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A Feasibility Study of Heart Rate Variability Biofeedback on Maternal Baroreceptor and Fetal Movement

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Abstract

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By Gershom T. Lazarus

The human baroreceptor response is crucial in maintaining homeostasis. During human pregnancy, baroreceptor functioning is attenuated, and this can lead to maternal hypertension and preeclampsia with implications for fetal development. There is limited research on the use of biofeedback to activate the baroreceptor reflex in pregnant women. The feasibility of using heart rate variability (HRV) biofeedback in a pregnant population to activate the maternal baroreceptor was investigated using an ABAB'A' design. The results indicated activation of the maternal baroreceptor that was associated with implementation of both the 1st and 2nd biofeedback. While there were initial increases in maternal HRV in association with the 1st biofeedback, the 2nd biofeedback was not associated with increased HRV. The overall fetal findings did not reveal any significant changes in fetal movement. Individual analysis of the fetal data revealed high variability of fetal movement changes that requires further investigation and currently there is no conclusive evidence to recommend the use of HRV biofeedback to target fetal movement changes. The results indicate the feasibility of using HRV biofeedback in a pregnant population to activate the maternal baroreceptor and the next step is to repeat this study in different pregnant populations.

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Introduction

Present Study Overview

The current study aims to test the efficacy of heart rate variability biofeedback as a manipulation to regulate short-term changes in the maternal autonomic nervous system function and fetal movements. Maternal shifts in homeostasis fluctuate according to the demands of pregnancy. During pregnancy normally adaptive autonomic and cardiovascular shifts in homeostatic control can become compromised by clinical factors that lead to maladaptive outcomes. A critical element in this cascade of physiologic and autonomic changes is the baroreceptor response. The baroreceptor response is a pressure sensitive signaling mechanism that helps optimize energy expenditure and metabolic efficiency. Baroreceptor attenuation has been linked to maternal hypertension and preeclampsia that can negatively impact fetal development (Brooks, Dampney, & Heesch, 2010; Lakhno, 2017). Interventions aimed at optimizing maternal energy expenditure and overall metabolic efficiency, such as *baroreceptor training* can attenuate or reverse the maladaptive pathways leading to maternal hypertension and its consequences.

Pregnancy affords an opportunity to observe how human physiology is altered to accommodate a growing fetus. Thus, physiologic adaptations provide a window into these regulatory systems which, under normal homeostatic loads, promote species survival but also include events that can lead to maternal and fetal death. Maternal heart rate variability biofeedback may help to activate the baroreceptor reflex via inducing an optimal respiratory rate. In addition, this activation should also increase heart rate variability, commonly known as beat-to-beat variability. Thus, baroreceptor activation at the optimal respiratory rate should optimize homeostasis, in part by increasing the efficiency of the autonomic nervous system. This hypothetical model, if supported by empirical study, should have beneficial effects on the growing fetus.

The maternal autonomic nervous system (ANS) can be viewed within the context of an integrator and regulator of information from various physiological systems that include the respiratory system, immune system, endocrine system, as well as cognitive areas (Cooke et al., 1998; Porges, 2007). The ANS has two main branches, the parasympathetic (PNS) and sympathetic. The resulting outflow of autonomic activity can be indexed by short-term heart rate

variability (HRV) or more commonly beat-to-beat variability (Shaffer & Ginsberg, 2017). Changes in short-term HRV can be attributed to two main sources: (1) the interplay between sympathetic and parasympathetic branches of the ANS and (2) the pathways that control heart rate through baroreceptors, respiratory sinus arrhythmia and vagal tone (Shaffer & Ginsberg, 2017). Baroreceptors form a key component in the regulation of heart rate that includes a feedback loop incorporating initial changes in blood pressure, neural integration of information from various physiological and neural areas, outflow of ANS via vagal efferent pathways, and heart rate changes to reset blood pressure. Given the importance of the maternal ANS as a diagnostic indicator as well as a key component of physiological regulation, an intervention to target optimizing ANS balance was selected. Specifically, based on the crucial role of HRV and the baroreceptor pathways, the intervention selected (HRV Biofeedback) was designed to target maternal baroreceptor activation and increases in short-term HRV (Shaffer & Ginsberg, 2017). Downstream from this cascade of regulatory metabolic processes within the pregnant mother are the possible effects of optimizing maternal ANS function on fetal viability and behavioral responses.

Measuring fetal behavior, in the form of overall general body movements, is a non-invasive index of neurological development (Hadders-Algra, 2007) with postnatal prognostic value in the domains of infant motor and temperament development (DiPietro et al., 2002). Changes in fetal general movements parallel developmental changes in the fetus, specifically neurological and autonomic nervous system changes. Activation of fetal neuro-motor pathways (initially from the subplate and later from the midbrain) sub-serves changes in fetal movement (Hadders-Algra, 2007; Lüchinger, Hadders-Algra, Van Kan, & de Vries, 2008; de Graaf-Peters, De Groot-Hornstra, Dirks, & Hadders-Algra, 2006; de Graaf-Peaters & Hadders-Algra, 2006; Hadders-Algra, 2007). Controlled interventions that produce changes in maternal homeostasis may also produce changes in fetal movement which reflect activation of fetal neuro-motor pathways.

The current project is therefore a feasibility study with two main aims: (1) To investigate the main effects of heart rate variability (HRV) biofeedback on maternal autonomic nervous system activity by measuring baroreceptor activation and increases in short-term HRV (SDNN) and (2) To investigate the effect of HRV biofeedback on short-term changes in fetal general

movements. The goal of the present study is to extend the literature on the effects of a maternal manipulation at both the maternal level (physiological) and fetal level (fetal behavior/development).

Review of Literature

Physiological changes during pregnancy (baroreceptor and heart rate variability).

This review will be restricted to activation of the baroreceptor response and the resulting changes in heart rate variability as important components in any methodology designed to optimize maternal autonomic tone and ideally, fetal responsiveness. During pregnancy there are a number of physiological shifts that occur as a compensatory response to the increased demands associated with pregnancy. The cardiovascular and autonomic nervous systems have noted increases in blood volume of approximately 1.5 liters and decreases in heart rate variability and baroreceptor sensitivity during pregnancy (Chandra, Tripathi, Mishra, Amzarul, & Vaish, 2012; Stein et al., 1999; Silver, Tahvanainen, Kuusela, & Eckberg, 2001). Pregnancy-induced hypertension was associated with reduced HRV (Flood et al., 2015). Changes in short-term HRV can be influenced by the pathways that control heart rate through baroreceptors, respiratory sinus arrhythmia, and vagal tone (Shaffer & Ginsberg, 2017). The current study will focus on testing the feasibility of HRV biofeedback to activate the maternal baroreceptor and increase maternal short-term HRV (SDNN).

HRV is primarily determined by extrinsic regulation of heart rate and has been defined as "the variation over time of the period between consecutive heartbeats" (Acharya, Joseph, Kannathal, Lim, & Suri, 2007). Heart rate examined over time (for example, when referring to an average heart rate of 60 beats per minute) does not factor into account the irregular beating of the heart at a physiological level, that is apparent when analyzing the time periods between each individual heart beat (ventricular systole). Heart rate variability has been implicated in widespread regulatory functions including neurocognitive (attention) (Ramirez, Ortega, & Del Paso, 2015), psychological (emotion regulation) (Francis, Penglis, & McDonald, 2016), and physiological (ANS balance) (Thayer & Brosschot, 2005). Heart rate variability biofeedback has demonstrated improvements in clinical and non-clinical (sports performance) (Paul & Garg, 2012) populations associated with the areas of functioning noted above. Overall, research suggests the use of HRV as an index of autonomic balance. However, heart rate variability biofeedback has not been extensively investigated during pregnancy. During pregnancy there

are various physiological adaptations occurring including decreased heart rate variability and attenuation of the baroreceptor (Silver, Tahvanainen, Kuusela, & Eckberg, 2001). While these changes provide homeostatic compensation for the additional demands of pregnancy on maternal physiological systems, the physiological shifts can also become maladaptive, for example, baroreceptor attenuation can lead to maternal hypertension and preeclampsia with implications for fetal development (Silver, Tahvanainen, Kuusela, & Eckberg, 2001; Lakhno, 2017). Therefore, investigating possible methods of increasing baroreceptor activation may mitigate some of these maladaptive physiological changes during pregnancy.

Baroreceptors are mechanosensors that functions in a closed loop system between the brain and the ANS to stimulate an increase or decrease in heart rate as a response to blood pressure changes (Prinsloo, Rauch, & Derman, 2014) and subsequently affect short-term heart rate variability (Shaffer & Ginsberg, 2017). Activity of the baroreceptor is crucial in regulating heart rate within an optimum range.

Baroreflex gain (baroreflex sensitivity), which refers to the efficiency of the baroreceptor system to regulate blood pressure, decreases during pregnancy and is further decreased in pathological conditions, for example, pre-eclampsia and gestational hypertension (Silver, Tahvanainen, Kuusela, & Eckberg, 2001; Lakhno, 2017). Approximately 2-8% of pregnant women are affected by preeclampsia. Preeclampsia has negative long-term consequences on the mother, such as the development of cardiovascular disease and stroke post-birth, as well as fetal preterm delivery and mortality (Amaral, Cunningham, Cornelius, & LaMarca, 2015). Even though baroreflex gain can be viewed as a typical physiological shift during pregnancy, the cross-over from normal blood pressure to the pathological range can occur relatively quickly (Silver, Tahvanainen, Kuusela, & Eckberg, 2001). In a study of pre-eclampsia, fetal autonomic control was positively correlated with maternal autonomic balance (Lakhno, 2017). Furthermore, fetal sympathetic activity was positively correlated and, fetal parasympathetic activity negatively correlated, with maternal pre-eclampsia severity. Mothers were treated pharmacologically for the pre-eclampsia, however this intervention had no effect in restoring ANS balance in the mother or fetus (Lakhno, 2017).

In addition to the subsequent negative effects of these clinical conditions in the mother, there are downstream maladaptive consequences for fetal development. Maternal pre-eclampsia has been associated with impairment of fetal sympathetic nervous system development (Lakhno,

2017). Currently, there are very few interventions to treat or prevent preeclampsia patients. In cases of mild preeclampsia, mothers are advised to get bed rest to lower the blood pressure and increase the blood flow to the placenta. In cases of severe preeclampsia, treatment includes intravenous medication to control maternal blood pressure and prevent seizures and other associated symptoms. The anticonvulsive medication used is magnesium sulfate, which helps prevent seizures. Steroid injections (corticosteroids) are also used to facilitate development of fetal lungs. It is important to stimulate development of the fetal lungs before the delivery in early-onset preeclampsia, due to high prevalence of preterm birth (NIH, 2018). Interventions with the potential for affecting the maternal cardiovascular system and enhancing maternal baroreceptor functioning during pregnancy necessitate more thorough research.

The limited treatment options in maternal ANS pathologies, suggest the need for development of more non-traditional, non-invasive interventions in this population that targets physiological pathways (baroreceptor pathway) with implications for affecting the maternal ANS. Heart rate variability (HRV) biofeedback was selected for this study because one of the primary mechanisms of action reported in the literature (Lehrer & Gevirtz, 2014) is through activation of the baroreceptor with subsequent impact on maternal short-term HRV (SDNN).

Relationship between fetal movements and maternal ANS sensitivity. Improvements in baroreceptor efficiency and increased HRV have been linked to a more optimally balanced ANS response in various clinical and non-clinical populations such as emotion regulation (Francis, Penglis, & McDonald, 2016) and sports performance (Paul & Garg, 2012). Within the context of pregnancy, changes in maternal physiological state has been linked to fetal cardiac and behavioral (movement) changes (DiPietro, Irizarry, Costigan, & Gurewitsch, 2004; Lakhno, 2017). Furthermore, there is evidence of a bi-directional maternal-fetal relationship with fetal movement preceding changes in maternal physiology (DiPietro, Irizarry, Costigan, & Gurewitsch, 2004). While there is no definitive evidence as to the specific pathways linking maternal state to fetal behavior, it has been proposed that immediate shifts in fetal movement are most likely the result of a fetal orienting response (in response to the auditory stimuli of changes in maternal HR) followed by the changes in levels of quicker acting maternal and fetal hormones (for example, maternal epinephrine and fetal noradrenaline and ß-endorphin) (DiPietro, Irizarry, Costigan, & Gurewitsch, 2004). Persisting changes in fetal movement may be maintained by

longer acting maternal hormonal changes (for example, cortisol) and continued fetal hormone release.

Given the evidence for fetal changes occurring through maternal stimuli, it is plausible that an intervention such as HRV biofeedback, with a mechanism of action that works through the maternal cardiorespiratory and ANS, can impact fetal movement. Fetal movements develop in conjunction with overall fetal neurological development. Fetal brain development is characterized by the interaction between transient and permanent neural structures throughout the fetal gestational period. Sensory stimulation is crucial in shaping cerebral structures and functional connections instigated by genetic factors. Fetal neuro-motor pathways are strengthened by exposure to stimuli and, utilizing the evidence of maternal-fetal synchrony, maternal interventions such as HRV biofeedback can be viewed as stimuli that can impact fetal neuro-motor pathway (neurological) development.

Heart Rate Variability Biofeedback. Biofeedback describes a collection of therapies, which provide a visual representation of an individual's underlying physiological indices. The addition of a visual stimulus is designed to help the patient bring into awareness underlying physiological processes with the goal of exerting control over these processes (Prinsloo, Rauch, & Derman, 2014). The basic mechanism of biofeedback may be reduced to a model of operant conditioning. The monitor provides information to the patient regarding the effects of their actions on physiological measures. This data results in feedback learning. When the patient is successful in influencing their physiological responses, they are positively reinforced by visual stimuli and continue the behavior that originally resulted in the desired effect (Frank, Khorshid, Kiffer, Moravec, & McKee, 2010). Heart rate variability (HRV) biofeedback was selected for use in this study owing to its specific mechanisms of action that target the autonomic nervous system.

Mechanisms of Action in HRV Biofeedback. The mechanisms of action in biofeedback extend beyond simply operant conditioning noted above and varies from one type of biofeedback to another. Research indicates that one mechanism of action of heart rate variability biofeedback is through activation of the baroreceptor (Lehrer et al., 2003). HRV biofeedback utilizes a breathing rate of 6 breaths per minute to stimulate the baroreceptor system with subsequent increases in short-term HRV (Lehrer & Gevirtz, 2014). Research investigating HRV

biofeedback and outcomes of HRV (SDNN) suggest improvements in overall HRV in healthy individuals (Lehrer et al., 2003), as well as individuals with asthma (Lehrer et al., 2004), fibromyalgia (Hassett et al., 2007), coronary artery disease (Del Pozo, Gevirtz, Scher, & Guarneri, 2004), major depressive disorder (Karavidas et al., 2007), and PTSD (Zucker, Samuelson, Muench, Greenburg, & Gevirtz, 2009). Long-term improvements in HRV were noted at 3 months' follow-up in individuals with coronary artery disease (Del Pozo, Gevirtz, Scher, & Guarneri, 2004). The literature review revealed no studies that investigated the use of HRV biofeedback on maternal HRV during pregnancy. One study showed increased HRV at 1-month post-partum in mothers who practiced HRV biofeedback after post-partum day 4 compared to a control group (Kudo, Shinohara, & Kodama, 2014).

Baroreceptors detect the pressure changes in blood flow and adjust the heart rate accordingly to within a set heart rate range. For example, high pressure in blood flow, stimulates the baroreceptors to send a message to the cardiovascular centers in the medulla to decrease heart rate. Breathing at 6 breaths per minute stimulates the baroreceptors to maximize baroreflex gain (Lehrer et al., 2003; Lehrer & Gevirtz, 2014). One method of inferring changes in baroreceptor activation/training in an individual is to analyze the frequencies of the power spectrum of the nervous system. Different areas of the nervous system operate at different speeds resulting in different frequencies and therefore an analysis of peak power at the different frequency ranges provide an indication as to the level of activation of the different components of the nervous system. The high frequency band is typically associated with activity of the parasympathetic nervous system (0.15-0.50 Hz) and the low frequency band (0.04-0.15 Hz) is associated with both sympathetic and parasympathetic influences (Xhyheri, Manfrini, Mazzolini, Pizzi, & Burgiardini, 2012). The beat-to-beat changes in heart rate discussed above (short term HRV) occur in a timeframe of milliseconds and are therefore largely reflected by parasympathetic nervous activity (specifically the vagal efferent nerve) responding significantly quicker (< 1 second) than sympathetic nervous activity (>5 seconds) (Nunan, Sandercock, & Brodie, 2010). Increased power within a specific frequency range suggests that the corresponding components of the nervous system are activated during this period and power is positively correlated with level of activation/training (McCraty & Shaffer, 2015; Lehrer & Gevirtz, 2014). The power spectrum provides a visualization of the degree to which each component of the nervous system (frequency range) is activated within a specified time (Figure 1) (McCraty & Shaffer, 2015).

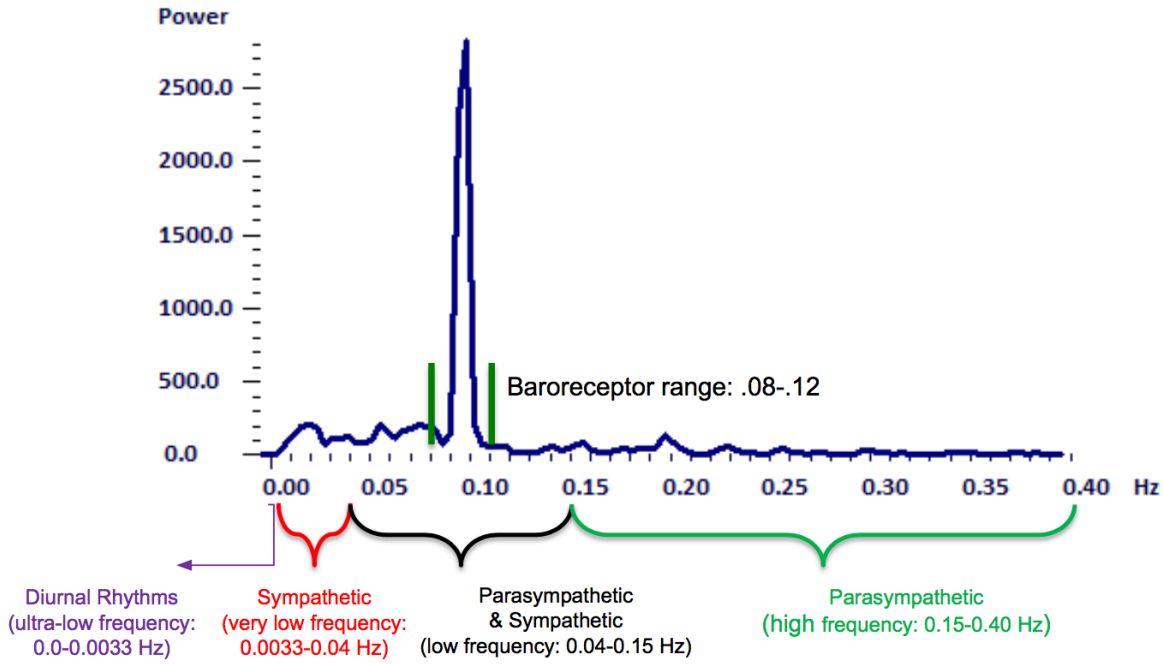

Figure 1. Components of the HRV power spectrum.

