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Whitney Lew 4/1/2014

# Early Neurobehavioral Development: Neuromotor Integration and Prenatal Markers of Growth

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An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
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Abstract

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The detection of neurobehavioral factors in the neonate that indicates normal fetal development has been vital in suggesting the potential neurological maturity of the infant into adulthood. Fetal measures are used as markers of nervous system development in pregnancies. The fundamental question of this study is specifically, what are the trends of cerebral blood flow and fetal movement across gestational age? In this study, cerebral blood flow and fetal movement are looked at as fetal markers of prenatal neurobehavioral development. The neuromotor integration period at around 28 gestational weeks is proposed as a point of dynamic change in development. Background literature suggests a lateralization of cerebral blood flow velocity (CBFV) trends, with the left hemisphere increasing at a faster rate of CBFV up to 28 weeks, and the right hemisphere having a higher CBFV starting from 28 gestational weeks. Research on fetal movement suggests that brief and spontaneous movements decrease over time and the frequency of whole-body movements increase. Results yielded no significant relationship between cerebral blood flow velocity and fetal movement, as the theoretical model would suggest. However, there was a relationship between the left and right hemisphere for cerebral blood flow, and the CBFV of left and right hemispheres crossed over at approximately 28-32 gestational weeks. There was a significant relationship among fetal movements, with 28-32 gestational weeks being a period of inflection for frequency trends. The results suggest that 28-32 gestational weeks may be a time of neuromotor integration.

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# Early Neurobehavioral Development: Neuromotor Integration and Prenatal Markers of Growth

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Abstract

The detection of neurobehavioral factors in the neonate that indicates normal fetal development has been vital in suggesting the potential neurological maturity of the infant into adulthood. Fetal measures are used as markers of nervous system development in pregnancies. The fundamental question of this study is specifically, what are the trends of cerebral blood flow and fetal movement across gestational age? In this study, cerebral blood flow and fetal movement are looked at as fetal markers of prenatal neurobehavioral development. The neuromotor integration period at around 28 gestational weeks is proposed as a point of dynamic change in development. Background literature suggests a lateralization of cerebral blood flow velocity (CBFV) trends, with the left hemisphere increasing at a faster rate of CBFV up to 28 weeks, and the right hemisphere having a higher CBFV starting from 28 gestational weeks. Research on fetal movement suggests that brief and spontaneous movements decrease over time and the frequency of whole-body movements increase. Results yielded no significant relationship between cerebral blood flow velocity and fetal movement, as the theoretical model would suggest. However, there was a relationship between the left and right hemisphere for cerebral blood flow, and the CBFV of left and right hemispheres crossed over at approximately 28-32 gestational weeks. There was a significant relationship among fetal movements, with 28-32 gestational weeks being a period of inflection for frequency trends. The results suggest that 28-32 gestational weeks may be a time of neuromotor integration.

# Early Neurobehavioral Development: Neuromotor Integration and Fetal Markers of Development

Markers of nervous system development during fetal life have been the focus of many studies (Amiel-Tison, Gosselin, & Kurjak, 2006; DiPietro, Borstein, Costigan, Pressman, Hahn, Painter, & Yi, 2002; Emory 2010; de Vries and Wong, 2006; Luchinger, Hadders-Altra, van Kan, & de Vries, 2008). Typical development of the prenatal nervous system includes closure of the neural tube, growth of sensory organs and limb buds (Tau & Peterson, 2010), proliferation and migration of cells to their final destination (Prayer, Kasprian, Krampl, Ulm, Witzani, Prayer, & Brugger, 2006), primitive movements, respiratory movements (Grant-Beuttler, Glynn, Salisbury, Davis, Holliday, & Sandman, 2011), and reactions of sensory stimuli (Picciolini, Porro, Meazza, Gianni, Rivoli, Lucco,, Barretta, Bonzini, & Mosca, 2014). These are just some of the developmental changes that occur across gestation.

Research on fetal development includes both observable and measurable factors of function. Emerging prenatal behaviors are considered to be markers for fetal nervous system development and fetal adaptation to conditions in the womb (Prechtl, 1984). Reliable functional aspects include heart rate (Signorini, Fanelli, & Magenes, 2014), motor behavior (Feng, Raynor, Fiano, & Emory, 1997), and reactivity to stimulation (Grant-Beuttler, Glynn, Salisbury, Davis, Holliday, & Sandman, 2011). These related domains develop in predictable ways over gestational age, with increasing complexity and coordination. An example is that although heart rate and motor behavior are expressed with coordination at the 20th gestational week, it's not until later that patterns of heart rate, eye movements, and fetal movements are combined into ways that reflect discernable sleep and waking states in the neonate (Mirro & Gonzalez, 1987). The individual functions coalesce into a phenotypic behavior.

