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Characterization of the Fetal Diaphragmatic Magnetomyogram and the Effect of Breathing Movements on Cardiac Metrics of Rate and Variability

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Abstract

Breathing movements are one of the earliest fetal motor behaviors to emerge and are a hallmark of fetal well-being. Fetal respiratory sinus arrhythmia (RSA) has been documented but efforts to quantify the influence of breathing on heart rate (HR) and heart rate variability (HRV) are difficult due to the episodic nature of fetal breathing activity. We used a dedicated fetal biomagnetometer to acquire the magnetocardiogram (MCG) between 36–38 weeks gestational age (GA). We identified and characterized a waveform observed in the raw data and independent component decomposition that we attribute to fetal diaphragmatic movements during breathing episodes. RSA and increased high frequency power in a time-frequency analysis of the IBI time-series was observed during fetal breathing periods. Using the diaphragmatic magnetomyogram (dMMG) as a marker, we compared time and frequency domain metrics of heart rate and heart rate variability between breathing and non-breathing epochs. Fetal breathing activity resulted in significantly lower HR, increased high frequency power, greater sympathovagal balance, increased short-term HRV and greater parasympathetic input relative to non-breathing episodes confirming the specificity of fetal breathing movements on parasympathetic cardiac influence. No significant differences between breathing and non-breathing epochs were found in two metrics reflecting total HRV or very low, low and intermediate frequency bands. Using the fetal dMMG as a marker, biomagnetometry can help to elucidate the electrophysiologic mechanisms associated with diaphragmatic motor function and may be used to study the longitudinal development of human fetal cardiac autonomic control and breathing activity.

Keywords

Autonomic Nervous System; Magnetocardiogram; Fetal Biomagnetometry; Diaphragm Activation

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Conflict of Interest Statement

The authors have no financial or personal relationships with other people or organizations that could inappropriately influence their work.

Introduction

Fetal heart rate (HR) and its pattern, in conjunction with body and breathing movements and measures of amniotic fluid volume, are the mainstays for clinical assessment of fetal well-being. As gestation progresses, fetal HR decreases while heart rate variability (HRV) increases, due in part to increased parasympathetic influence (1), maturation of central mechanisms that regulate autonomic control (2) and a higher incidence of breathing movements (3). Fetal breathing movements can be detected on ultrasound by 11 weeks of gestation (4), increase in number and duration with advancing gestational age (GA) (5) and are dependent on behavioral state (6-8).

Respiratory sinus arrhythmia (RSA) is a well documented and long recognized pattern of high frequency HRV associated with the respiratory phase (9). RSA is characterized by an increase in HR during inspiration and a decrease during expiration. Since RSA can be eliminated by vagal blockade, it is considered to be an indirect measure of parasympathetic influence on HR (10, 11). RSA has been documented in the human fetus during fetal breathing movements (12, 13) and is characterized by low amplitude, high-frequency fluctuations of HR (14-17). Analytical techniques routinely used to quantify RSA in adults such as time and frequency domain metrics spanning an interval of quiet rest are problematic in the fetus because fetal breathing movements are episodic in nature. Because it is difficult to obtain simultaneous, noninvasive measures of human fetal diaphragmatic movements and high resolution beat-to-beat cardiac intervals necessary to compute fetal HRV, the most comprehensive studies examining the relationship between breathing activity and HRV have been performed in chronically instrumented animals using the diaphragmatic electromyogram (EMG) as a marker of breathing activity. (18, 19).

Simultaneously measured tracheal fluid pressure and electrocardiograms (ECG) were measured in fetal baboons during breathing and non-breathing periods. Periods of fetal breathing lasted from a few seconds to several minutes and were bounded by periods of apnea. The power spectra derived from tracheal fluid pressure and the electrocardiogram (ECG) both showed a high frequency peak at 1 Hz, consistent with the respiratory rate of 60 breaths per minute. Fetal HR was lower during breathing periods and oscillated approximately 2 beats per minute with each fetal breath. During non-breathing periods, fetal HR was faster and tracheal fluid pressure and ECG spectral had significant power only at the low frequencies. The authors concluded that beat-to-beat variability in fetal HR is influenced by breathing movements and the interaction is equivalent to postnatal RSA (18).