Research indicates that the baroreceptor frequency range can vary from 0.075-0.108 Hz (4.5-6.5 BPM) and typically, HRV biofeedback intervention studies test individuals' baroreceptor resonance frequencies within this range (Vaschillo, Vaschillo, & Lehrer, 2006). During pregnancy, there are a number of physiological shifts that occur as a compensatory response to the increased demands associated with pregnancy. The cardiovascular and autonomic nervous systems have noted increases in blood volume of approximately 1.5 liters, and decreases in heart rate variability and baroreceptor sensitivity, respectively, during pregnancy (Chandra, Tripathi, Mishra, Amzarul, & Vaish, 2012; Stein et al., 1999; Silver, Tahvanainen, Kuusela, & Eckberg, 2001). There is no published research on what the baroreceptor resonance frequency range could be due to these physiological changes during pregnancy. Based on prior HRV biofeedback research I set the baroreceptor frequency range at 0.08-0.12 Hz (4.8-7.2 breaths per minute) (*participants that met final selection criteria for data analysis actually fell in a narrower range of 0.09-0.10, which also meets the range set by Vaschillo, Vaschillo, & Lehrer, 2006 noted above*). Therefore, those individuals that displayed a peak in the power spectrum in the range of 0.08-0.12 Hz (4.8-7.2 breaths per minute) were

considered as activating their baroreceptors during biofeedback, with higher peak power indicative of greater baroreceptor activation.

During biofeedback training it is common to see a prominent high-amplitude peak at around 0.10 Hz in the power spectral analysis suggesting activation of the baroreceptor and improvement in baroreflex gain, which refers to the efficiency of the baroreceptor system. The presence of this high-amplitude peak, as well as an increase in the LFHF ratio due to the shift to the range, provides an indication of baroreceptor activation (Lehrer & Gevirtz, 2014). An increase in baroreceptor gain indicates improved efficacy of baroreceptor functioning. An improvement in baroreceptor functioning suggests improved balance in the parasympathetic and sympathetic arms of the ANS, specifically focused on medullary input to the cardiovascular system (Lehrer & Gevirtz, 2014). Baroreflex gain is normally reduced during pregnancy and is further decreased in individuals with hypertension disorders of pregnancy (Silver, Tahvanainen, Kuusela, & Eckberg, 2001). HRV biofeedback, which stimulates the baroreceptors, has been shown to lower blood pressure in hypertensive patients (Gevirtz, 2013). Lehrer et al. (2003) revealed significant acute and chronic changes in baroreflex gain in a group of healthy adults using HRV biofeedback over 10 sessions. During the biofeedback sessions, baroreflex gain increased during the biofeedback procedure compared to baseline and post-biofeedback. Baseline baroreflex gain was elevated at session 10 compared to session 1, indicating long-term changes in baroreflex gain with the use of HRV biofeedback (Lehrer et al., 2003).

The effect on baroreceptor activation suggests that HRV biofeedback may have the potential to reduce baroreceptor attenuation during pregnancy. However, this intervention has not been tested extensively in a pregnant population and the potential impact on maternal baroreceptors has not been investigated. A decrease in baroreceptor attenuation may have possible long-term effects on pathologies such as preeclampsia (hypertension). In addition to the subsequent negative effects of these clinical conditions in the mother, there are downstream maladaptive consequences for fetal development. Maternal pre-eclampsia has been associated with impairment of fetal sympathetic nervous system development (Lakhno, 2017). Any intervention that demonstrates possible improvements in maternal baroreceptor functioning during pregnancy necessitates more thorough research.

Maternal "adaptive" interventions affecting change in maternal baroreceptor, SDNN or fetal behavior. The main aims of the current study are to test the feasibility of HRV biofeedback to target the activation of the maternal baroreceptor, enhancing maternal short-tern HRV (SDNN), and impacting the trajectory of fetal general movements. Research on interventions aimed at enhancing the maternal ANS (specifically focusing on the baroreceptor and short-term HRV) and fetal behavior (specifically focusing on general movements) is outlined below.

Research strongly motivates for the use of exercise as a non-invasive tool to manipulate cardiovascular indicators with positive health outcomes. The benefits of exercise on autonomic control have also been explored in pregnant populations. Throughout gestation, pregnant mothers who exercised regularly demonstrated higher maternal HRV (both SDNN and RMSSD) than mothers who did not exercise (May et al., 2016). HRV has been established as an indicator of autonomic balance and overall optimal health with higher HRV associated with more context-appropriate emotional responses in tests utilizing startle responses and self-reported emotional responses (Melzig, Weike, Hamm, & Thayer, 2009; c).

Gebuza, Dombrowska, Kaźmierczak, Gierszewska, & Mieczkowska (2017) demonstrated differential fetal responding to two types of classical music played to the mother. Music by Mozart and Strauss increased the number of fetal movements and short-term HRV compared to baseline in third trimester fetuses. DiPietro, Costigan, Nelson, & Laudenslager (2008) demonstrated the use of guided imagery relaxation in mitigating maternal stress response variables and enhancing autonomic balance. The positive maternal changes were associated with adaptive changes in fetal developmental indicators. This 18-minute guided imagery maternal relaxation intervention at 32 weeks' gestation was associated with decreased maternal levels of psychological tension, heart rate, skin conductance, respiration, and cortisol and increased respiratory sinus arrhythmia. The relaxation intervention was also associated with increased levels of coupling of fetal movement and fetal heart rate and decreased levels of fetal movement amplitude and fetal heart rate. Furthermore, fetal heart rate variability showed a linear increase from baseline to recovery following the maternal relaxation intervention (DiPietro, Costigan, Nelson, Gurewitsch, & Laudenslager, 2008).

HRV biofeedback was administered in a sample of women (N= 31) who were referred for severe anxiety and/or depression either during pregnancy or one-year post-pregnancy. Two

independent HRV biofeedback sessions (from 30 minutes to 1 hour) were administered. There were significant decreases in self-reported state anxiety and increases in self-reported quality of life and well-being when comparing pre and post scores for each biofeedback session (Beckham, Greene, & Meltzer-Brody, 2013). Another study reported that HRV biofeedback administered at postpartum day 4 at the hospital, and continued daily at home by the mothers, was associated with significant decreases in maternal depression scores and HR, in addition to increases in maternal HRV at 1 month postpartum. Specifically, SDNN was increased and maternal depression scores and HR were decreased in mothers in the HRV biofeedback group compared to a control group at 1-month post-partum (Kudo, Shinohara, & Kodama, 2014). Overall, there is preliminary evidence for the use of HRV biofeedback in a pregnant population to target ANS activity with possible effects on maternal psychological state. However, the use of HRV biofeedback as an intervention during pregnancy measuring maternal and fetal indices has not been extensively researched. A study assessing 7 maternal-fetal dyads revealed inconclusive findings on the effects of maternal heart rate variability biofeedback on fetal cardiac measures. In the seven-sample study, 2 subjects were classified as inconclusive while 1 subject showed an association between maternal heart rate variability biofeedback and increased fetal heart rate variability. The remaining 4 subjects did not show an association between maternal HRV biofeedback and fetal HRV: 1 noted being particularly stressed on the day of testing, 1 was unable to keep up with the biofeedback technique for the full exam time, 1 was diagnosed with gestational diabetes, and 1 with possible congenital abnormalities (Keeney, 2008).

Research suggests no known, significant, risks for HRV biofeedback (Eddie, Vaschillo, Vaschillo, & Lehrer, 2015) apart from one study in which 15% of patients with anxiety disorders experienced temporary dizziness (Reiner, 2008). Lehrer and Eddie (2013) theorize that excess use of HRV biofeedback, thereby constantly strengthening the associated physiological feedback loop and resonance frequency, may have collateral effects on other physiological feedback loops, for example diurnal rhythms (Figure 4). They therefore recommend that HRV biofeedback be used in relatively short durations (20 minutes per session) or as needed in response to a specific stimulus, for example, stress (Lehrer & Eddie, 2013).

Overall, the maternal interventions discussed have shown some evidence in targeting maternal physiological systems with either concurrent or subsequent effects on the fetus. The goal of the current research is to test the use of HRV biofeedback on the mother and investigate

associated short-term changes in maternal baroreceptor activation, HRV, and fetal general movements. The hypothesized mechanisms of action of HRV biofeedback suggest that this intervention directly targets specific physiological systems, for example, the baroreceptor, as compared to the other interventions noted above.

The current study utilized HRV biofeedback in a sample of pregnant women (N= 20) who followed a within-subject ABA design in order to test if the HRV biofeedback manipulation was associated with maternal baroreceptor activation. Given the associated effects on maternal baroreceptor and short-term HRV (SDNN) with the introduction of the HRV biofeedback manipulation, there is evidence for an experimental effect. The HRV biofeedback manipulation was then tested in an ABAB'A' within-subject design (N= 20) allowing for further manipulation of experimental and baseline phases.

Aims and Hypotheses

Aim 1: To investigate the effect of HRV biofeedback on maternal baroreceptor activation.
Hypothesis 1: Maternal baroreceptor activation will increase in response to the HRV biofeedback manipulation stages (biofeedback and biofeedback II) and decrease during the baseline/post-biofeedback (post-biofeedback and post-biofeedback II) stages; specifically, there will be a prominent peak noted around 0.10 Hz and a significant increase in LFHF ratio during biofeedback phases.

Aim 2: To investigate the effect of HRV biofeedback on maternal short-term HRV (SDNN).
Hypothesis 2: Maternal SDNN will increase in response to the HRV biofeedback manipulation.

Aim 3: To investigate the effect of HRV biofeedback on fetal behavior (fetal general movements: movement amplitude).
Hypothesis 3: Fetal movement amplitude will change significantly in response to the HRV biofeedback manipulation: fetal movement will either increase or decrease significantly in response to the manipulation.

Methods

Research Design

The study design was guided by the main purpose, APA guidelines, guidelines for single case research, and prior literature. The main purpose of the study was to test the feasibility of an intervention with the aim of establishing evidence for a causal relation between the manipulated/independent variable (maternal heart rate variability biofeedback) and two maternal dependent variables (maternal baroreceptor activation and heart rate variability) and one fetal dependent variable (fetal behavior indexed by measuring fetal general movement) (Kratochwill et al., 2010). In order to create the design of the study within the context of empirically supported therapies, the APA Division 12 criteria for Empirically Supported Therapies was used (Appendix A, copied directly from Chambless & Ollendick, 2001; pg. 689) and the areas in bold and italics were used as criteria to guide the current study design.

Research indicates high between-subject variability in maternal HRV and fetal behavior during pregnancy (Stein et al., 1999; Ekholm & Erkkola, 1996; DiPietro, Costigan, Nelson, Gurewitsch, and Laudenslager, 2008). Based on the literature review presented in the introduction, there are limited intervention studies that assess maternal and fetal outcomes. The study done by DiPietro, Costigan, Nelson, Gurewitsch, and Laudenslager (2008) on maternal relaxation during pregnancy was used as a template for this study because it matched closely with the objectives of the current study and was therefore used as a guide for the current study design. In this study on maternal relaxation and fetal movement, the authors selected a within subject design using a 'pre-baseline' period to act as a within-subject control. The authors noted in their conclusion that even though their results were significant (significant difference during intervention phases compared to non-intervention phases) they couldn't rule out the possibility of change in maternal posture affecting the fetal results. However, the study only had two phase transitions (A-B, B-A) from non-intervention to intervention and intervention to non-intervention (DiPietro, Costigan, Nelson, Gurewitsch, and Laudenslager, 2008). I address this design limitation by including 4 phase transitions (A-B, B-A, A-B, B-A). I chose to include additional intervention and post-intervention phases rather than a pre-baseline non-intervention phase as the main aims of this study is to test the feasibility of a manipulation. Based on the APA guidelines, prior research, characteristics of the data under investigation, a within subject (single case) design was determined to be the most appropriate model for this study in order to account for

individual differences in maternal-fetal dyads. A key criterium of the within-subject design is "replication of effect" that must be demonstrated across at least 3 phases. The ABABA class of single case designs was selected which allows for the examination of four different phase transitions (A-B, B-A, A-B, B-A) in order to establish replication of the proposed effect (Kratochwill et al., 2010).

Replication is also used in single case study designs to address the issue of external validity; using the findings to generalize to other populations (Stages of Generalizability outlined in Appendix B; Graham, Karmarker, & Ottenbacher, 2012). The Stages of Generalizability corroborate the two main types of replication outlined in *The Reviewer's Guide to Quantitative Methods in the Social Sciences* "direct replication and systematic replication" (Hancock, Mueller, & Stapleton, 2010) The current study tested the first stage of generalizability (Graham, Karmarker, & Ottenbacher, 2012) and direct replication (Hancock, Mueller, & Stapleton, 2010) within the context of all participants being referred by the Department of Family and Child Services to vocational services. In order to test one of the components of the intervention (duration), as noted in the third stage of generalizability (Graham, Karmarker, & Ottenbacher, 2012), the design was ABAB'A' (A= 10 minutes, B= 10 minutes, A= 10 minutes, B'= 5 minutes, A'= 5 minutes). I wanted to investigate if the shorter duration of biofeedback would still show a shift in the power spectrum, especially given that the effects of biofeedback on the baroreceptor are almost immediate (Lehrer, 2013). Five minutes was selected for the second biofeedback session because the minimum duration for measuring short-term HRV is 5-minute periods (Malik et al., 1996).

Participants

Individuals were drawn from a population attending vocational education classes at a local Occupational Skills Training Center in Decatur, GA. Participants were referred to the vocational education classes by Department of Family and Child Services (DFACS). Participants were not excluded based on number of pregnancies. Participants were African American, ages 20-34, within gestational weeks (GW) 20-38 of pregnancy, unemployed, education level mostly limited to mostly high school. Individuals with self-reported drug use during their pregnancy were excluded from the study. An ABA design was initially used in order to determine if the proposed manipulation was associated with maternal changes as

hypothesized and if further investigation was warranted. The ABA design was used in a sample of 20 participants. Once preliminary data in the ABA design indicated changes in maternal baroreceptor activation, the ABA design was expanded to an ABAB'A' design with 20 additional participants in order to test additional phase shifts between intervention and non-intervention phases. Demographic breakdowns of the participants in the ABA design and ABAB'A' design are shown in Tables 2 and 3.

Table 2.

Frequency and Percentage of Participant Demographic Information of Participants

	ABA; N= 20		ABAB'A'; N= 20	
Characteristic	*N*	%	*N*	%
Ethnicity				
African American	20	100	20	100
Asian	0	0	0	0
Caucasian	0	0	0	0
Hispanic	0	0	0	0
Other	0	0	0	0
Gestational Age				
20-23 weeks	4	20	2	10
24-27 weeks	6	30	9	45
28-31 weeks	4	20	5	25
32-38 weeks	6	30	4	20
Gender of Fetus				
Female	9	45	8	50
Male	11	55	10	40
Not Reported	0	0	2	10
Maternal Age				
18-20 years	0	0	1	5
21-25 years	10	55	10	50
26-30 years	5	25	5	25
31-35 years	5	25	4	20

Maternal Education				
Middle School (4-8 yrs.)	1	5	0	0
High School (9-12 yrs.)	16	80	17	85
College (13-16 yrs.)	3	15	1	5
Not Reported	0	0	2	10
Number of Prior Pregnancies				
0	0	0	0	0
1	6	30	1	5
2	2	10	6	30
3+	6	30	6	30
Not Reported	6	30	7	35

Table 3.

Mean and Standard Deviation of Participant Demographic Information of Participants

	ABA; N= 20		ABAB'A'; N= 20	
Characteristic	M	SD	M	SD
Maternal Age (years)	27.000	4.267	26.400	3.912
Gestational Age (weeks)	27.950	5.294	29.000	4.812
Number of Prior Pregnancies	2.210	1.424	2.615	1.044

Materials and Measures

Maternal assessments: physiological (*ANS)* indices: heart rate variability (SDNN), LFHF ratio, and power spectrum analysis. The NeXus-4 was used to record short-term HRV (SDNN), LFHF ratios, and produce a power spectrum analysis from the session data. The NeXus-4 is a four-channel system used for both biofeedback and neurofeedback, which connects with a user interface called the BioTrace+. The BioTrace+ software converts the raw physiological data into visual graphs. The NeXus-4 uses two sensors to collect data: a finger sensor to measure the individual's blood volume pulse, which is used to calculate heart rate and HRV, and a belt sensor placed around the diaphragm, which measures respiration rate. The NeXus-4 uses "active shielding for minimal noise and movement artifacts" ("NeXus-4," n.d.).

Maternal HRV is calculated by the standard deviation of normal-to-normal R-R intervals (SDNN), where R is the peak of the QRS complex that indicates ventricular depolarization. "The Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology define short-term heart rate variability as heart rate variability calculated over a minimum period of 5-minutes" (Malik et al., 1996). LFHF ratio is the ratio of low frequency to high frequency components in the HRV spectrum. The power spectrum analysis quantifies the degree to which each frequency occurs in a specified time. The frequencies correspond to various components of the nervous system. In addition to the LFHF ratio provided by BioTrace+ software, the peak that usually occurs in this graph during the biofeedback intervention (around 0.10 Hz) was plotted and the coordinates (frequency, power) were analyzed.