The term neurobehavior defines phenotypic, behavioral and autonomic expressions of basic human functioning (Brazelton, 1984). The early stages of pregnancy, from five to 24 gestational weeks, are particularly important for neurobehavioral development (Zhu, Sun, Hao, Chen, Jiang, Tao, Huang, & Tao, 2014; Prayer et al., 2006; Kasprian, Langsz, Brugger, Bitter, Weber, Arantes, Mavilde, Prayer, 2010). From the fifth gestational week, complex brain components begin to form, and by the ninth gestational week, general movements in the fetus can be observed, implicating the development of the neuromotor nervous system (Zhu et al., 2014; Prayer et al., 2006). During gestation the fetus is highly sensitive to maternal conditions, (Zhu et al., 2014; Prayer et al., 2006, DiPietro et al., 2002). For example, congenital anomalies and intrauterine growth restriction (Zhang, Merialdi, Platt, & Kramer, 2010; Reeves & Bernstein, 2008; Cooke, 2007) reflect exposure to maternal use of substances and diet (Gruszfeld & Socha, 2013; Merritt, Mazela & Merritt, 2013; Goettler & Tschudin, 2014), maternal stress (DiPietro, Costigan, & Gurewitsch, 2003), and environment contaminants (DiPietro et al., 2002). The focus of the current study is to characterize trends in cerebral blood flow and fetal movement during prenatal development.

**Sonograph Technology and Neurobehavioral Development**

A historical challenge in prenatal developmental studies is that the fetus cannot be directly touched, seen, or felt because it is in the womb. The detection of fetal maturation was done to the extent of measuring fetal heart rate through the stethoscope, and measuring fetal movement through a polygraph transducer (Sontag & Wallace, 1936). However, with these techniques, it was difficult to find accurate results because it required the amplitude of the heart rate and fetal movement to be strong enough for detection. This challenge caused many studies to rely on self-reporting from the mother (Welford & Sontag 1969). However, there were many

inaccuracies with self-reporting as well. The challenge of finding a window into the womb has been diminished with the development of ultrasound technologies in the 1970's (de Vries & Fong, 2006), which allowed for a controlled, accurate, and scientific approach to fetal visualization.

The initial use of 2-dimensional ultrasound scanning allowed researchers to view specific behavioral patterns across gestational age. These observations included fetal movements, which vary in length and strength across gestation. With the recent advent of 3-dimensional and 4-dimensional ultrasound scanning, sonographers have even detected facial expressions in a real time (de Vries & Wong, 2006; Filho, Araujo, Fernando de Mello, Nardozza, & Moron, 2012). Other newly developed functional tests such as the tilting test, CO2 reactivity, phase shift and m/r-wave analysis has further improved the understanding of the autonomic nervous system over the last few years (Ipsiroglu, Eichler, Stoeckler-Ipsiroglu, 1999). However, much of this technology is still being developed for more efficient application and use in research.

Ultrasound imaging is not limited to revealing fetal movement and behavior, but can be adapted to depict cerebral blood flow trends. Hemodynamics in the brain and vascular regions are most accurately measured by the Doppler ultrasound, (Wu, Hsieh, Hsu, Chiu, Chou, Chen, Peng, Hung, Chang, Chen, & Jeng, 2013), which allows investigators to get extensive information about blood flow in specific areas in the fetal brain (Wu et al., 2013; Emory, 2010; Feng et al., 1997).

This study uses 2-dimensional and Color Doppler ultrasound technology to visualize and record fetal nervous system development in normal and healthy populations. The current study describes a developmental trajectory of cerebral blood flow velocity in the major arteries of the Circle of Willis from the $20^{th}$ gw to the $40^{th}$ gw in a sample of healthy human fetuses. This

technology combines two-dimensional and Color Doppler ultrasonography, so that specific areas of the brain can be visualized.

**Cerebral Blood Flow**

There are a small number of studies that have examined fetal cerebral blood flow velocity across gestation in normal populations. However, there is a lack of definite conclusions primarily due to small sample sizes. The collective research to date supports a positive linear relationship between gestational age and cerebral blood flow velocity in the middle cerebral artery (Feng, et al., 1997; Kurmanavichius, Karrer, Hebisch, Huch, & Huch, 1991; see Emory, in press). There is also a significant pattern in the cerebral blood flow velocity (CBFV) between right and left hemispheres of the Circle of Willis as described in detail below.

