents average data from one infant. Compared to the breathing frequency in sitting, most infants showed an increase in breathing frequency during fast stepping, and a decrease in the breathing frequency during slow stepping. A small number of infants showed opposite trends (gray, dashed lines). Group averages are shown in the thick, dark lines. The “*” indicate pairs that were significantly different between movement states.

Figure 3. Example of a trial from a single subject (APG2), walking at a slow speed of 0.2 m/s. Convention of the figure is identical to Figure 1. Note that the breathing frequency decreases during slow stepping.

Figure 4. Time course of change in breathing frequency spanning the onset of stepping at fast (A) and slow (B) speeds, and the onset of standing in the control experiments (C). Each data point represents the average of 3 breaths from each infant, averaged across all subjects ± 1SEM. Negative breath groups correspond to breaths taken prior to stepping or standing, while positive groups represent those during stepping or standing, with group 0 being the first 3 breaths at the beginning of movement.

Figure 5. A. Breathing frequency during stepping is shown as a function of stepping frequency. B. The change in breathing frequency from sitting to stepping is shown as a function of the difference between the frequency of stepping and the frequency of breathing prior to stepping (i.e., sitting). Parameters for the best-fitting straight line are shown. The significant correlations indicate breathing frequency is related to the stepping frequency. Each data point represents averages from a trial, so some babies contribute more than one data point.

Figure 6. Raw data from individual trials in which an infant (CRS) changed his stepping rate during the trial because we changed the treadmill belt speed (vertical dashed line shows approximate time of speed change). A. Fast to slow transition. B. Slow to fast transition. Note that the rate of breathing varied with the rate of stepping.