Maternal assessments: breathing data. The belt sensor on the NeXus-4 continuously measured respiration rate data throughout the sessions. A Percent Physiological Breathing variable was created for each phase by calculating the percent of time the participant was breathing within the range of 4.8-7.2 breaths per minute. Breathing at 4.8-7.2 breaths per minute corresponds to 0.08-0.12 Hz, which is the frequency range where the baroreceptor system is stimulated. Therefore, those individuals that displayed a peak in the power spectrum in the range of 0.08-0.12 Hz and were breathing at 4.8-7.2 breaths per minute met criteria for activating their baroreceptors during biofeedback.

Fetal assessment: movement amplitude. Fetal data was collected from a fetal tracing using a Toitu fetal actocardiograph MT-516. This monitor detects and records fetal movement and fetal heart rate with a transabdominal Doppler transducer, which processes signals through a series of autocorrelation techniques. The fetal tracing from the Toitu generates fetal movement amplitude data in arbitrary units (A.U.) ranging from 0-50. Fetal movement amplitude was averaged for each session.

Procedure

Each new class of individuals at the Occupational Skills Training Center were presented with the purpose and the procedure of the research assessment. Consent was obtained from the individuals who volunteered to participate. They were provided brief information handouts as well as a copy of the consent form to take home.

A within-subject design, specifically ABA and ABAB'A', was selected based on prior research in the field as outlined in the methods above. There was one experimental group, and each maternal-fetal dyad served as their own control group using baseline, biofeedback, post-biofeedback, biofeedback II, and post-biofeedback II as time points for analysis. At 20 weeks of gestation or later, the individuals attended an in-lab assessment. Participants were instructed not to consume anything for 1 hour prior to the assessment and were assessed between 9:00 AM to 11:00 AM. Before beginning any assessments, all participants were given a brief description of the overall procedure (Figure 2). Next, participants filled out a biographical questionnaire.

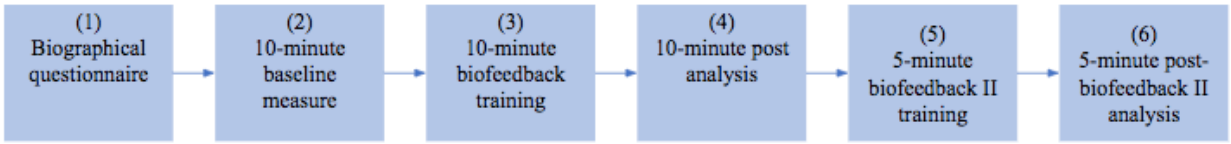

Figure 2. Outline of procedure for the ABAB'A' design. During Steps 2 to 6 data was collected from maternal indices (ANS) and fetal indices (general movements). Participants in the ABA design stopped after Step 4.

The functions of the sensors were explained to the individual and then attached: the finger sensor measured maternal blood flow; the belt sensor placed around the maternal diaphragm measured respiration rate; one sensor placed on the maternal abdomen measured fetal heart rate and fetal movement while the other sensor measured uterine contractions. Each participant was briefed prior to the 10-minute baseline; they were instructed to simply relax and rest semi-recumbent on a cushioned chair with minimal movement. The proctor made sure to not instruct participants to breathe normally in order to prevent them from drawing attention to their own breathing during the baseline period. The proctor stated that the purpose of the baseline session was to provide insight into the normal somatic activity of the mother and her fetus.

During the biofeedback sessions, a laptop displaying the maternal physiological signals was placed in clear sight 2 feet in front of the mother on a chair. The only instructions given were to follow the breathing pacer as close as possible to the best of the mothers' ability. Participants were instructed to let the proctor know immediately if they experienced any discomfort. Post-biofeedback and post-biofeedback II sessions followed the same protocol as

the baseline. Throughout the entire session, the mother was instructed to remain silent to avoid introducing maternal voice as a stimulus to the fetus. The apriori sample size (derived by G*Power 3.1) and post-hoc power for each statistical test is outlined in Table 4.

Table 4.

Apriori and Post-hoc G Power

Variable	Statistical Test	Apriori Sample Size	Post-hoc Observed power (N= 20; ABA)	Post-hoc Observed power (N= 20; ABAB'A')	Reference
LF/HF Ratio Across 3 Time Points (ABA)	Repeated measures ANOVA	No applicable research	1.000	N/A	N/A
LF/HF Ratio Across 5 Time Points (ABAB'A')	Repeated measures ANOVA	No applicable research therefore used N from ABA as guideline given adequate power in ABA.	N/A	0.986	N/A
SDNN Across 3 Time Points (ABA)	Repeated measures ANOVA	No applicable research	0.999	N/A	N/A

SDNN Across 5 Time Points (ABAB'A')	Repeated measures ANOVA	No applicable research therefore used N from ABA as guideline given adequate power in ABA.	N/A	0.987	N/A
Movement Amplitude Across 5 Time points (ABAB'A')	Repeated measures ANOVA	9	N/A	0.272	DiPietro, Costigan, Nelson, Gurewitsch, & Laudenslager, 2008 (η_p^2= 0.216)

Data Analysis Plan

The BioTrace+ software provides SDNN and LFHF ratio values. The raw data output from the BioTrace+ software was cleaned of any artifacts and unanalyzable data before statistical analysis. Artifacts included areas of the data where accidental maternal movement or actions (i.e. maternal laughing, coughing, accidental moving of sensors) or environmental sounds occurred that might have led to inaccurate data measurement. Any complete sessions that contained significant portions of missing data (>5%) were omitted. The data was then transferred to SPSS where outliers that did not fall within 3 standard deviations were removed. Log-transformed data was used for analysis for maternal SDNN and LFHF ratios; however, the means and standard deviations are reported as the actual values.

In order to meet compliance criteria for HRV biofeedback the following criteria had to be met: A peak in the power spectrum in the frequency range of 0.08-0.12 Hz during both biofeedback sessions and a breathing rate of 4.8-7.2 breath per minute for 1% of the time for biofeedback and 2 % of the time for biofeedback II. The percent breathing rate within the range was used based on research that indicates the rapid shift to baroreceptor range under conditions of HRV biofeedback (Lehrer, 2013).

Repeated Measures ANOVA was selected to analyze the changes in dependent variables across the 5 timepoints for N= 20 and 3 timepoints for N= 20. Research has established that HRV biofeedback is associated with an increase in baroreflex gain (Lehrer et al., 2003) and HRV (Lehrer et al., 2003; Lehrer et al., 2004; Hassett et al., 2007; Del Pozo, Gevirtz, Scher, & Guarneri, 2004; Karavidas et al., 2007; Zucker, Samuelson, Muench, Greenburg, & Gevirtz, 2009) therefore motivating the directional hypotheses provided above for these two indices (LFHF and SDNN). There is however, a lack of established research on HRV biofeedback on fetal movement to suggest an apriori specific direction of fetal movement changes that should be reflected in the hypotheses. Research on maternal interventions, such as relaxation, Stroop, and music interventions, show varied effects on fetal movement (DiPietro, Costigan, Nelson, Gurewitsch, & Laudenslager, 2008; DiPietro, Costigan, & Gurewitsch, 2003; Akbarzade, Rafiee, Asadi, & Zare, 2015).

Results

Breathing Data (N= 20; ABA)

An ABA design was used to document an effect on maternal baroreceptor and HRV in pregnant women. Descriptive statistics for percent breathing at the physiological range (4.8-7.2 breaths per minute) across time points are reported in Table 5. A repeated measures analysis of variance for maternal percent physiological breathing across 3 time points (baseline, biofeedback, and post-biofeedback) revealed a **statistically significant difference** over time ($F(2, 30)= 30.294$, $p< 0.001$, $\eta_p^2= 0.669$; observed power= 1.000). Post hoc analysis indicated that maternal percent physiological breathing increased from baseline to biofeedback, which was statistically significant ($p< 0.001$, $d= 101.795$, large effect size). Maternal percent physiological breathing decreased from biofeedback to post-biofeedback ($p< 0.001$, $d= 2.844$, large effect size).

Table 5.

Descriptive Statistics for Percent Physiological Breathing

	M	SD	N
Baseline	0.984	2.979	16
Biofeedback	35.290	23.362	16
Post-Biofeedback	4.152	13.091	16

Maternal Autonomic Nervous System (N= 20; ABA)

Maternal SDNN and LFHF ratio were all skewed distributions and therefore log-transformations were applied. The log-transformed data was used for analysis; however, the means and standard deviations are reported as the actual values. Descriptive statistics for maternal indices across time points are reported in Table 6. A repeated measures analysis of variance for maternal LFHF ratio across 3 time points (baseline, biofeedback, and post-biofeedback) revealed a **statistically significant difference** over time ($F(1.172, 8.201)= 41.038$, $p< 0.001$, $\eta_p^2= 0.854$; observed power= 1.000; Figure 3). Post hoc analysis indicated that maternal LFHF ratio increased from baseline to biofeedback, which was statistically significant ($p= 0.001$, $d= 48.977$, large effect size). Maternal LFHF ratio decreased from biofeedback to post-biofeedback ($p= 0.001$, $d= 2.188$, large effect size).

Table 6.

Descriptive Statistics for Maternal Indices

	LFHF Ratio			SDNN		
	M	SD	N	M	SD	N
Baseline	1.086	0.633	8	40.285	29.121	8
Biofeedback	5.562	2.522	8	61.568	23.505	8
Post-Biofeedback	1.300	0.570	8	44.855	25.593	8

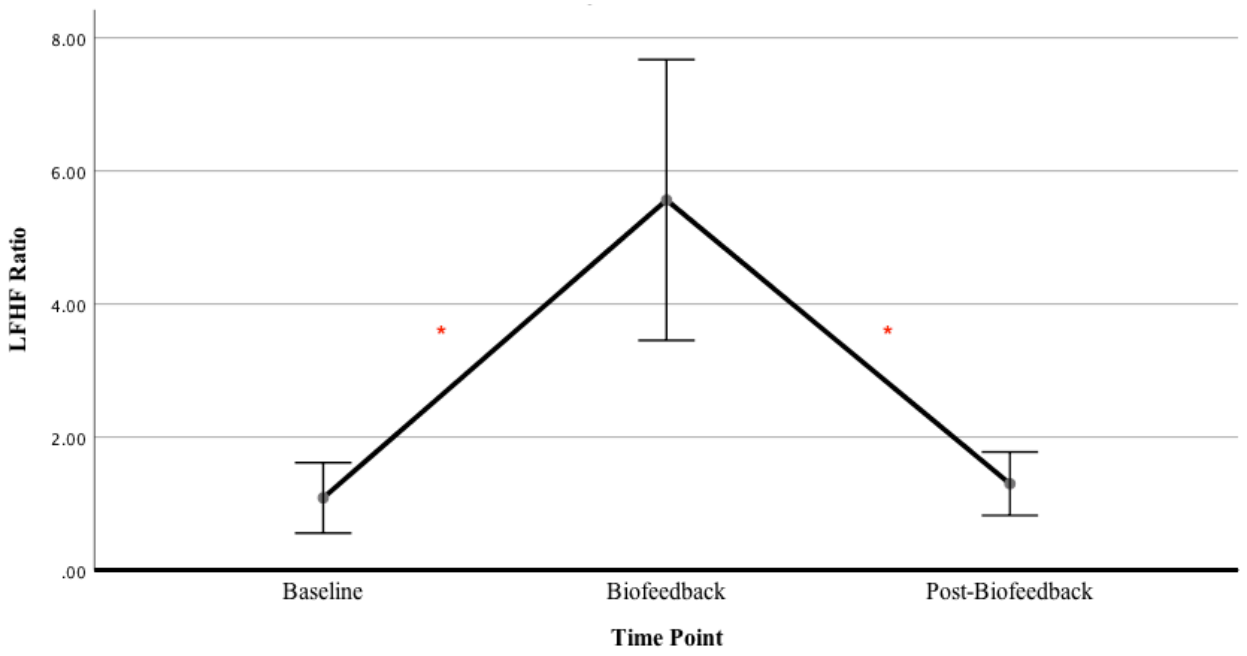

Figure 3. Comparison of mean values of maternal LFHF ratio at baseline, biofeedback, and post-biofeedback time points. Stars (*) on a line indicate a significant difference between the time points connected by the line. Stars (*) of the same color indicate a significant difference between the two time points.

A repeated measures analysis of variance for maternal SDNN across 3 time points (baseline, biofeedback, and post-biofeedback) revealed a **statistically significant difference** over time ($F(2, 14)= 18.354$, $p< 0.001$, $\eta_p^2= 0.724$; observed power= 0.999; Figure 4). Post hoc analysis indicated that maternal SDNN increased from baseline to biofeedback, which was statistically significant ($p= 0.003$, $d= 2.244$, large effect size). Maternal SDNN decreased from biofeedback to post-biofeedback ($p= 0.027$, $d= 1.893$, large effect size).

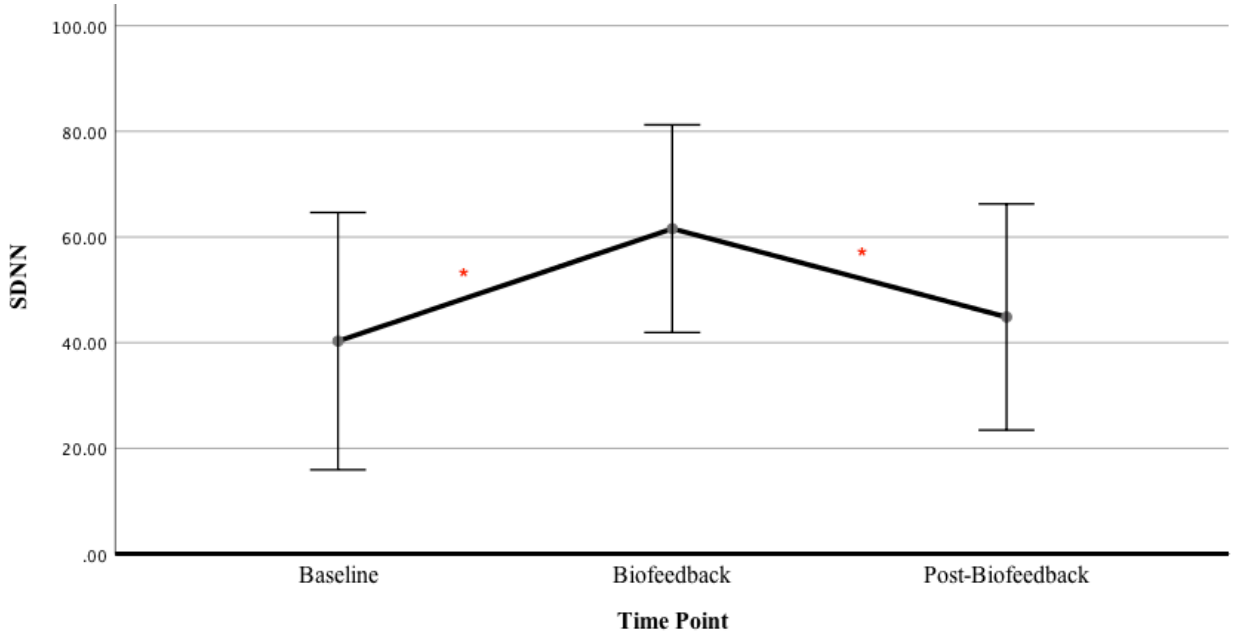

Figure 4. Comparison of mean values of maternal SDNN at baseline, biofeedback, and post-biofeedback time points. Stars (*) on a line indicate a significant difference between the time points connected by the line. Stars (*) of the same color indicate a significant difference between the two time points.

Breathing Data (N= 20; ABAB'A')

Descriptive statistics for percent breathing at the physiological range (4.8-7.2 breaths per minute) across time points are reported in Table 7. A repeated measures analysis of variance for percent physiological breathing across 5 time points (baseline, biofeedback, post-biofeedback, biofeedback II, and post-biofeedback II) revealed a **statistically significant difference** over time ($F(1.409, 26.766)= 16.544$, $p< 0.001$, $\eta_p^2= 0.465$; observed power= 0.993; Figure 5). Post hoc analysis indicated that percent physiological breathing ratio increased from baseline to biofeedback, which was statistically significant ($p= 0.001$, $d= 3.761$, large effect size). Percent physiological breathing significantly decreased from biofeedback to post-biofeedback ($p= 0.002$, $d= 0.737$, medium effect size). Percent physiological breathing increased from post-biofeedback to biofeedback II ($p= 0.022$, $d= 2.873$, large effect size). Percent physiological breathing decreased from biofeedback II to post-biofeedback II ($p= 0.009$, $d= 0.767$, medium effect size). Biofeedback II percent physiological breathing increased significantly compared to baseline ($p=$

0.009, *d*= 3.500, large effect size). Percent physiological breathing during post-biofeedback II also decreased significantly compared to biofeedback (*p*= 0.001, *d*= 1.029, large effect size).

Table 7.