The Circle of Willis is an arterial ring located at the base of the brain that serves as a protective barrier from hemodynamic pressure. Intracranial pressure and volume of blood have an exponential relationship, meaning that increased intracranial pressure will cause an increase in blood flow (Vrselja, Brkic, Mrdenovic, Radic, & Curic, 2014). This may be one of the reasons that velocity of cerebral blood flow shifts in dominance between the right and left hemispheres throughout gestation.

The left hemisphere is dominant in cerebral blood flow at 20-22 week gestation, whereas right hemisphere CBFV is dominant at parturition (Hering-Hanit, Achiron, Lipitz & Achiron 2001). Once the fetus is at term, cerebral blood flow velocity significantly decreases (Greisen, 1997; Kurmanavichius, et al., 1991). There is a shift in cerebral dominance from the right to left hemisphere after birth (Feng, et al., 1997; Lust, et al., 2011). The timing of this postnatal shift corresponds well with functional limb asymmetries and the emergence of complex linguistic abilities in young children (Previc, 1991; Lust, et al., 2011). There seems to a relationship

between the hemispheres in blood flow dominance, as well as shift in motor functioning around the same time those shifts occur. Fetal movements describe motor functioning in the neonate, which is addressed in the next section.

Evidence supports the idea that the shifts of brain laterality underlie the development of the cognitive processes and motor control. Examples of hemispheric specialization showing this brain-behavior relationship include the preference of hand dominance in early life (Kaufman, Zalma, & Kaufman, 1978) and the development of language skills (Previc, 1991). Cerebral blood flow velocity is also correlated with behavioral state changes in newborns, with blood flow velocities increasing when the child is awake, and decreasing when asleep (Hill, Hogan, Onugha, Harrison, Cooper, McGrigor, Datta, & Kirkham, 2006). These findings draw attention to fundamental changes in the pattern of cerebral blood flow velocity dominance, as it may underlie lateral shifts related to important cognitive and behavioral developments in early life.

Cerebral blood flow velocity is sensitive to environmental conditions in the womb and to the overall health of the mother. Normal fetal brain development relies on sufficient intracranial blood flow, which depends on specific mechanisms such as carbon dioxide, oxygen, and hydrogen ions to guarantee perfusion of the central nervous system (CNS) (Feng et al., 1997). Placental insufficiencies are related to anomalies in fetal brain circulation, which is related to fetal hypoxia, fetal anoxia, neonatal death, and fetal growth restriction (Hernandez-Andrade, Serralde, Cruz-Martinez, 2012; Nomura, Niigaki, Horigome, Francisco, and Zugaibm, 2013; Nardozza, Araujo, Barbosa, Caetano, Lee, and Moron, 2012). Most of these are related to complications with the cerebrovascular resistance. Thus, most studies are inclined to focus upon abnormal or at-risk populations and not normal healthy individuals due the clinical relevance of fetal cerebral blood flow for neurologic deficits (Veille, Hanson, & Tatum, 1993).

**Fetal Movement**

Fetal movement (FM) is a basic marker of individual differences in temperament after birth as it prepares the infant for postnatal adaptation in neurologic circuitry and musculature (Saudino & Eaton, 1991). Fetal movements can be a strong indicator of fetal neurodevelopment. Abnormal movements in preterm infants are related to worse motor, language and cognitive outcomes in ages 2 and 4 (Spittle, Spencer-Smith, Cheong, Eeles, Lee, Anderson & Doyle, 2013). Abnormal movements are defined as rigid, not very complex and unsynchronized, chaotic fetal movements (Spittle et al., 2013).

The earliest movements of the fetus consist of slow, small, noncomplex, and isolated movements of proximal parts of body. The first sign of movement begins at the 7$^{th}$ gw when the fetus is able to make a small movement of the head (Luchinger et al., 2008). From the 9$^{th}$ gw there is an increase of general FM with variation and complexity (Luchinger et al., 2008). From the 13$^{th}$ to 16$^{th}$ gw, there is a surge of strong and sudden movements and low and inert movements in the body and extremities at the 17$^{th}$ to 18$^{th}$ gw (de Vries and Fong, 2008). By the 18$^{th}$ gw, the mother can begin to feel the fetal movement (Fai, Singh, Malcus, Biswas, Arulkumaran & Ranam, 1996). Evidence suggests that the overall activity level (movement x duration) of movements declines over gestation (DiPietro et al., 2002). There is also a decrease in the length of time each movement is exhibited from the 20$^{th}$ to 26$^{th}$ gw (Roodenburg, Wladimoroff, van Es, & Prechtl, 1991). Along with the decrease in the length of FM, there is an increase in the interval time between each FM (D'Elia, Pighetti, Moccia, & Santangelo, 2001).