In a review of methods used to measure fetal breathing, Jansen et al. described breathing behavior in fetal lambs and found that the diaphragmatic electromyogram (EMG) provided a stable and reliable record of fetal breathing activity (19). Because these invasive methods are impossible in the human fetus, efforts to characterize fetal HRV during breathing periods have been limited.

Using real-time sonography and transabdominal maternal ECG, Groome, et. al., investigated the contribution of fetal RSA to overall HRV spectral power in quiet sleep (17). They observed a high frequency peak during fetal breathing that accounted for approximately 20% of the total power. While high frequency amplitude was reduced in non-breathing periods, power in the RSA defined frequency band (0.4-1.0 Hz) did not disappear.

Hirsch and colleagues (14) used simultaneous ultrasound and maternal abdominal electrocardiogram (ECG) and then extracted the fetal ECG for spectral analysis of short, partially overlapping traces of the fetal HR time-series. Increased high frequency power

between 0.6 and 0.7 Hz was always observed during fetal breathing movements; sometimes preceding the breathing movement, or decreasing before the cessation of breathing movements. They also described periodic elevations in high frequency activity during non-breathing periods. This led to the conclusion that fetal breathing was closely related to changes in fetal autonomic activity.

More recently, fetal HR power was characterized in specific frequency bands as a function of GA using time-frequency analysis of fetal HRV (20). Fetal ECG, extracted from the maternal abdominal ECG, was analyzed using a time-frequency decomposition based on a continuous wavelet transform. The authors observed increased high frequency power (0.4-1.7 Hz) with advancing GA and suggested an association with fetal breathing periods. Because fetal breathing is episodic and irregular in frequency, they suggested that a “correct method to investigate the respiratory peak” and to estimate fetal vagal control would be to separate and compare assumed fetal breathing periods (based on increased high frequency power in the continuous wavelet transform) and periods of “apnea” (areas with reduced high frequency power). Even as informative as the continuous wavelet transform is with regard to showing differences in power across time and various fetal behaviors, selecting regions based only on increased or decreased power in frequency band is problematic, not only because the outcome is predictable but also because of the inconsistencies reported by Groome and Hirsch, et. al. (14, 17).

Noninvasive and precise measures of fetal HR and movements are required in order to elucidate the mechanisms surrounding the development and maturation of fetal breathing movements, RSA and cardiac autonomic control. Using a dedicated fetal biomagnetometer that detects magnetic fields produced by electrical currents in the maternal-fetal bodies, we previously described the biomagnetic signals and characteristic waveforms associated with fetal hiccups and non-nutritive suck and the modulation of HR around these events (21, 22). Now we report a waveform seen in fetal biomagnetometry recordings that has a temporal frequency and duration consistent with fetal breathing movements. During contractions, the diaphragm is an electrically active region (23-26). We provide evidence that the biomagnetic signals characterized here are generated by activation of the fetal diaphragm, which we have termed the diaphragmatic magnetomyogram (dMMG). In an effort to assess whether respiratory-linked parasympathetic cardiac control could be observed *in utero*, we used the dMMG as a marker of fetal breathing and non-breathing epochs and then measured the effect of fetal breathing movements on time and frequency domain metrics of fetal HRV.

Methods

Study Population

Maternal-fetal data were part of a retrospective analysis of the records of pregnant women who participated in a longitudinal prenatal assessment involving simultaneous maternal-fetal magnetocardiogram (MCG) recordings. All protocols were approved by the University of Kansas Medical Center Human Subjects Committee and informed consent was obtained from each participant. Women were tested between 10:00 and 17:00 hours. Since this was a retrospective analysis of data collected during various protocols designed to characterize the biomagnetometer recordings, no specific instructions were given to women in respect to timing of meals prior to the recording. An ultrasound was obtained immediately prior to the MCG recording in order to document the position of the fetal body and note the presence or absence of fetal movements (breathing, mouthing or body movements).