Descriptive Statistics for Percent Physiological Breathing

	M	SD	N
Baseline	1.989	8.893	20
Biofeedback	39.618	35.468	20
Post-Biofeedback	2.420	8.234	20
Biofeedback II	37.694	42.917	20
Post-Biofeedback II	1.436	4.496	20

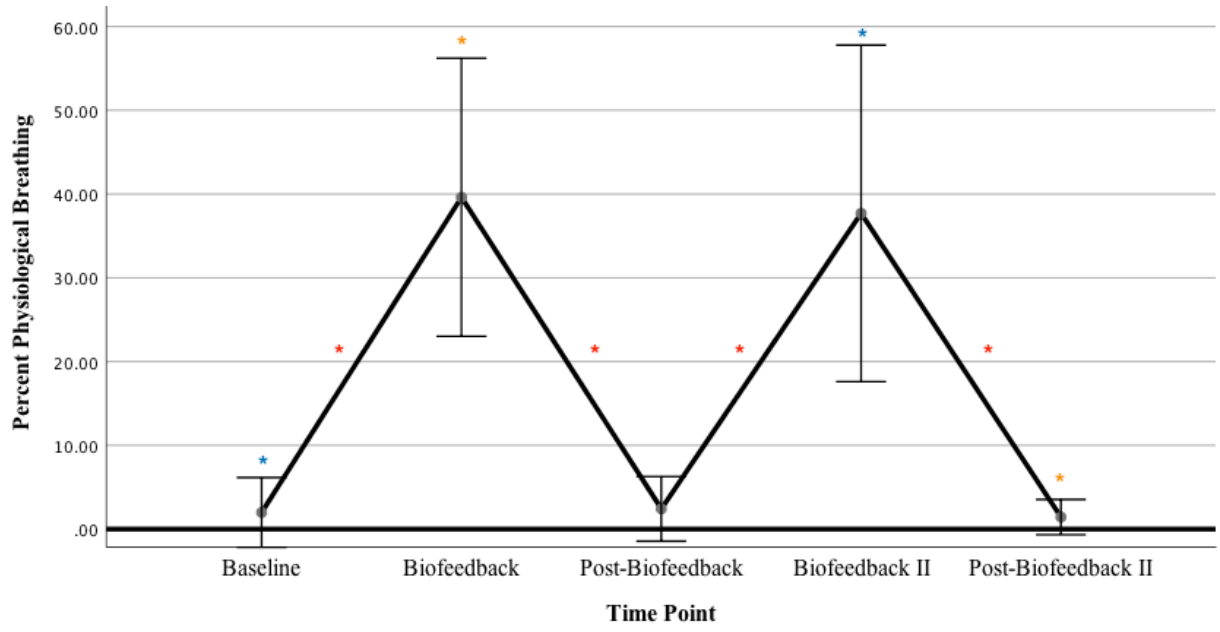

Figure 5. Comparison of mean values of maternal percent physiological breathing at baseline, biofeedback, post-biofeedback, biofeedback II, and post-biofeedback II time points. Stars (*) on a line indicate a significant difference between the time points connected by the line. Stars (*) of the same color indicate a significant difference between the two time points.

Compliance Data

Based on prior HRV biofeedback research, I set the baroreceptor frequency range at 0.08-0.12 Hz, corresponding to 4.8-7.2 breaths per minute. Therefore, those individuals that displayed a peak in the power spectrum in the range of 0.08-0.12 Hz and were breathing in these ranges were considered as activating their baroreceptors during biofeedback.

Of those participants undergoing the ABAB'A' protocol, during Biofeedback I, 18/20 mothers were breathing at the rate of 4.8-7.2 for a minimum of 1% of the time over the 10-minute session. During Biofeedback II, 15/20 mothers were breathing at the rate of 4.8-7.2 for 2% of the time or more over the 5-minute session. Breathing in this range demonstrates a significant shift in breathing rates to the intended breathing range of the intervention to activate the baroreceptor at the resonance frequency. Amount of time people spend in the breathing range differed per participant; the percentages ranged from 0-100%. In current research, there are no established norms for how long a participant needs to breathe within the desired breathing range in order to activate the baroreceptor effects. The 1% and 2% criteria to qualify as breathing in the physiological range for biofeedback and biofeedback II were used because research shows immediate effects in ANS changes at the onset of HRV biofeedback (Lehrer, 2013). During Biofeedback I, 17/20 mothers demonstrated a peak in the baroreceptor range, while during Biofeedback II, 14/20 mothers demonstrated this peak.

In order to meet compliance criteria for HRV biofeedback the following criteria had to be met: A peak in the power spectrum in the frequency range of 0.08-0.12 Hz during both biofeedback sessions and a breathing rate of 4.8-7.2 breath per minute for 1% of the time for biofeedback and 2 % of the time for biofeedback II. The percent breathing rate within the range was used based on research that indicates the rapid shift to baroreceptor range under conditions of HRV biofeedback (Lehrer, 2013). 14/20 maternal-fetal dyads met compliance criteria for final data analysis.

Table 8.

Overview of Compliance Data

	Physiological Breathing Criteria	Peak within Baroreceptor Range	Both Physiological Breathing and Baroreceptor Range

ABAB'A'; Biofeedback	18/20	17/20	17/20
ABAB'A'; Biofeedback II	15/20	14/20	14/20
Combined Biofeedback and Biofeedback II	15/20	14/20	**14/20**

Maternal Autonomic Nervous System (N= 20; ABAB'A')

Maternal SDNN and LFHF ratio were all skewed distributions and therefore log-transformations were applied. The log-transformed data was used for analysis; however, the means and standard deviations are reported as the actual values. Descriptive statistics for maternal indices across time points are reported in Table 9. A repeated measures analysis of variance for maternal LFHF Ratio across 5 time points (baseline, biofeedback, post-biofeedback, biofeedback II, and post-biofeedback II) revealed a **statistically significant difference** over time ($F(1.514, 19.685)= 14.303$, $p< 0.001$, $\eta_p^2= 0.524$; observed power= 0.986; Figure 6). Post hoc analysis indicated that maternal LFHF ratio increased from baseline to biofeedback, which was statistically significant ($p= 0.007$, $d= 0.96$, large effect size). Maternal LFHF ratio decreased from biofeedback to post-biofeedback ($p= 0.026$, $d= 0.795$, medium effect size). Maternal LFHF ratio increased from post-biofeedback to biofeedback II ($p= 0.029$, $d= .812$, large effect size). Maternal LFHF ratio decreased from biofeedback II to post-biofeedback II ($p= 0.019$, $d= 0.836$, large effect size). Biofeedback II maternal LFHF ratio increased significantly compared to baseline ($p= 0.012$, $d= 0.941$, large effect size). Biofeedback maternal LFHF ratio was significantly increased compared to post-biofeedback II ($p= 0.010$, $d= 0.913$, large effect size).

Table 9.

Descriptive Statistics for Maternal Indices

	LFHF Ratio			SDNN		
	M	SD	N	M	SD	N
Baseline	0.950	0.457	14	36.987	8.828	14
Biofeedback	4.957	4.368	14	58.859	21.117	14
Post-Biofeedback	1.414	0.815	14	47.045	12.941	14
Biofeedback II	6.900	7.701	14	56.808	18.123	14
Post-Biofeedback II	1.107	0.573	14	44.535	11.918	14

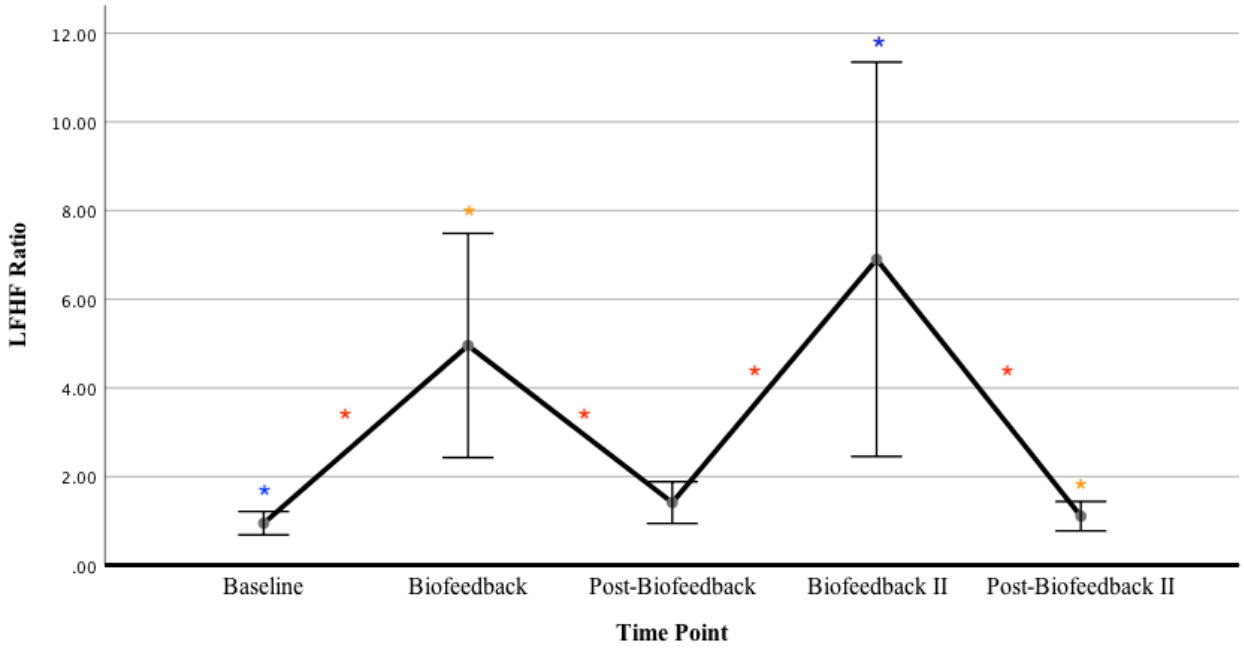

Figure 6. Comparison of mean values of maternal LFHF ratio at baseline, biofeedback, post-biofeedback, biofeedback II, and post-biofeedback II time points. Stars (*) on a line indicate a significant difference between the timepoints connected by the line. Stars (*) of the same color indicate a significant difference between the two time points.

A repeated measures analysis of variance for maternal SDNN across 5 time points (baseline, biofeedback, post-biofeedback, biofeedback II, and post-biofeedback II) revealed a **statistically significant difference** over time ($F(2.393, 31.104)= 10.023$, $p< 0.001$, $\eta_p^2= 0.435$;

observed power= 0.987; Figure 7). Post hoc analysis indicated that maternal SDNN increased from baseline to biofeedback, which was statistically significant (p= 0.002, d= 1.245, large effect size). Maternal SDNN increased from baseline to biofeedback II (p= 0.014, d= 1.024, large effect size).

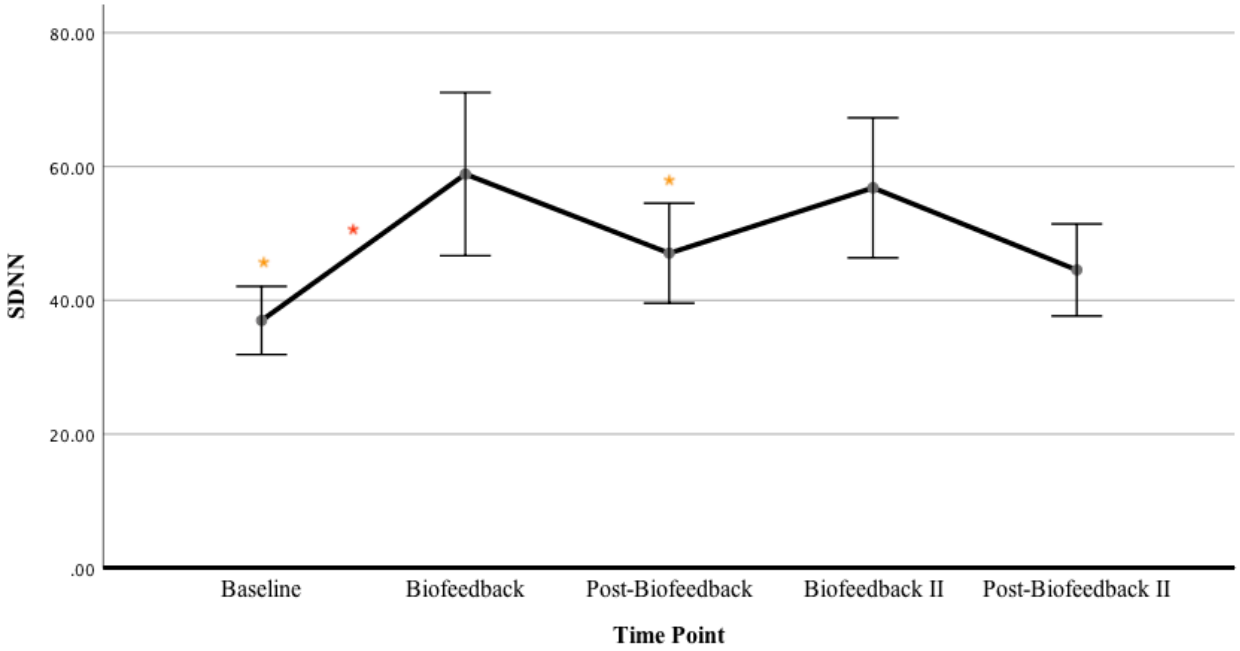

Figure 7. Comparison of mean values of maternal SDNN at baseline, biofeedback, post-biofeedback, biofeedback II, and post-biofeedback II time points. Stars (*) on a line indicate a significant difference between the timepoints connected by the line. Stars (*) of the same color indicate a significant difference between the two time points.

Fetal Movement Amplitude (N= 20; ABAB'A')

Descriptive statistics for fetal movement amplitude across time points are reported in Table 10. A repeated measures analysis of variance for fetal movement amplitude across 5 time points (baseline, biofeedback, post-biofeedback, biofeedback II, and post-biofeedback) revealed there was not a statistically significant difference over time ($F(2.617, 34.023)= 1.184$, p= 0.327, η_p^2= 0.083; observed power= 0.272).

Table 10.

Descriptive Statistics for Fetal Movement Amplitude (Fetal Movement ranges from 0-50 arbitrary units)

	M	SD	N
Baseline	16.810	4.257	14
Biofeedback	15.810	5.300	14
Post-Biofeedback	17.200	5.730	14
Biofeedback II	15.160	5.880	14
Post-Biofeedback II	16.700	5.771	14

Case-by-case Individual Analysis: Mean and Trend

Given the overall lack of significance in the repeated measures ANOVA, coupled with prior literature that suggests high variability in fetal movement changes in response to a stimulus (DiPietro, Costigan, Nelson, Gurewitsch, & Laudenslager, 2008; DiPietro, Costigan, & Gurewitsch, 2003; Akbarzade, Rafiee, Asadi, & Zare, 2015), a follow up case-by-case analysis of fetal movement for each individual fetus was conducted. Based on the visual analysis criteria for single-case designs (Hancock, Mueller, & Stapleton, 2010), a mean and trend analysis was conducted for each fetus across the 5 time points. The visual analysis reveals wide spread differences among the individual fetuses. Given the large volume of data that this analysis generated, 5 sample fetuses are presented below in order to demonstrate the visual analysis: one demonstrating fetal movement increase in response to the biofeedback (Figures 8a - 8b), one illustrating fetal movement decrease in response to the biofeedback (Figures 9a - 9b), one representing varying responses in fetal movement to the biofeedback (for example, increase in response to the first biofeedback and decrease in response to second biofeedback) (Figures 10a - 10b), and one exemplifying no significant movement changes in response to the biofeedback (Figures11a-11b). Figures 12a-12b represent a fetus' movement across time points with a mother who did not meet any inclusion criteria (did not breathe in the physiological range during biofeedback and biofeedback II and did not have a peak in the baroreceptor range in their power spectrum at biofeedback and biofeedback II). "Biofeedback physiological breathing percent" refers to the percent of time that the mother spent breathing within the HRV biofeedback

breathing range. The remainder of the visual analysis for each individual fetus is presented in Appendix C.

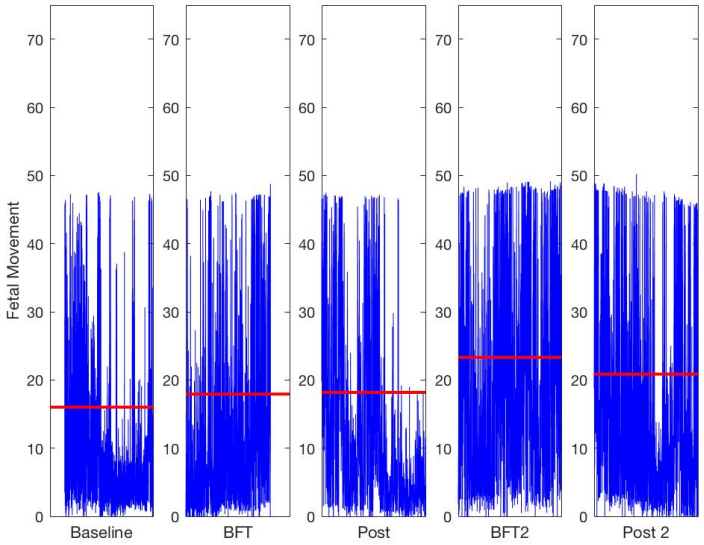

Figure 8a. Mean analysis for fetus 1 (28 weeks) that increased movement in response to the biofeedback. Biofeedback physiological breathing percent = 95.84, biofeedback II physiological breathing percent = 97.84. Peak power biofeedback= 1312.59, peak power biofeedback II= 1575.50.

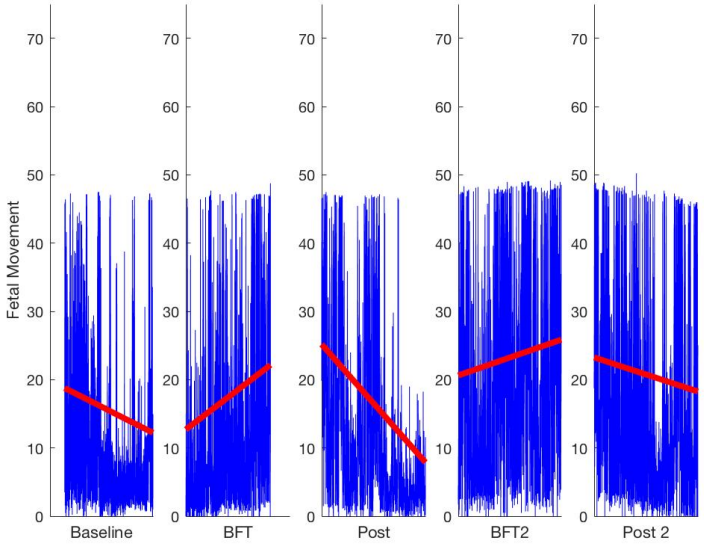

Figure 8b. Trend analysis for fetus 1 (28 weeks) that increased movement in response to the biofeedback.

When analyzing the mean and trend (slope) of fetal movement across phases for fetus 1 represented in Figures 8a-8b the data suggests that the introduction of the biofeedback stimulus is associated with a slight increase in mean fetal movement and a significant change in the trajectory of fetal movement (slope); the non-intervention phases are characterized by a decreasing fetal movement trajectory while the intervention phases are characterized by an increasing fetal movement trajectory (Figures 8a - 8b).