Throughout gestation, FM progresses from uncoordinated movements to more integrated and fluid behavior patterns, and there is a decrease in motor vigor over time. Movements at the 36$^{th}$ gw are not as sporadic and frequent compared to that of those at the 28$^{th}$ gw (Grant-Beuttler

et al., 2011). The decrease of FM correlates with the increase of gestational age and maturation (Grant-Beuttler et al., 2011). It is important to understand that short-duration isolated movements dominate fetal movements in early gestation, and whole-body movements dominate fetal movements in late gestation (Hata, Kanenishi, & Sasaki, 2010). Whole-body movements are conceptualized as a combination of isolated body movements (Hata et al., 2010). Moreover, over time, motor responses are more distinguished and complex, suggesting greater influence of higher order processes, such as learning (Grant-Beuttler, 2011). Jerky, large movements are characteristic of earlier gestation (before and around 26 gw), while smooth, large limb movements can be exhibited in later gestation (36 gw) (Grant-Beuttler, 2011). Thus, as gestational age increases, there is a decrease in shorter duration, high magnitude, jerky movements, and an increase in longer duration, fluid, smooth movements. There are more complex and coordinated movements as fetus comes to term, which is a crucial indicator of motor development and neurological development.

In this study, we are categorizing each movement by the length of time it is observed. The observations of fetal movements are categorized into four types: the isolated spike, isolated cluster, epoch, and episode. There is the isolated spike, which is a quick, jerky movement exhibited for less than 3 seconds. The isolated cluster is a movement that lasts from 3 to 10 seconds. The epoch lasts from 10 to 30 seconds, and lastly, the episodes are movements longer than 30 seconds. Unlike, previous research, our method of fetal movement measurement does not look at the vigor or define types of movements based on vigor. Rather, we are strictly looking at the frequency of each type of movement. If we compare our measurements to previous research we can say that the frequency of movements short in length (isolated spike and isolated cluster)

would decrease over gestation, while the frequency of movements longer in length (episodes and epochs) would increase over gestation.

There are limitations of using FM as a clinical indicator of neurobehavioral development because the inconsistent measuring techniques across studies make it difficult to compare results (ten Hof et al., 1999; Winje, Roislien, & Froen, 2012; Luchinger et al., 2008; de Vries and Fong, 2006). The way in which fetal movement is measured varies according to how each researcher chooses to categorize certain movements. Some studies focus on the number of movements (Winje et al., 2012), while others focus only on the strength of the movement (Luchinger et al., 2008). The lack of consistency makes it difficult to see clear trends across studies. From the collected background literature, we have not been able to find another study that has used our method of fetal movement measurement. It is possible that the absence of supporting literature in regard to the time length of movements may have resulted from findings in contention with our proposed hypothesis.

**Neuromotor Integration Period**

The neuromotor-integration period is defined by a period of rapid growth of major biological functions like heart rate, muscle flexion, and respiration, as well as evolving patterns of fetal movement (Emory & Israelian, 1998). It is therefore hypothesized that developmental changes in cerebral blood flow velocity and fetal movement may be directly related to one another or dependent on one another. Emory and Israelian suggest the existence of a neuromotor integration stage as a critical period beginning at the $26^{th}$ gw and ending around the $28^{th}$ gw. Starting from the $28^{th}$ gw, the fetus begins to assume more coordinated movements. DiPietro (2005) also mentions a transitional period starting at 28 gestational weeks (gw), during which the fetus becomes more neuro-behaviorally stable. Starting from the $28^{th}$ gw, phases of bodily

activity and rest become increasingly linked to definite parameters of fetal heart rate and eye movements, which result in fetal behavioral states (Nijuis & ten Hof, 1999). From the 28th to 32nd gw, fetal EEG patterns are the same to that of a newborn (Emory & Israelian, 1998). This demonstrates that cerebral functioning of the infant does not change drastically through the transition from prenatal to postnatal life. At birth, the full term fetus demonstrates same neurobehavior as the newborn, and these patterns are supported throughout gestation (Krasnegor, Fifer, Maulik, McNellis, Romero, & Smotherman, 1998).

Also, it is at 28 weeks that the six layers of the cerebral cortex begin to take form (Huttenlocher, 1991), and a period of rapid dendritic differentiation in the cortical plate takes place (Mrzljak, Uylings, Van Eden, & Judas, 1990). During this time, the cerebral cortex is maturing and becoming more connected and dense. It is at this time when the brain can start to perceive and control movement. Figure 1 illustrates the formation of the prefrontal cortex throughout gestation. At 26 to 29 gestational weeks, the cortex has matured to form six layers. This is evidence of a transition in the neuronal cellular level through the growth of the cortical layers.