Pregnancies with fetal cardiac arrhythmias, fetal growth retardation, twins, maternal gestational diabetes and maternal smoking were excluded from consideration. The aim of this retrospective study was to characterize the fetal dMMG and then to determine the effect

of fetal breathing movements on measures of HR and HRV. To achieve this aim, we limited the variables that have been shown to influence HR and HRV; fetal state and GA. In order to maximize our chances of observing breathing movements and to eliminate differences in HRV by comparing data from less mature fetuses, the analysis was limited to 36-38 weeks GA (n=88). While fetal breathing occurs in both active (2F, 4F) and quiet (1F, 3F) states, the chances of observing these movements are significantly higher in the active states (7). Also, fetal HR and HRV are significantly different between active and quiet states (27), therefore; only HR patterns associated with an active fetal state were inspected for breathing epochs and considered for further HR and HRV analysis (n=43). Since it is unlikely that the fetus would maintain a continuous state for the duration of the recording (18 minutes), fetal state was determined by visual inspection of each 6 minute IBI-time series by two independent investigators. If state determination differed between investigators, a third opinion was sought and consensus was reached.

All women whose recordings were part of this retrospective analysis delivered healthy term infants.

Data Acquisition

Biomagnetic signals were recorded using an investigational 83 channel dedicated fetal biomagnetometer (CTF Systems, Inc), housed in a magnetically shielded room. The axial gradiometer sensors are spatially distributed to cover the gravid maternal abdomen. Pregnant subjects were comfortably seated, slightly reclined and in contact with the surface of the biomagnetometer interface without applying pressure to the abdomen. The data were acquired in a continuous 18 minute recording using a 300 Hz sampling rate and recording filter of 0-75 Hz. Data were digitally filtered between 1 and 40 Hz offline (bidirectional fourth-order Butterworth filter). The raw data were divided into 3 consecutive 6 minute sections for independent component analysis (ICA).

Data Processing

The data in each sensor (channel) is a mixture of biomagnetic signals originating from various electrophysiological sources. The multivariate data were presented to an Infomax ICA algorithm implemented in EEGLAB toolbox (version 4.311) (28) in order to segregate the contributions from spatially distinct electrophysiological sources into individual components.

The ICA data were visually inspected for a clearly identifiable sinusoidal component with a temporal frequency consistent with fetal breathing movements. In every case, this component was ranked within the first 10 components. The duration (time onset and offset) of the sinusoidal activity was marked and only breathing epochs greater than or equal to 1 minute in duration were used in the analysis of HR and HRV. Non-breathing epochs were determined by selecting a region in the same component that immediately preceded or followed the breathing epoch that was as close to the duration of the breathing epoch as possible without exceeding it. The mean duration of breathing and non-breathing epochs used in the analysis was 104.1 seconds $\pm$ 41.8 and 92.3 seconds $\pm$ 22.6 respectively. Heart rate and HRV were determined from one breathing and one non-breathing epoch per fetus. Of the 43 records that qualified for analysis based on GA and activity state, 20 records met the criteria we established. The fetal MCG was identified and fiducial R-peaks were automatically detected using a template-matching algorithm described in detail in May, et. al (29).

We generated topographical magnetic field maps and power spectra of maternal and fetal MCG and fetal breathing components. From the IBI time-series, we generated a time-

frequency analysis implemented in EEGLAB 4.311 (28), modified to provide a specified time (6 minutes) and frequency resolution (0.0–1.7 Hz). The output from time-frequency analysis (power integral of defined frequency bands) was based on the work of David, et. al(20), using the following frequency bands: very low frequency (VLF) [0.02-0.08 Hz], low frequency (LF) [0.08-0.2 Hz], intermediate (Int) [0.2-0.4 Hz], high frequency [0.4-1.7 Hz] and total power (TP), [0.02-1.7 Hz].