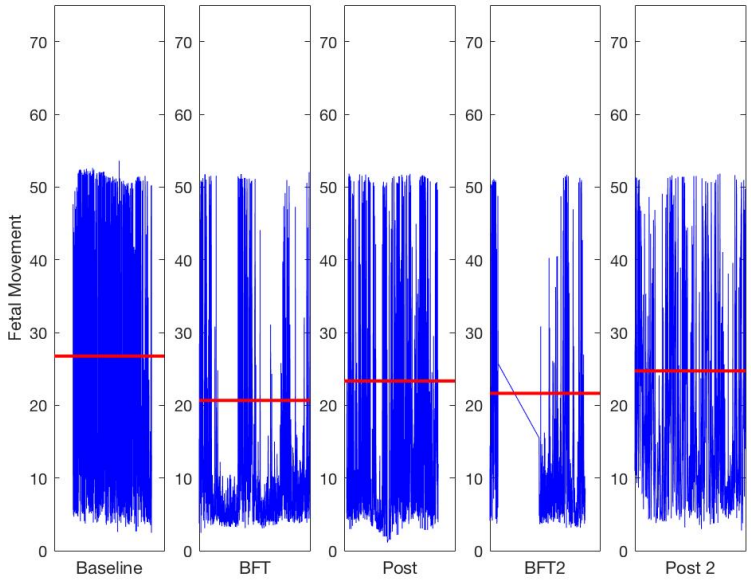

Figure 9a. Mean analysis for fetus 2 (28 weeks) that decreased movement in response to the biofeedback. Biofeedback physiological breathing percent = 53.94, biofeedback II physiological breathing percent = 100. Peak power biofeedback= 949.39, peak power biofeedback II= 827.59.

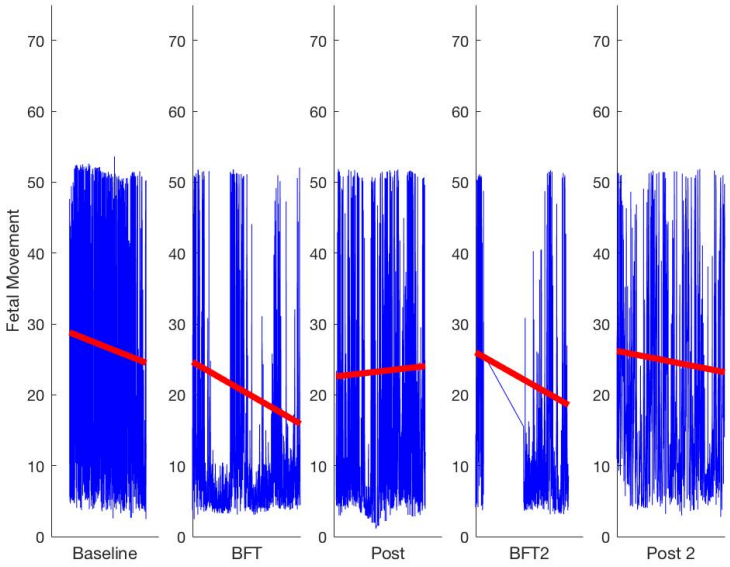

Figure 9b. Trend analysis for fetus 2 (28 weeks) that decreased movement in response to the biofeedback.

Analysis of mean and trend of fetal movement across phases for fetus 2 represented in Figure 9a-9b indicates that the introduction of the biofeedback stimulus is associated with a decrease in mean fetal movement and a continued downward trajectory of fetal movement. The introduction of the second biofeedback is associated with a slight decrease in mean fetal movement and a significant change in fetal movement trajectory from an increasing slope during post-biofeedback to a decreasing slope during biofeedback II. Fetal movement returns to a higher amplitude following removal of the second biofeedback (Figures 9a-9b).

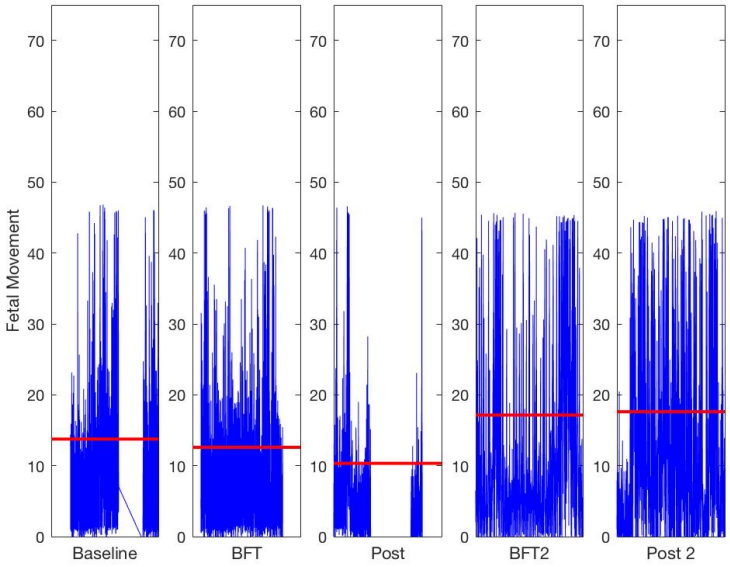

Figure 10a. Mean analysis for fetus 3 (36 weeks) that showed varying responses in response to biofeedback and biofeedback II. Biofeedback physiological breathing percent = 25.80, biofeedback II physiological breathing percent = 37.74. Peak power biofeedback= 463.95, peak power biofeedback II= 725.47.

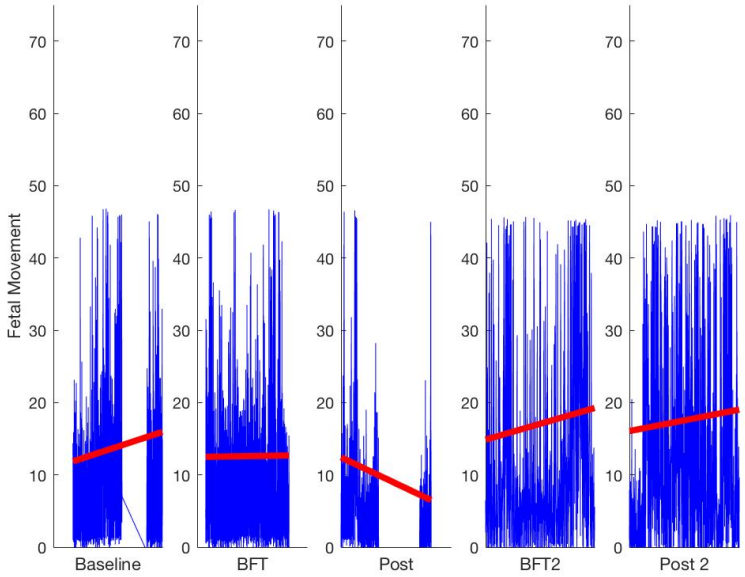

Figure 10b. Trend analysis for fetus 3 (36 weeks) that showed varying responses in response to biofeedback and biofeedback II.

Fetus 3 represented in Figures 10a -10b varied in its response to the biofeedback stimulus. While mean movement did not change significantly, the increasing slope during baseline leveled off during the biofeedback. However, during the second biofeedback there was an increase in mean fetal movement as well as a shift in trajectory from a downward to upward trajectory of fetal movement that was maintained post-biofeedback II.

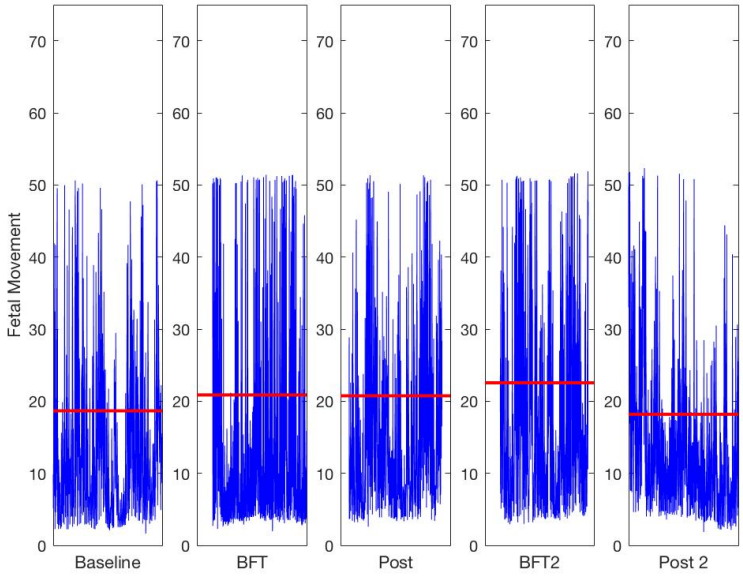

Figure 11a. Mean analysis for fetus 4 (26 weeks) that did not exhibit significant change in response to the biofeedback. Biofeedback physiological breathing percent = 36.17, biofeedback II physiological breathing percent = 7.28. Peak power biofeedback= 812.55, peak power biofeedback II= 55.5.

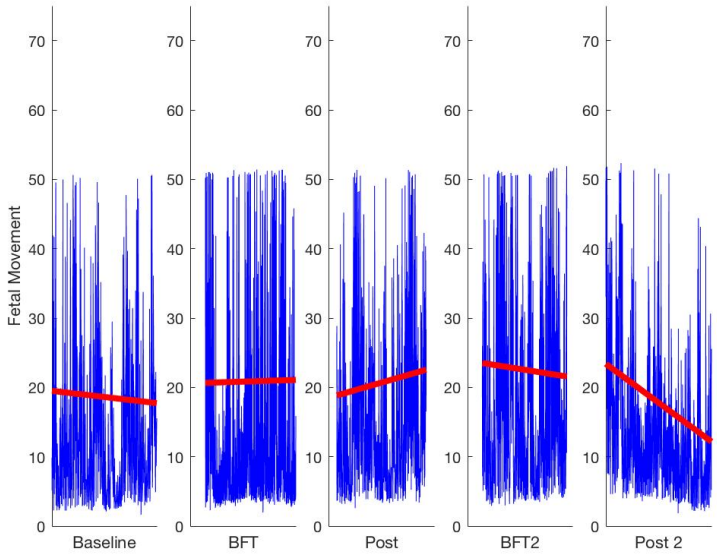

Figure 11b. Trend analysis for fetus 4 (26 weeks) that did not exhibit significant change in response to the biofeedback.

The mean and trend analysis for fetus 4 represented in Figures 11a-11b does not exhibit notable changes until the introduction of the second biofeedback. The second biofeedback is associated with a change in movement trajectory from an upward slope to a downward slope that persists post-biofeedback II.

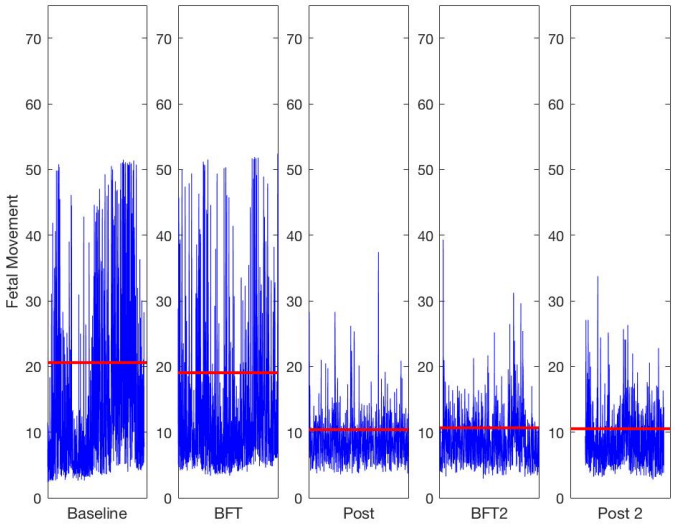

Figure 12a. Mean analysis for fetus 5 (24 weeks) whose mother did not meet any inclusion criteria. Biofeedback physiological breathing percent = 0.63, biofeedback II physiological breathing percent = 0.

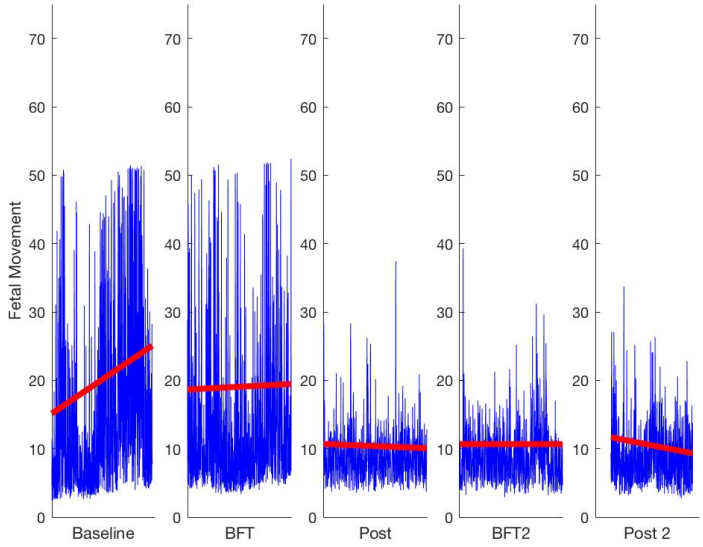

Figure 12b. Trend analysis for fetus 5 (24 weeks) whose mother did not meet any inclusion criteria.

The mother for fetus 5 represented in Figures 12a-12b did not meet any inclusion criteria, indicating that the mother did not breathe in the predefined physiological range of breathing and did not activate her baroreceptor system during biofeedback due to a lack of a peak in the power

spectrum. However, the fetal movement across time points shows a significant decrease from biofeedback to post, which persists throughout biofeedback II and post-biofeedback II. In addition, the trend analysis reveals changes in movement trajectory from an upward trend during baseline which level off during biofeedback. A possible explanation for these changes in fetal movement without the inclusion criteria could lie in maternal breathing changes. The mother's mean breathing rate decreased from 17.39 breaths per minute at baseline to 13.66 breaths per minute during biofeedback. Though this does not fall in the predefined breathing range in this study it is possible that the decrease in breathing rate had a stimulus effect on fetal movement that persisted across the following time points.

Overall, the visual case-by-case analysis of fetal movement indicates differential responding to maternal biofeedback among individual fetuses with varied movement trajectories. The case-by-case analysis reveals that decreasing maternal breathing rate may impact fetal movement. Comparison of additional fetuses within the same gestational age are required to make interpretations linking fetal movement changes with specific gestational age. Further investigation is required to determine if the specific breathing criteria facilitated by HRV biofeedback adds any additional effect to fetal movement than lowered breathing rates.

Discussion

Maternal Implications

The main goal of the current study was to test the feasibility of a manipulation to effect changes in (1) maternal autonomic nervous system (specifically, activation of the baroreceptor and increases in HRV) and (2) fetal behavior (fetal general movements). Current findings provide preliminary evidence that HRV biofeedback is associated with increased maternal baroreceptor activation and increases in short-term HRV. In previous research, it is common to see a prominent power peak around 0.10 Hz in the HRV power spectrum analysis during HRV biofeedback (McCraty & Shaffer, 2015; Lehrer & Gevirtz, 2014). Figures 13, 14, and 15 illustrate the power spectrum analysis of one individual in the current study during baseline, biofeedback, and post-biofeedback, respectively. The results of the change in LFHF ratio corroborate the shifts in frequency towards a peak around 0.10 Hz indicating activation of the baroreceptor during the biofeedback condition and the shift back to baseline levels during the

post-biofeedback condition. In the ABAB'A' procedure, the shift demonstrated in Figures 13-15 occurred in 17/20 participants during the first biofeedback, and in 14/20 participants during the second biofeedback that met breathing criteria. The significant increase in LFHF ratio from baseline to biofeedback, provides a statistical corroboration of baroreceptor activation in these individuals.

The association between application of HRV biofeedback and baroreceptor activation was confirmed in the ABAB'A' (N= 20) design. Furthermore, there were 4 phase shifts from decreased baroreceptor activation to increased baroreceptor activation corresponding with non-intervention and intervention phases respectively (baseline to biofeedback; biofeedback to post-biofeedback, post-biofeedback to biofeedback II, and biofeedback II to post-biofeedback II) therefore meeting the criteria for experimental control (Kratochwill et al., 2010). None of the published studies reviewed included a non-invasive manipulation with the potential for directly activating the maternal baroreceptor. Baroreceptor attenuation is common during pregnancy and leads to increased hypertension (Brooks, Dampney, & Heesch, 2010). Pregnant women had increased total sympathetic activity compared to a control group of non-pregnant women; this effect is attributed to attenuation of the baroreceptor during pregnancy (Usselman et al., 2015). While increased sympathetic activity during pregnancy provides compensatory benefits, shifts in maternal ANS functioning can lead to pathological conditions such as hypertension.

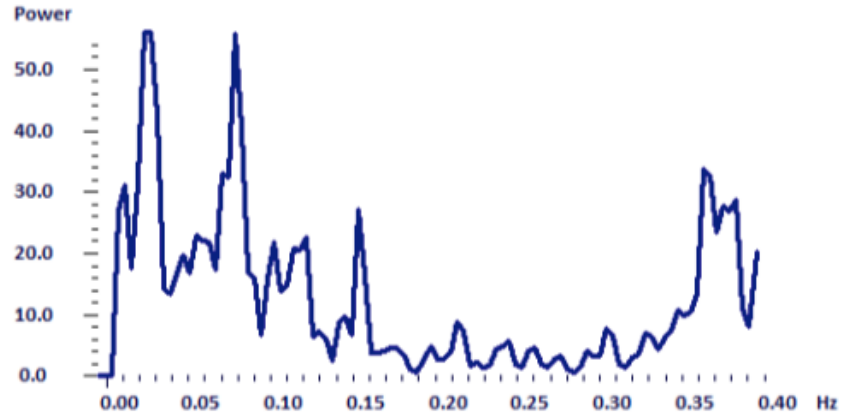

Figure 13. Single participant's power spectrum graph during baseline.