The integration period coincides with specific CBFV dominant hemisphere trends. Previous to the integration period, the left side has a greater dominance in CBFV, but at around the 29-30 gw, dominance is shifted to the right side (see Emory, in press). By the third postnatal year this pattern of dominance in blood flow velocity is reversed and again favors the left hemisphere(see Emory, in press). This phenomenon is regarded as the *double shift hypothesis* in early brain organization. It describes cerebral blood flow velocity patterns originating from prenatal life through childhood.

We learn from the Grant-Beuttler et al.'s (2011) research that muscle stimulation becomes more synchronized and complex with gestation. In this study, a 10 second vibration was applied to the abdomen of the mother. The vibration stimulated the fetal movement. The frequency of movement was recorded before, immediately, and after the stimulation. The results showed that there is a significant decrease in frequency and type of movement between 26 and 36 gestational weeks. The research suggests that movements become more specific to certain areas of the body, which may be related to the decrease in intrauterine space, as the fetus grows bigger (D'Elia et al., 2001). Also, there is an increase in fetal breathing movement from 26-36 gestational weeks, which suggests lung maturation. This time period also coincides with the proposed theory of the neuromotor integration period.

**Statement of Purpose**

The fundamental question of the current study asks, what is the topography of normal prenatal neurobehavioral development? Unlike much of the previous studies in the field of prenatal development, this study focuses on neurobehavioral trends in normal healthy populations. The study of normal healthy subjects may serve as a theoretical basis and model for atypical populations. Essentially, we are interested in testing the significance of the neuromotor integration period in relation to CBFV and FM.

Specifically, we are testing the following hypotheses:

1. There is a relationship between cerebral blood flow velocity and fetal movement across gestational age.
2. There will be a greater dominance of blood flow velocity in the left hemisphere up to 28 weeks, and after the 28$^{th}$ gw, the right hemisphere will have dominant CBFV. We predict

the 28 gestational week to be an inflection point for the change in velocity between the right and left hemisphere.

3. There will be a decrease in the frequency of isolated spikes and isolated clusters, and an increase in the frequency of epochs and episodes across gestation. We predict that there will be a shift in the frequency of each type of movement at the 28th gestational week.

## Methods

### Sample

The data used in this study was collected as part of a larger study (see Emory, in press) using a sample of 143 healthy right-handed mothers and their singleton fetuses attended one to six prenatal visits (*M*=2.0, *SD*=1.3) yielding 284 total observations. Volunteers were recruited through local print media in the greater Atlanta area. Each observation represents a visit to the research laboratory. Number of visits ranged from one to six. Maternal age ranged from 16-42 years (*M*=29.8, *SD*=5.6). The sample included 46 (32.2%) African-American mothers, 73 (51.0%) Caucasian mothers, 22 (15.4%) Asian mothers, and two (1.4%) mothers from an "Other" ethnic category. The sample included 70 (49%) female fetuses and 73 (51%) male fetuses. None of the women had a history of major psychiatric illness.

Expectant mothers had low-risk pregnancies, benign obstetrical histories, and were clinically healthy. Mothers were excluded from participation if:

1.) They were carrying multiple fetuses

2.) The gestational age of the fetus was less than 20 weeks or greater than 40 weeks

3.) If they used illicit substances, cigarettes, or alcohol

4.) The fetus exhibited any abnormalities on ultrasound exam

5.) There were obstetrical complications (e.g., maternal diabetes, hypertension, placenta previa)

6.) The mother was prescribed any medications excluding prenatal vitamins.

**Procedures**

Expectant mothers participated in the current study during visits to the Behavioral Perinatology Suite at Crawford Long Hospital in Atlanta, Georgia. The Institutional Review Board and all appropriate committees of Emory University approved this research. The procedure consisted of two phases: 1) obstetrical examination; 2) cerebral blood flow velocity measurement.

During the Obstetrical examination, abdominal symmetry, gestational age and heart rate were examine. All fetuses were determined to be healthy and of appropriate weight for gestational age, gender, and race. Then a certified ultrasound technician, under supervision of a board certified maternal-fetal medicine physician assessed fetal position, collected biometric measurements to rule out fetal abnormalities, confirmed gestation age, and measured placental and amniotic fluid volumes.

Mothers gave informed consent and were examined in a semi-recumbent position with an ACUSON 128XP10 OB Imager using either a 3.5 or 5 MHz ultrasound transducer as dictated by maternal body habits. Once the fetus showed no gross body movements or breathing movements, the examiner placed the ultrasound transducer on the fetal head to locate a transverse section of the fetal brain. Cerebral vessels in the Circle of Willis were then identified, including the anterior, middle and posterior cerebral arteries using Color Doppler Energy (CDE) technology. This technology combines two-dimensional and Color Doppler ultrasonography. Figure 2 illustrates a CDE image of the Circle of Willis in a 28 week-old fetus. Once a vessel

was identified, a gated cursor was centered on the vessel to sample flow characteristics. The angle of insonation was always less than sixty degrees to ensure accurate peak velocity. Using a 50 MHz low pass filter, pulsed Doppler velocity was recorded. Waveform spectral analysis was used to calculate flow velocities, time average velocities, and pulsatility indices. The average coefficient of variation for the blood flow calculations was 0.03. The current study examined blood flow velocity in the left and right anterior, middle, and posterior cerebral arteries of the human fetus.