To compare time-domain metrics during breathing and non-breathing periods, the fetal QRS component was imported into QRSTool and CMetX, a suite of freely available tools for transforming ECG/MCG data to metrics of HRV (30) (<http://www.psychofizz.org>). QRSTool provides a graphical user interface that allows extraction of the interbeat interval (IBI) series from the MCG data. CMetX calculates several metrics of cardiac chronotropy based on the imported IBIs (IBI values in milliseconds). The IBI series is first converted to an IBI time-series by linear interpolation at 10 Hz, resulting in a time-series that is appropriate for frequency analyses. Epochs comprising fetal breathing were analyzed separately from those comprising non-breathing. We used the following metrics from the CMetX output:

1. metrics of rate (mean IBI, mean HR) which are influenced by both parasympathetic and sympathetic activity
2. metrics summarizing total HRV influenced by both parasympathetic and sympathetic activity, which included:
 - a. the natural log of the variance in the IBI time series(log HRV)
 - b. the standard deviation of IBI series (SDNN, as NN is the acronym for “normal-to-normal intervals”)
 - c. the root mean square of successive differences between consecutive IBIs, (RMSSD)
 - d. mean absolute successive IBI difference (MSD)
3. a putative parasympathetic metric, the natural log of the variance in the band-limited IBI time-series(log RSA). The band-limited signal was selected to capture the breathing frequency of the neonate (0.4-1.7 Hz; (30), and following filtering of the IBI time series in this band, the natural log of the variance in this filtered time series yields the metric logRSA

In this small sample size of 20 subjects, normality was not assumed in the outcome measures and the differences between the fetal breathing and non-breathing periods were compared by the Wilcoxon signed-rank test. The distribution of some of the variables was skewed, even after log transformation. For those variables that were not skewed, the small sample size limits us from asserting that the distribution is symmetric, therefore, median and inter-quartile ranges are presented in Table 1. All significance values reflect the Wilcoxon signed-rank test.

Results

Descriptive Characterization of the Magnetic Signals Generated by Fetal Breathing Movements

An example of biomagnetometer channel data from a 36-week pregnancy is illustrated in Figure 1A. The data in each sensor (channel) is a mixture of biomagnetic signals originating from various maternal and fetal electrophysiological sources. Maternal and fetal MCG are recognizable in the raw signal. In addition, an oscillating baseline can be seen in channel MRK1 (maternal right, channel K1). After ICA decomposition (Figure 1B.), the components

that contribute to the maternal cardiac signal (1, 4), fetal cardiac signal (2, 3) and putative fetal breathing component (5) are separated. Component 5 (red) is superimposed over channel MRK1 to demonstrate that the oscillation seen in the raw data is identical in temporal frequency and phase. Next, topographical magnetic field maps of the components of interest are generated (Figure 1C.). The maps corresponding to independent components 1, 2, and 5 are consistent with the position of maternal heart, fetal heart and diaphragm, respectively. The map associated with component 5 has a dipolar pattern that is orthogonal in direction to the fetal cardiac component (IC-2), a finding we described previously in fetal hiccups, another signal with a diaphragmatic source (21). The power spectra for the MCG components are characteristic of their sources. Maternal and fetal MCG components have a more complex profile with multiple harmonics. Component 5 generates a single peak ~1.2 Hz, consistent with the temporal frequency of the oscillation seen in the waveform and consistent with the reported frequency of fetal breathing movements. In some cases, as seen with maternal and fetal cardiac components, the putative fetal breathing component was found in 2-3 independent components. After identifying the components consistent with fetal breathing movements, we removed any unrelated components (maternal-fetal cardiac, various artifacts) and reconstructed the signal in sensor (channel) space.