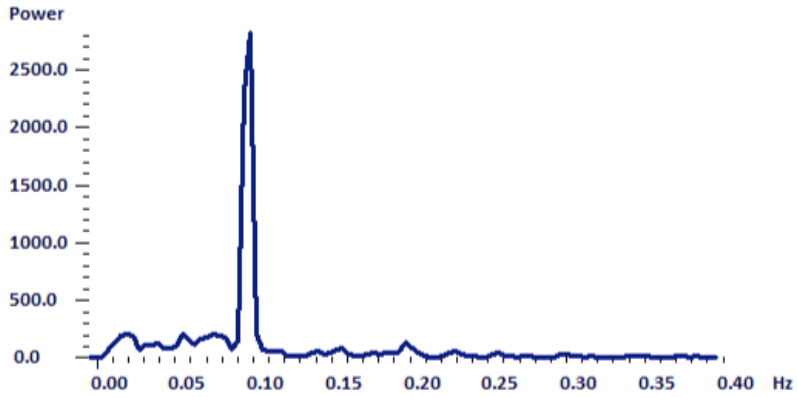

Figure 14. Single participant's power spectrum graph during biofeedback manipulation.

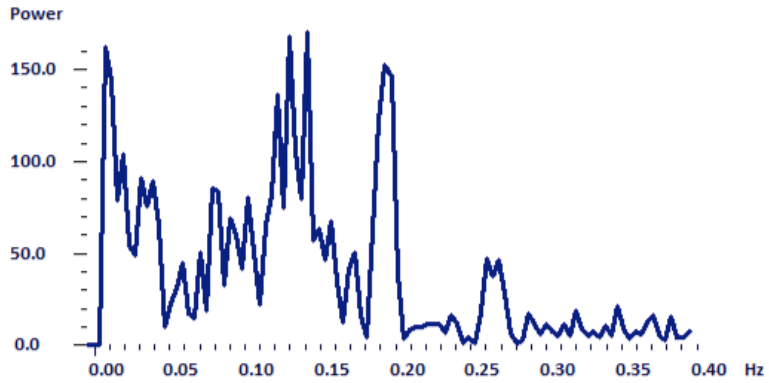

Figure 15. Single participant's power spectrum graph during post-biofeedback.

Maximized baroreceptor activation has been noted to occur at approximately 0.10 Hz and is associated with increased HRV (McCrathy & Shaffer, 2015; Lehrer & Gevirtz, 2014). Previous research on HRV biofeedback noted increases in HRV during the intervention in a wide range of populations including healthy participants and in participants with a variety of psychological or medical disorders (Wheat & Larkin, 2010; Lehrer et al., 2003; Lehrer et al., 2004; Hassett et al., 2007; Karavidas et al., 2007). However, HRV biofeedback has not been extensively studied as an intervention in a pregnant population. In fact, only one study investigated the effects of the intervention on maternal physiology. The study revealed that the intervention increased maternal HRV coherence, indicating improved interactions between the parasympathetic and sympathetic nervous systems (Keeney, 2008). The results from the present study extend previous research to suggest that HRV biofeedback is associated with short-term increases in maternal HRV in a pregnant population.

In the ABAB'A' design (N= 20), HRV increased during biofeedback and remained increased during post-biofeedback compared to baseline, suggesting persisting effects of the HRV biofeedback manipulation on maternal HRV. The lack of significance noted in response to biofeedback II, compared to post-biofeedback, could be attributed to a persisting elevation of maternal HRV from biofeedback. The shortened duration of biofeedback II (biofeedback II was 5 minutes compared to biofeedback that was 10 minutes) may have contributed to the lack of significance in increase of maternal HRV however, this interpretation is not conclusive given the elevated state of HRV following the initial biofeedback. Overall, the results suggest that maternal baroreceptor activation falls under direct and immediate experimental control of the HRV biofeedback manipulation. Maternal HRV appears to be linked to baroreceptor activation, however the changes in maternal HRV that arise from changes in baroreceptor activation may persist and require a HRV biofeedback stimulus for a duration greater than 5 minutes.

Increased HRV is linked to greater variability in inputs from the sympathetic and parasympathetic nervous systems, indicating increased ability of the ANS to quickly shift between the two branches. Increasing HRV through biofeedback has been associated with improved outcomes in patients with anxiety, depression, anger management, and other psychological disorders (Gevirtz, 2013; Francis, Penglis, & McDonald, 2016). The current study investigated short-term, immediate increases in HRV, rather than long-term effects. However, even short-term changes to HRV can have an effect on behavioral outcomes. Previous research suggests an association between short-term increases in HRV and the ability to manage anger (Francis, Penglis, & McDonald, 2016). Pregnancy-induced hypertension was associated with reduced HRV (Flood et al., 2015). The use of HRV biofeedback to target and train baroreceptors to maximize efficiency has the potential to alleviate baroreceptor attenuation during pregnancy and improve maternal cardiovascular and ANS allostasis thereby optimizing conditions for maternal health and fetal development.

The results of the current study provide preliminary evidence that HRV biofeedback is associated with changes in ANS indices that can help target ANS pathologies such as pre-eclampsia in a pregnant population. This intervention is particularly appealing in this population because it is non-invasive, low risk, low cost and can be applied cross-culturally with limited language barriers. Furthermore, the primary technique of HRV biofeedback could be implemented on a smartphone thereby allowing for increased access to the intervention.

Tentative findings from the use of HRV biofeedback in perinatal depression suggests that the majority (81.9%) of mothers continued use of the biofeedback technique at home without the biofeedback equipment (Beckham, Greene, Meltzer-Brody, 2013). This speaks to the wide-ranging accessibility of the underlying technique behind HRV biofeedback. Physiological and psychological changes during pregnancy can cause significant acute and chronic conditions in pregnant women, including hypertension and postpartum depression. The current findings demonstrate activation of the baroreceptor and increases in short-term maternal HRV during biofeedback. Increased activation of the baroreceptor has been associated with improvement in baroreflex gain. Enhancing baroreceptor gain indicates more long-term adaptations of baroreceptor functioning (Lehrer et al., 2003). Increases in HRV have been associated with significant improvements in mental and physical health including reduced depressive symptoms, improved emotion regulation, and more efficient cardiovascular, HPA axis, and ANS functioning (Wheat & Larkin, 2010).

Fetal Implications

The data on HRV biofeedback and fetal behavior (fetal general movement) is characterized by individual differences therefore limiting the scope of generalizing interpretations. The theory underlying the use of maternal HRV biofeedback to affect fetal movement stemmed from the research on maternal-fetal synchrony. The goal was to stimulate the fetal neuro-motor pathways. There was no apriori direction of fetal movement change hypothesized as increases and decreases in fetal movement provide an indication of neuro-motor pathway activation. The initial data analysis revealed no overall change in fetal movement amplitude across 5 time-points (ABAB'A'). The ABAB'A' (4 phase shifts A-B, B-A, A-B', B'-A') did not demonstrate significance and therefore the "3 phase shift" criteria required to demonstrate experimental control was not met (Kratochwill et al., 2010). Taking into account the impact that individual differences may have on fetal movement, individual visual analysis was done for each fetus.

Visual analysis forms part of the APA Division 12 standards and guidelines for single-case research (Chambless & Ollendick, 2001; Hancock, Mueller, & Stapleton, 2010; Cohen, Feinstein, Masuda, & Vowles, 2013). The mean and trend across each of the 5 time-points for each fetus was graphed. Visual analysis revealed varying responses according to the individual

fetus. The main goal of testing the HRV biofeedback manipulation was to determine the feasibility of shifting baseline fetal movement trajectory thereby stimulating fetal neuro-motor pathways. The case-by-case individual analysis of fetal movement revealed shifts in mean and trend of fetal movement associated with implementation and withdrawal of the biofeedback stimulus, however these changes were not consistent across the 5 time-points or across all fetuses. Furthermore, visual analysis of a fetus in which the mother did not meet the breathing or baroreceptor activation criteria of the biofeedback, revealed changes in fetal movement. In this case, even though biofeedback criteria were not met, maternal breathing was decreased during biofeedback compared to baseline and this may account for the fetal movement changes.

The fetal movement findings necessitate further investigation on the implications of using HRV biofeedback as a stimulus to activate fetal neuro-motor pathways. The neurotrophic hypothesis outlines a balance between programmed cell death and strengthening of neural pathways during fetal development (Buss, Sun, & Oppenheim, 2006). Given the association between HRV biofeedback and changes in fetal movement, it is feasible that this manipulation can be used to activate fetal neural-motor pathways, thereby shifting the balance towards strengthening of these pathways and increasing the fetus' range of responding to stimulus. Given the changes in fetal movement noted in a mother that did not meet biofeedback criteria, it is plausible that simply decreasing maternal breathing can impact fetal movement. Although, our results indicate that the biofeedback technique (a stimulus technically applied to the mother) can induce shifts in fetal movements, further investigation is necessary to determine if the biofeedback provides additional changes to fetal movements beyond simply decreasing maternal breathing. Greater activation of a neural pathway provides these pathways a greater chance of survival during the prenatal period, allowing the fetus a broader repertoire of neurobehavioral responses with the potential of more adaptive functioning post-birth. A broader range of neurobehavioral responding post-birth has the potential to impact areas of physiological and psychological adaptation such as emotion regulation and cognitive flexibility.

Limitations and Future Directions

The current study did not investigate possible links between maternal and fetal reactions to the biofeedback stimulus. While maternal-fetal synchrony is often viewed in terms of maternal effects on the fetus, research indicates a bi-directional relationship. Research revealed

that fetal movement consistently preceded generation of a maternal sympathetic response. Maternal heart rate and skin conductance were positively correlated with fetal movement at a 2 and 3 second delay respectively (DiPietro, Irizarry, Costigan, & Gurewitsch, 2004). Research investigating maternal-fetal synchrony must factor in a bi-directional relationship with time lag effects. The bi-directional and time-dependent nature of the maternal-fetal relationship indicate that maternal-fetal synchrony cannot be simplified to a "stimulus-effect" relationship. The methodology of the current study was designed to investigate "direct replication" and Stage I of generalizability as outlined in guidelines for single case study design (in Hancock, Mueller, & Stapleton, 2010; Graham, Karmarker, & Ottenbacher, 2012*)*. In order to place the results of the current study within the context of empirically supported therapies the APA Division 12 criteria for Empirically Supported Therapies was used (Chambless & Ollendick, 2001). There are 3 main areas to place within the context of these guidelines:

1. Maternal Baroreceptor activation
2. Maternal SDNN increase
3. Fetal Movement (Behavioral) Changes

APA Division 12 guidelines for testing an intervention (Chambless & Ollendick, 2001; pg. 689) are outlined in Table 11.

Table 11.

Outline of APA Division 12 Task Force Criteria

	Maternal Baroreceptor Activation	Maternal SDNN Increase	Fetal Movement (Behavioral) Changes
Well-established treatments			
II. A large series of single-case design experiments (n = 9) must			

demonstrate efficacy with:			
A. Use of good experimental design (See Table 15) and	Within subject design outlined in methods and supported by guidelines and previous research	Within subject design outlined in methods and supported by guidelines and previous research	Within subject design outlined in methods and supported by guidelines and previous research
B. Comparison of intervention to another treatment	no published research found	no published research found	Compared to DiPietro, Costigan, Nelson, Gurewitsch, & Laudenslager, 2008; No significant findings in current study
III. Experiments must be conducted with treatment manuals or equivalent clear description of treatment	Outlined in methods section	Outlined in methods section	Outlined in methods section
IV. Characteristics of samples must be specified	Outlined in methods section	Outlined in methods section	Outlined in methods section
V. Effects must be demonstrated by at least two different	Next phase of	Next phase of	Next phase of

investigators or teams	study	study	study	

For II A ("good experimental design"), the single-case (within-subject) design was employed in the current study and the APA Division 12 Task Force on Psychological Interventions analysis standards and guidelines for Single Case Research was used as a guide. According to APA Division 12 Task Force on Psychological Interventions (Table 12) (Cohen, Feinstein, Masuda, & Vowles, 2013; pg. 128).

Table 12.
Analysis Standards and Guidelines for Single Case Research (Cohen, Feinstein, Masuda, & Vowles, 2013; pg. 128)

	APA Division 12 Task Force on Psychological Interventions	**Maternal Baroreceptor Activation**	**Maternal SDNN Increase**	**Fetal Movement (Behavioral) Changes**
1. Visual analysis	Acceptable (no specific guidelines or procedures offered)	N/A	N/A	MATLAB (changes in mean and trend; no consistent pattern across fetuses)
2. Statistical Analysis Procedures	Preferred when the number of data points warrants statistical procedures (no specific guidelines or procedures offered)	Repeated measures ANOVA	Repeated measures ANOVA	Repeated measures ANOVA
3. Demonstrating an Effect	ABAB- stable baseline establishing during first A period, data must show improvement during the first B period, reversal or leveling of improvement	Demonstration of effect across four phase shifts (6 non-sequential phase shifts)	Demonstration of effect across one phase shift (2 non-sequential phase shifts)	No effect

	during the second A period, and resumed improvement in the second B period (three phase shift) (no other guidelines offered)			
4. Replication	1. 3 replications of ≥ 3 subjects each *2. Replications conducted by ≥ 2 independent research groups	1. Satisfied (Repeated measures ANOVA) *2. Not satisfied	1. Not satisfied *2. Not satisfied	1. Mixed Findings (MATLAB) *2. Not satisfied

*Next phase in study

Within subject designs testing an intervention are composed of three stages of "generalizability" in order to strengthen the applicability of findings (Table 13).

Table 13.

Outline of 3 Stages of Establishing Generalizability in Small-N Research (Graham, Karmarker, & Ottenbacher, 2012)

	Stage Definition	Current Study
Stage I	"…the accumulation of a number of direct replication of specific treatment effect on 1 well-defined outcome measure within a defined clinical setting. In this form of replication, participants are matched as closely as possible on subject characteristics. The aim is to establish, as clearly as possible, that a given intervention can have an effect on a certain kind of patient within a specific setting. If a series of direct replication small-N	Criteria met for maternal baroreceptor activation and increase HRV to move to Stage II. Criteria not met for fetal movement to move to Stage II.

	studies produces consistently positive results, then the replication process moves to the next level."	
Stage II	"… the systematic replication of the treatment across various participants, settings, clinicians, or a combination of these. Systematic replication helps to establish the generality of the findings over a wider range of situations than direct replication."	Next stage of study.
Stage III	"Clinical replication involves establishing the generality of related components of the intervention. These might include issues such as intensity or duration or combing multiple components of the intervention and testing them across various patients and settings."	Duration component investigated in this study. Baroreceptor activation associated with 5- and 10-minutes biofeedback. Evidence suggests that HRV increase requires 10 minutes biofeedback.

Stage I-III of the generalizability outlined above match the guidelines for replication according to *The Reviewer's Guide to Quantitative Methods in the Social Sciences* (Hancock, Mueller, & Stapleton, 2010). Specifically, Stage I encompasses the "direct replication" while Stages II-III deal with the "systematic replication." The current findings allow for progression to Stages II and III in order to test systematic replication of the effects of HRV biofeedback on the maternal baroreceptor. However, the fetal findings from the current data are not as conclusive as the maternal findings and therefore warrant further investigation at Stage I (direct replication).

Conclusion

Optimizing maternal physiological conditions during pregnancy can have a significant positive impact on maternal health as well as fetal development. Adaptability in fetal movement, particularly in response to stimuli and the ability to control movement, parallel developmental changes in the fetus, specifically neurological changes. The current findings provide evidence that HRV biofeedback can be used as a non-invasive, low-cost intervention to activate the maternal baroreceptor with implications for restoring baroreceptor attenuation during pregnancy and mitigating the maladaptive effects of maternal hypertension and preeclampsia. The findings for the effects of HRV biofeedback on fetal movements reveal high variability between fetuses and even across non-intervention and intervention phases within individual fetuses, necessitating further research in this area before promoting the use of this intervention to stimulate fetal movement changes.