Fetal movement was measured by a vibrating stimulus, which was put on the abdomen of the mother. Fetal movement was recorded through ultrasound imaging before and after the stimulation. Gestational age was recorded into three categories: (1) 20-28 weeks; (2) 28-32 weeks; and (3) 32-40 weeks.

## Results

Data analysis was performed using SPSS for Windows Vista (SPSS Inc., Chicago, IL). Normality of the continuous variables (blood flow and movements) was tested by means of frequency distribution and the variables were logarithm transformed prior to analysis. Table 1 presents the descriptive statistics for all variables. Bivariate correlations were run for the variables within each gestational age category (noted as G1, G2, and G3) because the curve for fetal movement across gestational age was not linear. Within each gestational age category, the left and right hemisphere velocities had a significantly positive correlation, and fetal movements have a significant positive correlation to each other. There were no significant correlations between fetal movement and blood flow. Hypothesis 1 was not supported.

Hypothesis 2 stated that there will be a greater dominance of blood flow velocity in the left hemisphere up to 28 gw, and then a dominance of blood flow velocity in the right

hemisphere starting from 28 gw. The paired *t* test was used to compare left and right hemisphere differences in peak mean velocities within each gestational age category. Specifically, at G1, the blood flow velocity of the LMCA (*M*=.345, *SD*=.074) was higher compared to the blood flow velocity of the RMCA (*M*=.325, *SD*=.062). Results of the *t* test comparing these means were significant, $t(81)=2.39$, $p<.05$. At G2 (28-32 gw) and G3 left and right hemisphere CBFV were not significantly different, $t(81)=-1.49$, $p<.05$, $t(81)= -1.59$, $p<.05$, respectively. A *p* value of <0.05 was considered statistically significant. Figure 3 illustrates a bar graph of the blood flow velocities for the left and right MCA at each gestational age category. Hypothesis 2 was partially supported.

Hypothesis 3 stated that there would be a decrease in isolated spikes and isolated clusters, and an increase in epochs, and episodes across gestation. For hypothesis 3, a one-way repeated measures analysis of variance was used to compare mean values of the four different types of movements across gestational age. There were four levels for fetal movement type: isolated spike (is), isolated cluster (ic), epoch (epch), and episode (epi); and three levels for gestational age: G1, G2, and G3. The assumptions for movement type were violated through Mauchy's Test of Sphericity; hence, we looked at the Greenhouse-Geisser statistic. There was a significant main effect for gestational age, $F(2,37)= 18.8$, $p=.00$, movement type, $F(3,37)= 17.7$, $p=.00$, and a significant interaction between gestational age and movement type, $F(3.5,37)= 5.48$, $p=.00$. The effect size for gestational age level as a main effect was small ($\eta^2=-0.01$). The effect size for movement type as a main effect was large ($\eta^2=0.12$)

A comparison of changes in mean between the groups was done through dependent paired *t* tests to find which specific movements were significant. We specifically looked at the relationships that we hypothesized would have the most stark contrasts. The results yielded

significance between episodes and isolated spikes at each gestational age level (Table 3). There was also significance between epochs and isolated spikes, isolated clusters, and isolated spikes, and episodes and epochs. Due to the possibility of gestational age levels contributing to fetal movement frequencies, post-hoc analyses of the Bonferroni method were conducted but the results remained significant, except for isolated clusters and isolated spikes at G3.

Additionally, a dependent paired *t* test was done to show mean differences between gestational age categories for each movement. The means showed that episodes significantly decreased from G1 to G2, $t(57)=3.507$, $p=.00$, and significantly increased from G2 to G3, $t(57)=-2.632$, $p=.011$. Epoch movements, isolated clusters, and isolated spikes did not have statistically significant mean differences across gestational age categories. Figure 4 is a bar graph of the movements at each gestational age category.