Example dMMG data from 4 fetuses are seen in Figure 2. Six minutes of continuous recording are shown for each fetus. The waveforms show periods of oscillations that are rhythmic and organized, lasting for nearly two minutes in some cases. Amplitude is variable, averaging approximately 200 femtoTesla.

A six minute recording from a 36 week GA fetus is shown in figure 3. The time-frequency analysis (top), compressed fetal dMMG (middle) and IBI time-series (bottom) are shown. The dMMG is decompressed into two, 180 second sections in panel B. At the beginning of the recording when there is no dMMG activity, power is concentrated in the lower frequency bands (<0.4 Hz). There is a spike at 108 seconds noted by the green asterisk. At that time, increased high frequency power between 0.5 and 1.0 Hz is observed in the time frequency plot. Low amplitude oscillations appear in the dMMG at 140 seconds and evolve into organized oscillatory activity with variable amplitude (blue asterisk) lasting approximately 145 seconds. This is followed by 13 seconds of no activity. Another short burst of activity lasting about 35 seconds is marked by the purple asterisk. Notice that power in the high frequency band corresponding to the rate of fetal breathing fluctuates and, where there are brief periods of apnea, high frequency power is diminished but not completely abolished. This is not unexpected in the fetus where episodic periods of breathing of variable temporal frequency are common. Similar to the findings of Hirsch, et. al.(14), it was not uncommon to observe increased high frequency power preceding the appearance of organized dMMG activity or the appearance of RSA in the IBI time-series as illustrated figure 3. During short bursts of breathing activity, changes in power are less clear. This would limit the sole use of the time-frequency analysis as a way to estimate vagal control during fetal breathing, as suggested by David, et. al. because the actual onset of breathing activity may not be appreciated if it is preceded by high frequency power. For example, including the data between the green and blue asterisks in Figure 3 based on the increased high frequency power alone would have included approximately 30 seconds of apneic activity as evidenced by the dMMG. Similarly, selected periods of high frequency activity could include bursts of breathing activity bounded by apneic periods. In contrast, the observation of RSA in the IBI time-series was more consistently linked with the onset and offset of fetal breathing movements.

Forty-five seconds of fetal MCG, dMMG and the corresponding IBI time-series are shown in Figure 4. During fetal breathing activity, RSA is observed in the IBI time-series. Fetal HR is lower during breathing movements and begins to accelerate about 5 seconds prior to the

offset of fetal breathing. HR is higher and no RSA is seen during the apneic period when no activity is seen in the dMMG. For comparison, the dMMG during paced (~1 Hz), maximal activation of the diaphragm in an adult, non-pregnant subject is also shown. This is to demonstrate the ability to record the dMMG in a compliant adult and to show the concurrent RSA in the IBI time-series. Using the dMMG as a marker, we next compared time and frequency domain metrics of fetal HRV during breathing and non-breathing periods.

Statistical Comparison of Fetal Heart Rate Variability during Breathing and Non-Breathing Epochs

Metrics of Rate—Fetal HR was significantly lower during breathing epochs ($p=0.0001$) and accordingly, the IBI was longer ($p=0.0001$). Fetal HR ranged from 115 to 158 during breathing epochs and from 122 to 171 during non-breathing epochs.

Metrics Summarizing Total HRV—There was no significant difference in overall HRV between breathing and non-breathing epochs. (SDNN: $p=0.08$, Log HRV: $p=0.08$). Short-term variability (RMSSD, MSD) was significantly higher during breathing epochs (RMSSD $p=0.006$, MSD, $p=0.0007$). Since short-term HRV is inversely correlated with HR (14) this outcome is not unexpected.

Parasympathetic Metric—Respiratory-linked variability in heart rate (logRSA) was significantly higher during breathing epochs (LogRSA, $p=0.0021$), consistent with increased parasympathetic input during breathing movements.