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Appendix A

APA Division 12 Criteria for Empirically Supported Therapies (Chambless & Ollendick, 2001; pg. 689)

Well-established treatments

I. At least two good between-group design experiments must demonstrate efficacy in one or more of the following:

A. Superiority to pill, or psychotherapy placebo, or to other treatment

B. Equivalence to already established treatment with adequate sample sizes

OR

II. A large series of single-case design experiments must demonstrate efficacy with:

A. Use of good experimental design and

B. Comparison of intervention to another treatment

III. Experiments must be conducted with treatment manuals or equivalent clear description of treatment

IV. Characteristics of samples must be specified

V. Effects must be demonstrated by at least two different investigators or teams

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Probably efficacious treatments

I. Two experiments must show that the treatment is superior to waiting-list control group

OR

II. One or more experiments must meet well-established criteria IA or IB, III, and IV above but V is not met

OR

III. A small series of single-case design experiments must meet well-established-treatment criteria

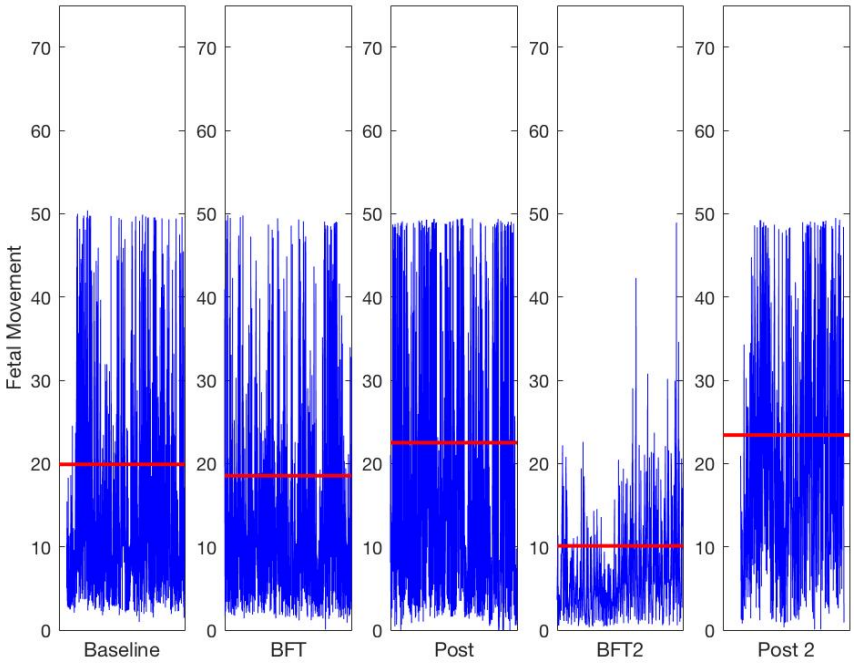
Appendix B

Outline of 3 Stages of Establishing Generalizability in Small-N Research (Graham, Karmarker, & Ottenbacher, 2012)

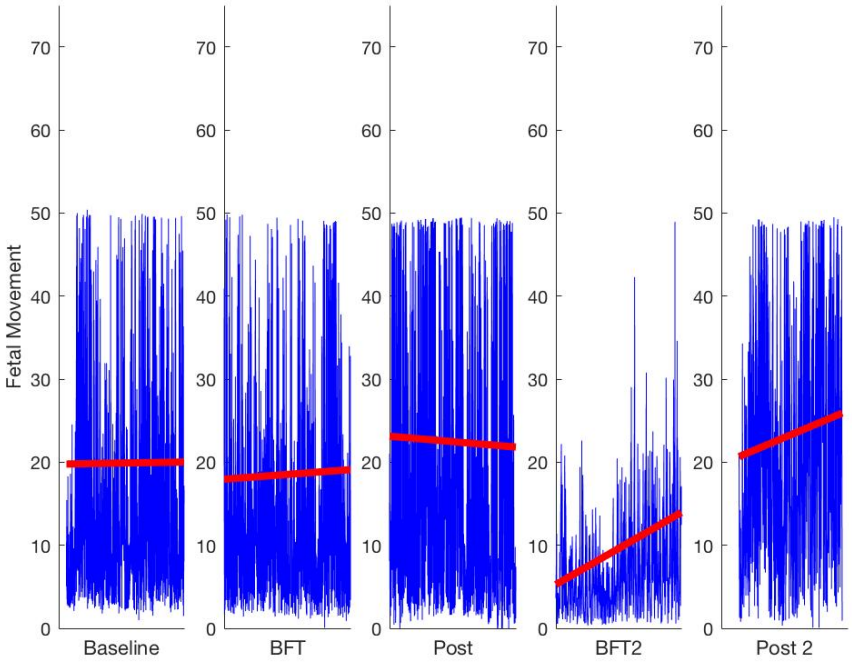
	Description
Stage I	***"…the accumulation of a number of direct replications of specific treatment effect on 1 well-defined outcome measure within a defined clinical setting. In this form of replication, participants are matched as closely as possible on subject characteristics. The aim is to establish, as clearly as possible, that a given intervention can have an effect on a certain kind of patient within a specific setting. If a series of direct replication small-N studies produces consistently positive results, then the replication process moves to the next level."***
Stage II	"… the systematic replication of the treatment across various participants, settings, clinicians, or a combination of these. Systematic replication helps to establish the generality of the findings over a wider range of situations than direct replication."
Stage III	"Clinical replication involves establishing the generality of related components of the intervention. These might include issues such as intensity or duration or combing multiple components of the intervention and testing them across various patients and settings."