## Discussion

The fundamental question that this study asks is what are the trends of cerebral blood flow and fetal movement across gestational age? More specifically, is the time period between 28 and 32 gestational weeks a critical neuromotor period, defined by changing patterns of cerebral blood flow and fetal movement. I hypothesized that cerebral blood flow velocity would be faster in the left hemisphere until 28 weeks, and the right hemisphere cerebral blood flow velocity would be faster after 28 weeks. Additionally, I hypothesized that epoch and episode movements would increase over time, and isolated spike and isolated cluster movements would decrease over time. The results suggested that CBFV is significantly higher in the left MCA at prior to 28 weeks gestation, but that there were no significant differences in the right and the left MCA from 28 weeks onward. In addition, episodes were the only movements to have a significant frequency difference between each gestational age category. The other movements

had no significant difference in mean frequency. Contrary to what was hypothesized, there was no relationship between cerebral blood flow and fetal movement, even within later gestational windows.

For cerebral blood flow velocity, there was significant difference at G1 between right and left hemispheres, with the right hemisphere at a faster mean speed compared to the left hemisphere. This was consistent with previous literature that supports a positive linear relationship between gestational age and cerebral blood flow velocity in the middle cerebral artery. The right hemisphere had a slightly faster mean velocity at 28 gw compared to the left, but it was not significant. The velocity had increased but not at the same accelerating rate. This may be related to the fact that neurobehavioral maturation begins to slow down after approximately 30 to 32 gestational weeks (see Emory, in press). Children who are born extremely preterm have significantly lower cognitive, language and motor function levels (Mansson & Stjerngvist, 2014), but premature infants born after 30 weeks have a greater chance of successful cognitive development as they are born closer to full term gestational age (Poulsen, Wolke, Kurinczuk, Boyle, Field, Alfirevic, & Quigley, 2013). This shows the significance of the neuromotor integration period as a period of dynamic change from the neuronal level to the functional level prenatally and postnatally.

In Figure 3, it appears that the velocity rates for the left and right hemispheres cross over at approximately 28 gestational weeks, around the time of the neuromotor integration period. Based on the *double shift hypothesis* by Emory and Israelian (1998), there may be support in the idea that the shifts of brain laterality may underlie the development of the cognitive processes and motor control.

Hemispheric specialization can influence a broad spectrum of learning and development, from early visual processing to prosody perception (Park, Chiang, Brannon & Woldorff, 2014; Witteman, Goerlich-Dobre, Martens, Aleman, Van Heuven, & Schiller, 2014). Lateral brain differences in cerebral blood flow velocity suggest that hemispheric specialization may begin during the prenatal period. Furthermore, the shift in cerebral blood flow velocity from left to right around 28 gestational weeks may indicate the separation of discrete motor functions to either the right or left hemisphere. Hemispheric specialization is not fully evident and visible until the child is born; however, based on the previous literature that mature infants exhibit similar behavioral patterns as newborns it seems plausible that the mature fetus may also exhibit hemispheric specialization.

For fetal movement, there was a main effect for gestational age category and fetal movement type, as well as a significant interaction between gestational age category and movement type. Previous literature has not examined length of time as a measurement of fetal movement. Grant-Beuttler et al., (2011) stated that reflexive, jerky movements decreased across gestation. We applied this definition to the movements shortest in length, which were isolated spikes (less than 3 seconds) and isolated clusters (3-10 seconds). The previous literature stated that whole body movements increased in frequency across gestation, which we loosely applied to the definition of episode (greater than 30 seconds) and epoch (10-30 seconds) movements. Despite the lack of predetermined definitions and supporting studies with this measuring technique, there was indeed a significant decrease in isolated spikes and isolated clusters, and a significant increase in episode and epoch movements.

Although there was no statistical evidence of significant mean differences between gestation age categories in isolated spikes, isolated clusters and epochs, the following is a

conjecture based on visual trends shown on Figure 4. According to Figure 4, G2 seems to be a point of change for the mean frequencies of fetal movements across time. Figure 4 shows that between G1, G2, and G3, the mean values for each movement get closer in value to each other. For an example, the mean frequencies between isolated spikes ($M$=10.08) and epoch ($M$=5.8) are far apart at G1. By G2, the mean for isolated spike ($M$=8.2) decreases from G1, and mean for epoch ($M$=4.6) decreases slightly as well. Lastly, at G3, the mean frequency for isolated spike ($M$=7.8*)* decreases again, and the mean frequency for epoch at G3 ($M$=6.14) increases. The difference in means at G1 to G3 between isolated spikes and epochs goes from 5 to 1.66. The means seem to get closer at every time period, which corresponds with our theoretical basis that shorter movements decrease as longer movements increase. The movements shorter in length (isolated spike, isolated cluster) end (G3) up lower than where they start (G1), while the longer movements (epoch, episode) end up higher. It is not apparent on Figure 4 that the frequency of Episodes are higher at G3 compared to G1. However, the background literature supports the notion that whole-body movements should increase through gestation. Perhaps length of time does not correspond well to whole-body movements.