Frequency Domain Metrics—There were no significant differences between breathing and non-breathing epochs were found for total power, or power in the VLF, LF or Int frequency bands or VLF/LF ratio. As predicted, however, there was a significant increase in HF power (<0.0001) during breathing epochs. Moreover, the ratios of VLF/HF and LF/HF were significantly higher (0.0007, 0.004) during breathing epochs suggesting greater sympathovagal balance during fetal breathing movements. Similar to the report of David, et. al., we found the distribution of power was greatest in the VLF and LF bands. We also observed what David, et. al. described as a “power gap” between 0.2-0.4 Hz. Frequency in this band was not influenced by fetal breathing movements. Groome, et. al., found that HF power (0.2-2.5 Hz) and “RSA” which they defined as power between 0.4-1.0 Hz, accounted for approximately 20% of the total power (17). We found that mean HF power (0.4-1.7 Hz) accounted for 21.5% of the total power during fetal breathing epochs compared to 7.1% during non-breathing epochs.

Discussion

As part of a retrospective analysis of data recorded with a dedicated fetal biomagnetometer, we describe a waveform seen in the sensor raw data and ICA components that has a temporal frequency, duration, spatial location and episodic activity pattern consistent with fetal diaphragmatic movements associated with *in utero* breathing activity. This waveform, along with the concomitant presence of RSA and increased high frequency power leads us to conclude that the observed signal is the magnetic correlate of the diaphragmatic EMG, the dMMG.

Using the dMMG as a marker, we isolated epochs of breathing and non-breathing activity and compared metrics of fetal HR and HRV in the active fetal state between 36-38 weeks GA. Fetal HR was significantly lower during breathing epochs. Short-term HRV (RMSSD, MSD), a metric of respiratory-related HRV that putatively reflects parasympathetic influences (Log RSA), and HFpower and ratios describing sympathovagal balance (VLF/

HF, LF/HF) were all significantly higher during breathing epochs. There were no differences in metrics that summarized total HRV or power in VLF, LF or INT frequency bands or total power.

Possible Sources of the dMMG Signal

The biomagnetometer detects magnetic fields generated by electrically active tissue. Fetal tissues which have been shown thus far to contribute to biomagnetic recordings are heart (31), muscle (21, 22) and brain (32). While intercostal muscles contribute primarily during inspiration in adults, the fetus and newborn have poorly developed intercostal muscles and breathing is essentially diaphragmatic. (33). The human fetal dMMG we have characterized here shows strong similarity to the morphology of the diaphragmatic EMG in chronically prepared near-term fetal lambs (19). Furthermore, the observation of RSA in the IBI-time series and lower fetal HR during breathing activity is identical to that shown in chronically instrumented fetal baboons (18). The rhythmic movement of abdominal muscles in concert with fetal breathing movements could potentially produce a signal, although one would expect the electrically active diaphragm to make a greater contribution to the biomagnetic response as seen in the adult recording during maximal diaphragmatic activation. The topographical map of the fetal dMMG we describe here is consistent with the spatial location of the fetal diaphragm and orthogonal to the fetal cardiac map, similar to our previous report of fetal hiccup activity (21). The temporal frequency, periodic nature, spatial location and influence on cardiac autonomic control effectively rule out any other electrically active source of fetal activity other than breathing movements. Fetal non-nutritive suck activity has a shorter burst duration with 6-12 cycles per burst and a temporal frequency of approximately 3 Hz (22) which results in a slow, oscillatory fetal HR pattern consistent with the 1F state (34). Contrary to fetal breathing movements, fetal HR increases during non-nutritive suck (22), consistent with reports in full term newborns during simultaneous recordings of sucking and HR (35). It is possible that rolling eye movements would generate a detectable signal since the eye is an electrically active moving dipole, however, rolling eye movements characteristic during eye lid closure are slower and differ in spatial location; moreover, they would not be expected to be linked to fetal RSA (36). Maternal uterine contractions generate a magnetomyogram (37) with a temporal frequency that could potentially overlap that of fetal breathing (0.34-1.0 Hz) (38), however, uterine contractions would not be expected to induce fetal RSA. While maternal breathing and the subsequent diaphragmatic activity is seen in the unfiltered raw data during the recording (data not shown), the maternal diaphragm is located superior to the sensor array and the continuous breathing pattern, lower temporal frequency and spatial location of the electrically active maternal diaphragm is not consistent with the fetal dMMG. Finally, since this writing, the biomagnetic signature of human fetal breathing movement was confirmed by Ulusar, et. al., using a dedicated fetal biomagnetometer with simultaneous ultrasound [39]. The characteristic waveform, spatial location and periodic activity described are identical to our findings. These findings strengthen our assertion that the waveform we observed in both the raw data and ICA components and characterized as fetal dMMG is generated by fetal breathing movements.