Appendix C

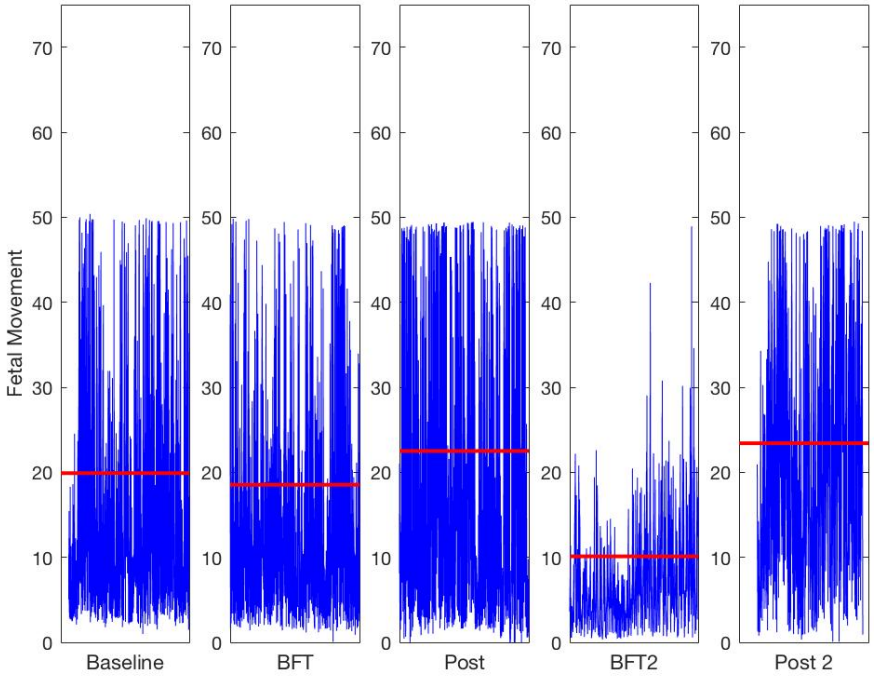
Fetus 6 (23 weeks)- Mean



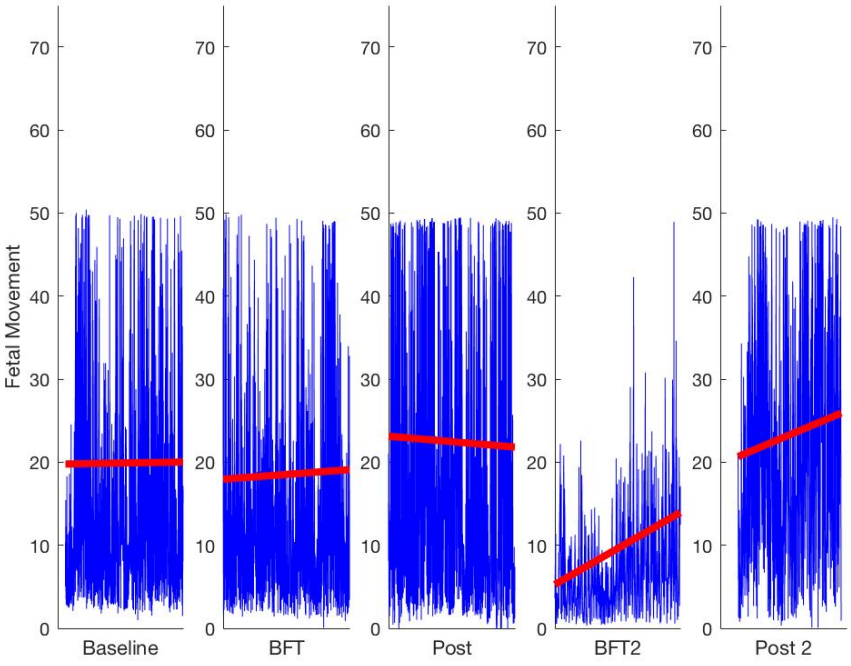
Fetus 6- Trend



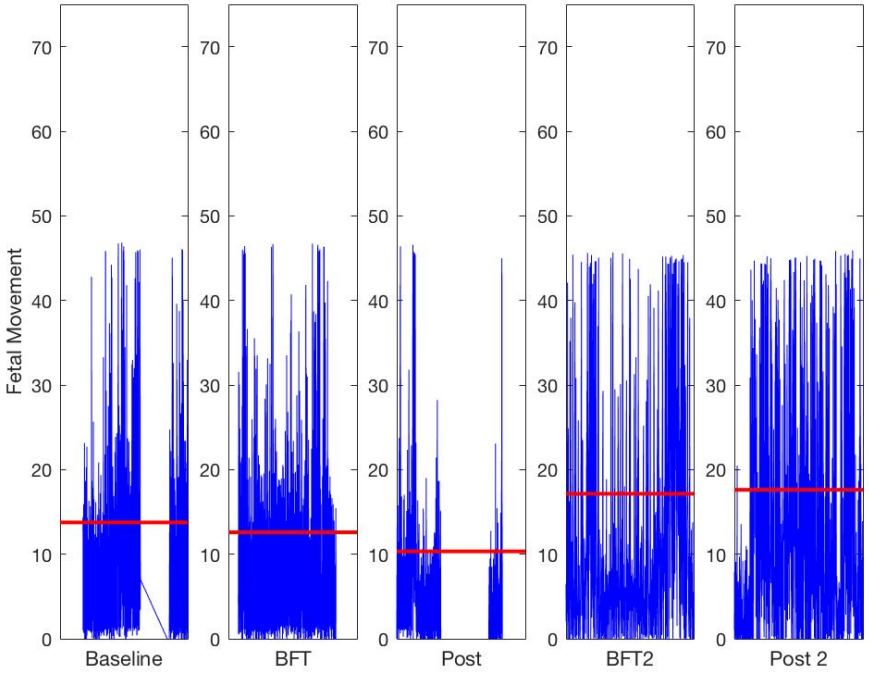
Fetus 7 (26 weeks)- Mean



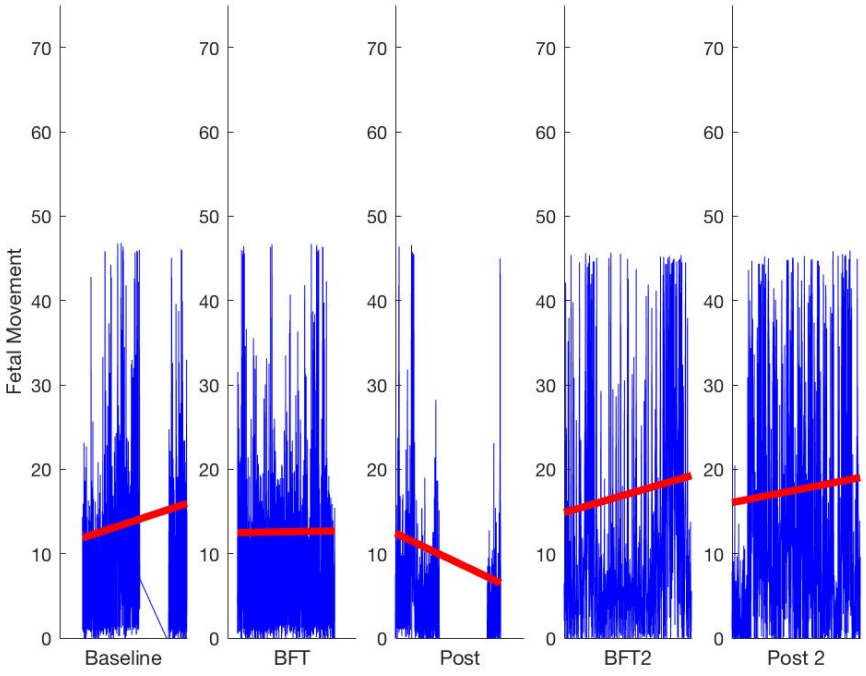
Fetus 7- Trend



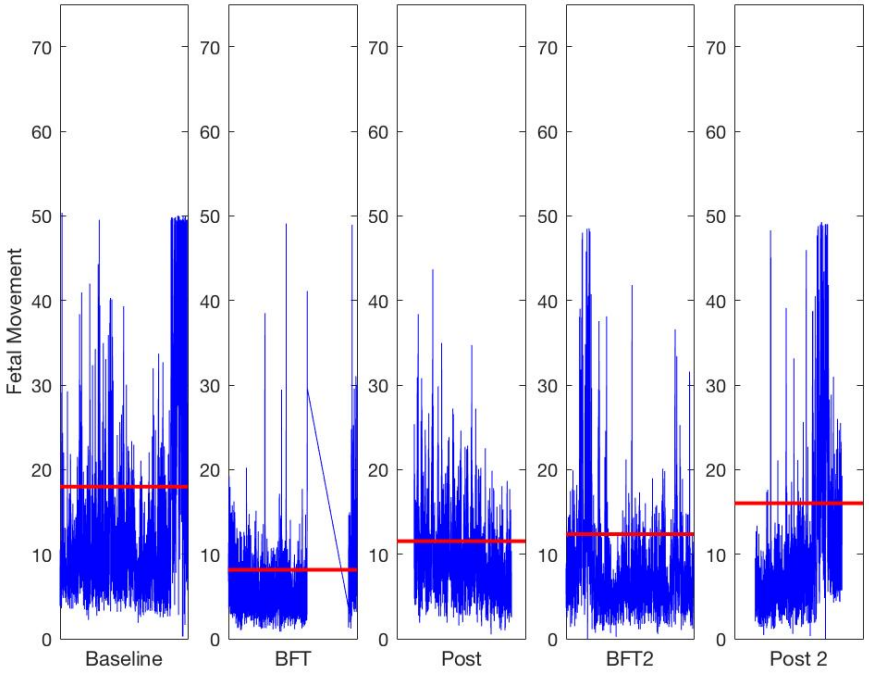
Fetus 8 (33 weeks)- Mean



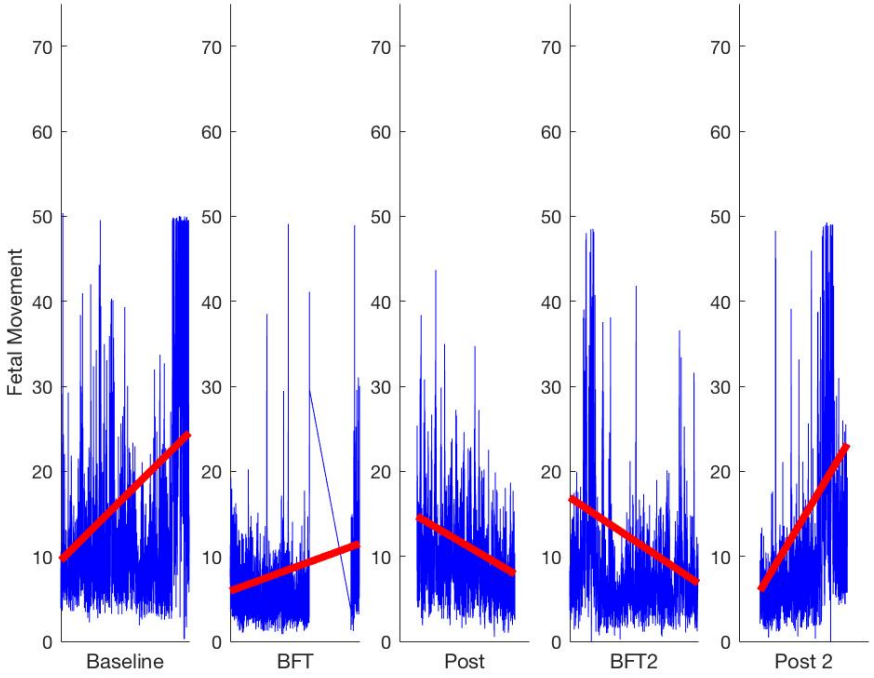
Fetus 9- Trend



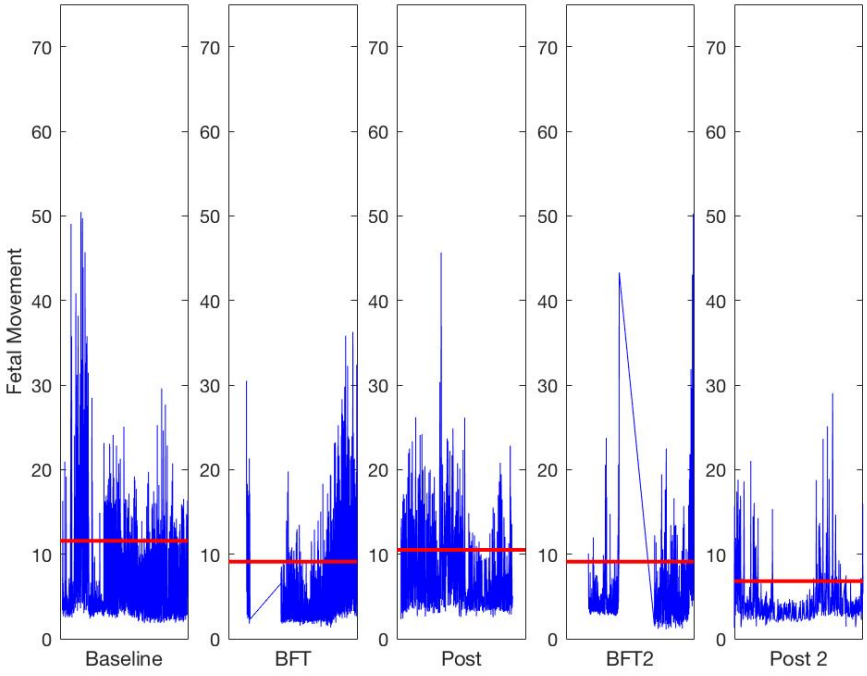
Fetus 9 (31 weeks)- Mean



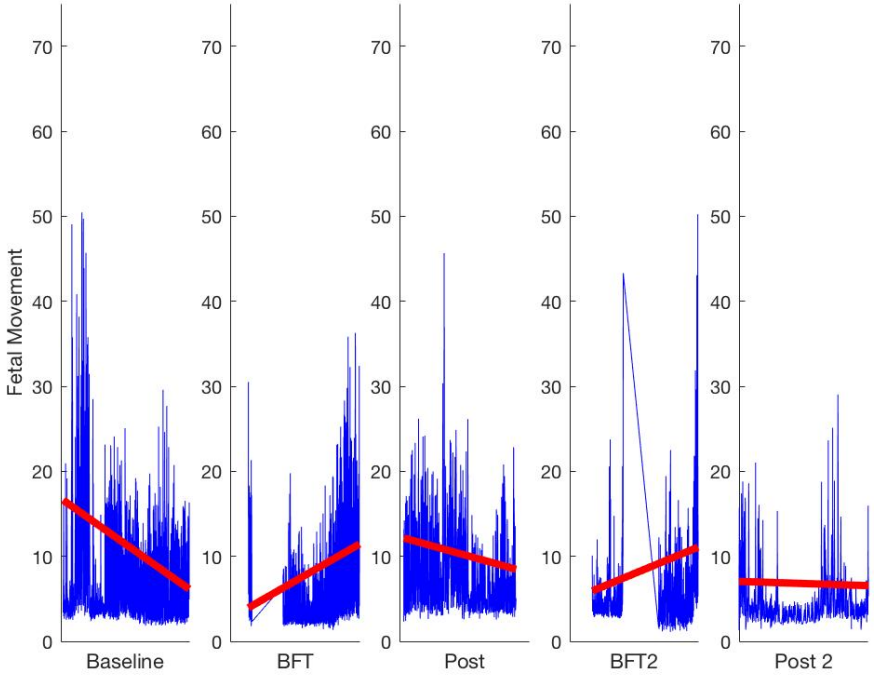
Fetus 9- Trend



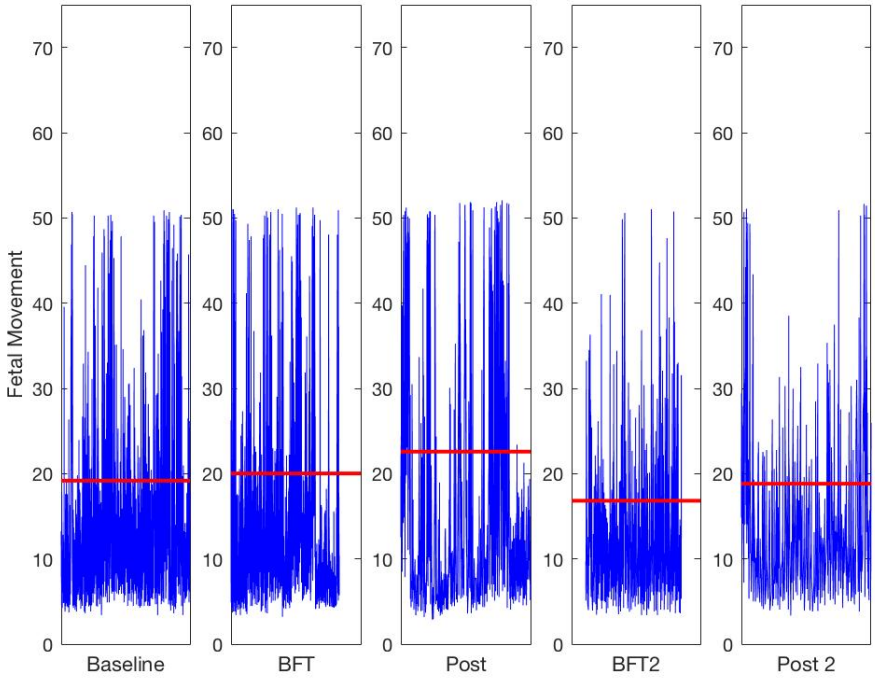
Fetus 10 (24 weeks)- Mean



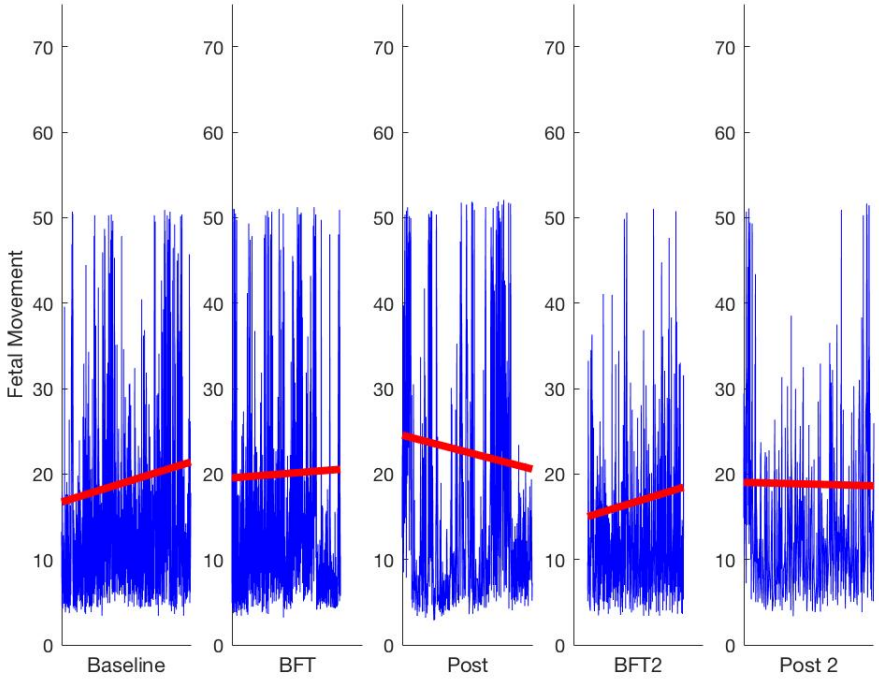
Fetus 10- Trend



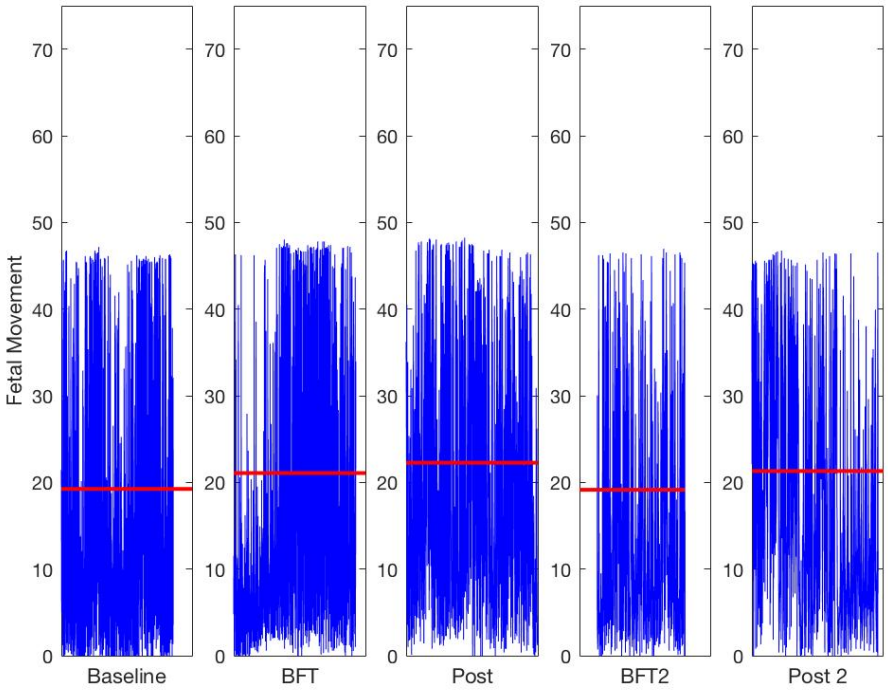
Fetus 11 (38 weeks)- Mean



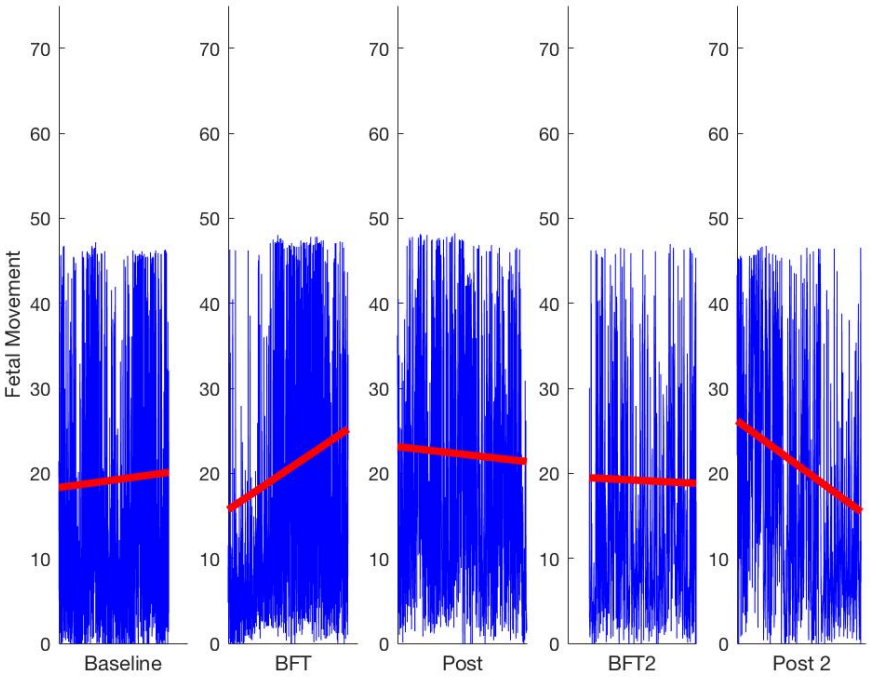
Fetus 11- Trend



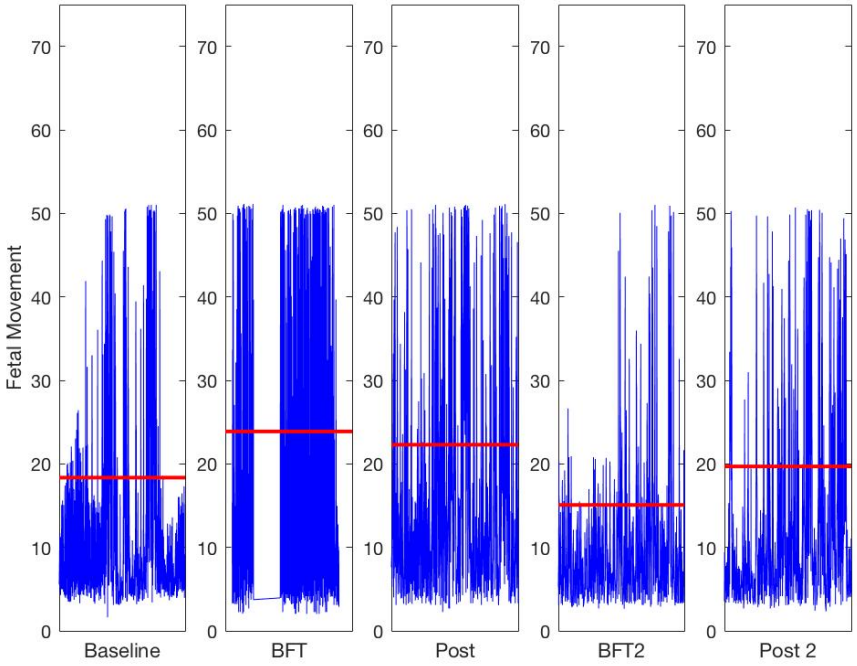
Fetus 12 (23 weeks)- Mean



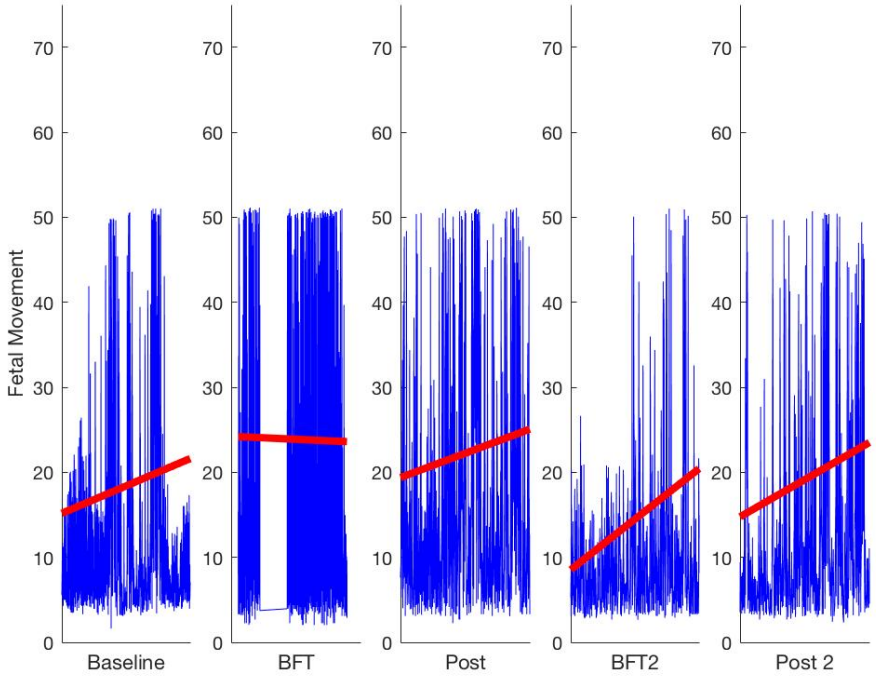
Fetus 12- Trend



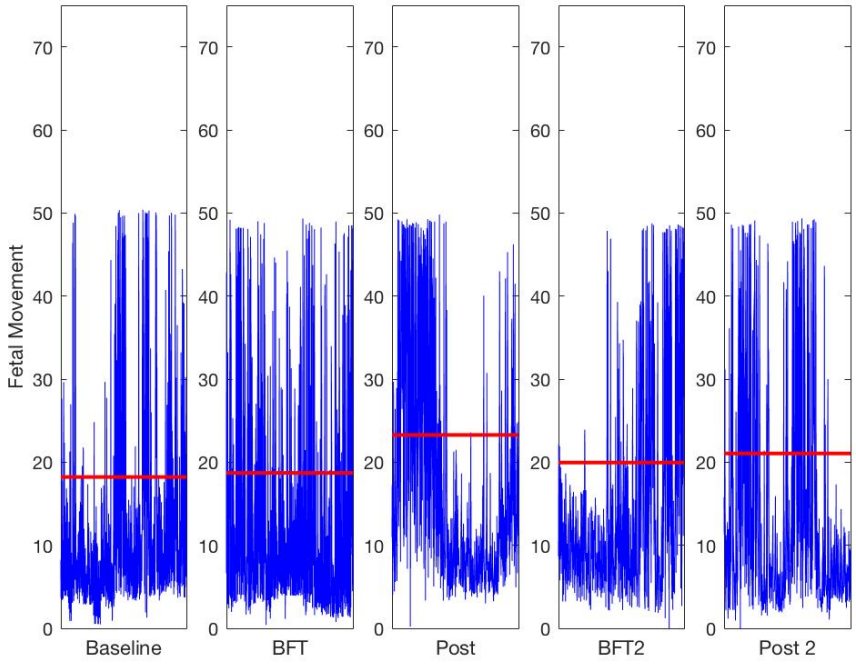
Fetus 13 (27 weeks)- Mean



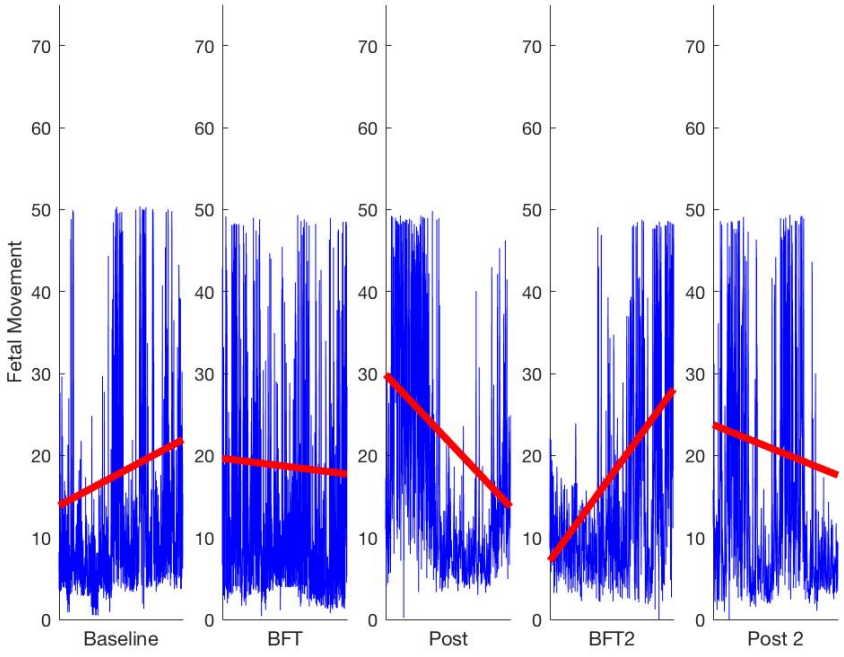
Fetus 13- Trend



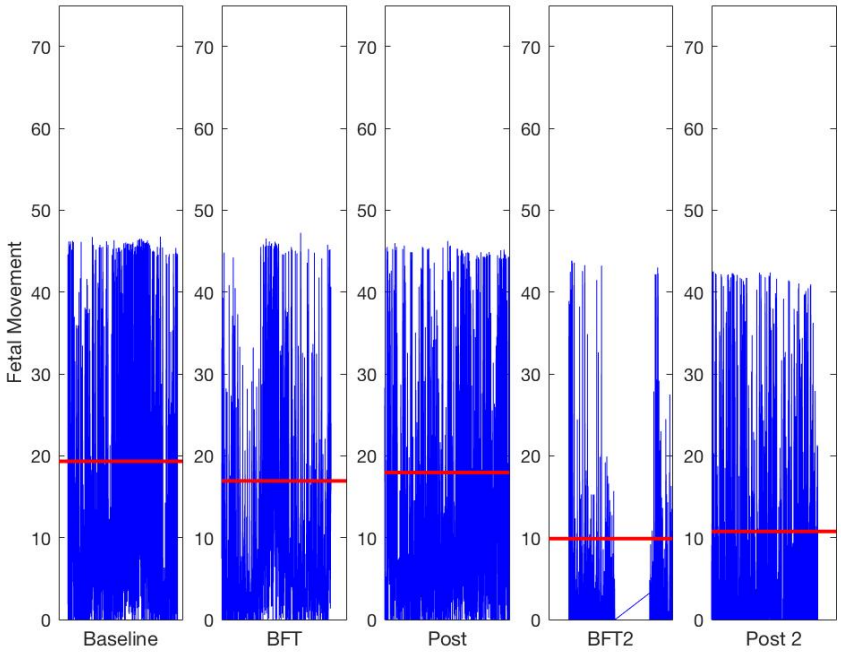
Fetus 14 (29 weeks)- Mean



Fetus 14- Trend



Fetus 15 (29 weeks)- Mean



Fetus 15- Trend