Limitations of the Present Sample

We imposed stringent criteria for this retrospective analysis by limiting the GA to 36-38 weeks, activity state to 2F or 4F and duration of breathing and non-breathing epochs to 1 minute or greater. This reduced the number of datasets available for analysis but also reduced potential differences that might be influenced by fetal maturation or state so that we might better understand how fetal breathing movements influence the autonomic control of heart rate and variability. The biomagnetometer is sensitive to the position and distance of the magnetic field of interest therefore; successful recording of the fetal dMMG is

influenced by fetal size, fetal trunk movements, timing of maternal meals and maternal obesity. These factors could limit the ability to acquire quality dMMG recordings. We plan to continue these investigations at earlier developmental time points when fetal behavioral states begin to emerge and increase the duration of our recording time to maximize the likelihood of capturing fetal breathing activity.

Implications

There is increasing awareness that an adverse *in utero* environment can have long-term consequences on adult health (40). Significant differences in fetal breathing patterns have been reported in women with pregnancy induced diabetes (41), a condition that places the offspring at greater risk for development of childhood obesity, insulin resistance, diabetes and cardiovascular disease (42). This technology may be used to study the longitudinal development of fetal cardiac autonomic control and breathing activity especially as it relates to maternal nutrition and exercise during pregnancy (29). Moreover, in neonates, respiratory-linked and vagally-mediated HRV has been shown to provide a continuous index of the severity of neuropathology (43).

To our knowledge, this is the first report of human fetal dMMG with cardiac metrics of rate and variability during breathing and non-breathing epochs. Fetal biomagnetometry is a non-invasive technology that, when combined with advanced techniques of signal-interference separation, may contribute substantially to the investigation of fetal neurophysiologic development. We were able to detect significant differences in cardiac metrics of rate and variability during breathing and non-breathing epochs in the late-term human fetus using the dMMG as a marker. This approach can extend the investigation of neuropathology to the prenatal period.

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Figure 1. Biomagnetometry raw data, ICA decomposition and Magnetic Field Maps with Corresponding Power Spectrum

Ten seconds of channel data (A.), the ICA decomposition (B.) and topographical magnetic field maps with their respective power spectra (C.) are shown. In figure 1A., the raw data are shown with R-peaks marked by colored triangles. The fetal MCG (blue) can be seen imbedded in the maternal MCG (green). Note the undulating baseline in channel MRK1. In section B., the individual ICA components are displayed. Maternal (green) QRS is seen in components 1 and 4. Fetal QRS (blue) is seen in components 2 and 3. The fetal breathing component (red) is in component 5. The breathing component was superimposed over the same time period in raw data (Panel A) to demonstrate that the undulating baseline seen in channel MRK1 is the waveform generated by fetal breathing movement. In section C., the magnetic field maps of the primary components of interest are shown. The magnetic field distribution for the maternal cardiac component is typical of the maternal heart positioned above the sensor array. The fetal cardiac component map shows a dipolar source. The power spectra for both cardiac components are complex with multiple harmonics. The map for the fetal diaphragm is dipolar and orthogonal to the fetal cardiac map. The power spectrum for this component has a single peak consistent with the temporal frequency (~ 1.2 Hz) in component 7.

Figure 2. Four examples of fetal dMMG

Each section represents 360 seconds of dMMG in 4 individual cases to demonstrate the various morphologies and patterns of fetal dMMG activity. The first section shows data from a 38 week fetus, the remaining 3 blocks are from 36 week fetuses.

Figure 3. Time-frequency analysis, fetal dMMG and IBI time-series

Time (X-axis, 0-360 seconds) by frequency (Y-axis, 0.02-1.7 Hz) is shown in the top panel. Power (dB) is represented on the scale to the right. The fetal dMMG is compressed in the middle panel for the 360second (6 min) period and decompressed in the lower panel so that the specific waveforms can be compared to the compressed sample. Colored asterisks mark periods noted in the text to allow comparison of the dMMG between the compressed and decompressed samples. During periods of fetal breathing (oscillations in the dMMG), power increases in the band between 0.4 and 1.7 Hz. Concurrent with the increased high-frequency power is the presence of fetal RSA seen in the IBI time-series (expanded in the inset).

Figure 4. Fetal and Adult MCG, dMMG and Concurrent IBI Time-series

The fetal MCG is shown in the top panel (left). The bottom panel shows the IBI time-series (green) between 45-90 seconds. Fetal dMMG (red) is plotted above the IBI time-series to show the presence of fetal RSA during the breathing epoch. At 65 seconds, fetal heart rate begins to accelerate and the breathing epoch ends at ~72 seconds. RSA is not seen in the IBI time-series after 72 seconds. For comparison, the right panel demonstrates a paced breathing exercise in an adult, non-pregnant subject. In this case, the dMMG (red) is shown below the IBI time-series (green) and the resulting RSA is seen in the IBI time-series.

Table 1

Comparison of fetal cardiac metrics during breathing and non-breathing epochs.

Variable	Breathing Epoch			Non-Breathing Epoch			p-value
	Q1	Median	Q3	Q1	Median	Q3	
IBI (ms)	425.5	437	462.9	383.45	418.75	433.85	0.0001
HR (bpm)	130	138	141	139	143.5	156.5	0.0001
SDNN	8.4	9.9	17.8	10.7	18.0	22.9	0.08
RMSSD	6.3	8.2	9.95	3.65	5.6	7.85	0.006
Log HRV	4.2	4.65	5.7	4.70	5.8	6.2	0.09
MSD	4.85	6.5	7.9	2.55	3.8	5.65	0.0007
Log RSA	1.90	2.25	2.75	0.85	1.25	1.85	0.0021
VLF	1.65	3.63	6.08	2.04	4.91	7.67	0.33
LF	0.77	1.42	2.24	0.67	1.39	3.02	0.81
Int	0.25	0.33	0.44	0.12	0.35	0.54	0.31
HF	0.95	1.31	2.23	0.33	0.63	0.98	< 0.0001
VLF/LF	1.18	2.75	4.20	1.81	2.94	4.51	0.28
VLF/HF	1.08	2.75	4.34	4.42	6.70	9.61	0.0007
LF/HF	0.55	0.98	1.74	1.57	2.23	3.51	0.004
Total power	4.03	7.44	10.55	3.25	7.44	13.23	0.70

Note: Measures include time domain assessments of rate (IBI=Mean Interbeat Interval; HR=Mean Heart Rate), total variability (SDNN = Standard Deviation of IBIs; RMSSD = Root mean Square of Differences between IBIs; (MSD = Mean of absolute value of consecutive IBI differences; Log HRV = Natural Log of variance of IBI time series), an estimate of parasympathetically-controlled variability Log RSA = Natural Log of variance of filtered (0.4-1.7 Hz) IBI time series). Frequency domain measures of band-specific power (integrals in bpm²) include very low frequency (VLF) [0.02-0.08 Hz], low frequency (LF) [0.08-0.2 Hz], intermediate (Int) [0.2-0.4 Hz], high frequency (0.4-1.7 Hz).