The fact that no previous published study has used length of time as a measurement of movement is significant to this study. From the data we know that different types of movements have a higher frequency depending on gestational age. Perhaps the frequency of specific movements during specific times conveys the progression of the prenatal neuromotor system. This is consistent with the background literature that fetal movement is a marker of neurobehavioral development. Postnatal temperamental patterns lie in the differences in patterns of neuromotor activity during the prenatal stage (Degani, Leibovitz, Shapiro, & Ohel, 2009). Movement patterns in utero predetermine postnatal motor activity levels. Therefore, the length of

time may have been a more objective measurement of fetal movement, compared to movement type.

Although there was no significant relationship between CBFV and FM, there was an evident shift in configuration for both systems at approximately 28 gw. At 28 weeks, there was a dynamic change in CBFV between hemispheres and in mean frequency of movements between fetal movements. As mentioned in the previous literature, around 28 weeks is also when migrating neurons in the prefrontal cortex have differentiated the cortical plate, giving rise to the six layers of the cortex. Simultaneous to this time there is an elevated level of glucose metabolism and blood-oxygen levels, which are processes related to cerebral blood flow (Kurjak, Zudenigo, Predanic, & Kupesic, 1993). By the late third trimester, at around 36 gestational weeks, fetal movements, eye movements, and heart rate become even more tightly linked as behavioral states (Martin, 2008). Perhaps this is an indication that all systems of the brain are changing together at a specific time because the simultaneous actions coalesce into one function.

**Strengths, Problems and Limitations**

This study's strengths lie in repeated measures available for analysis and in the focus on a typically developing population. The neuromotor integration period is a significant point of dynamic change during the prenatal stage, and the current study adds to the literature on this integration period. The study's limitations are also important to consider. Limitations rise from statistical testing, number of observations, and ambiguous definitions.

A limitation of this study was that we did not use the Hierarchical linear modeling (HLM) technique for statistical testing. HLM improves estimation of effects within individual units by appropriating statistical strength from the entire sample of data. Also, HLM techniques permit the modeling of cross-level effects, or how variables measured at one level influence

relationships occurring at another level. The extra *t* test after the repeated measures ANOVA would not have to be done under HLM. A third advantage is that HLM provides increased flexibility in handling missing data in longitudinal designs. Participants who have been seen at least twice can be incorporated, though optimally most participants will have visited on multiple occasions. Unlike repeated measures ANOVA, data for an entire subject does not have to be deleted due to one missing observation. The number of analyses done increased the possibility for a Type 1 error, but the results may also be affected by a Type 2 error due to the sample size. Thus, HLM could have been a better for the theoretical model we were hoping to create.

Methodological flaws of this study include the way in which data was recorded. One issue was the fact that gestational age was divided into three levels. The data set that I used did not have gestational age as a continuous variable, but merely separated into G1, G2, and G3. Perhaps if the actual gestational age was recorded as a number rather than a category for a time range, the results could have yielded more information regarding the integration period as well as overall trends across time. DiPietro's study presents a neuromotor transitional phase as occurring between 28 to 32 gestational weeks, whereas Emory and Israelian's theory of neuromotor integration states that the neurobehavioral effects of the integration manifests around 28 gestational weeks. Although the numbers may be similar, the slight difference in methodological approach could have greater implications regarding developmental changes.

Another potential flaw was that mothers did not participate in laboratory sessions at a consistent rate. There may have been a risk of non-response bias. For example, some mothers came twice for one time range, but none for another. Also, the numbers of visits were not consistent between mothers. A complete set of data was not available for statistical testing, resulting in lower statistical strength.

Some ethical issues that were raised were the actual health of the mother as well as what the established definition of 'normal and healthy' might be. The previous literature briefly talks about the contentious definition of healthy. Due to this discrepancy, that the guidelines for healthy pregnancy are not established, there might have been confounding factors that have not been addressed. The fetus is sensitive to environmental factors in utero and behaviors engaged in by the mother. A doctor or health professional is not with the mother 24/7 to keep track of every potential substance she may consume. They could only trust the information they take from the mother during the check up.

**Implications for Future Research**

There are several implications for both theoretical basis of hemispheric specialization and the validity of the neuromotor integration phase for this study. The neonate rapidly develops into full term fetus in a matter of weeks. Every phenomenon during the prenatal phase occurs to facilitate a type of development once the child is born. There are many systems working and changing at one critical time. There is much more to be explored in this sense, and with the ongoing progression of sonographic technology, future studies may have far more evidence and better tools to come up with more defined and established outcomes.

Future studies could attempt to replicate the findings of this study and feature longitudinal designs many more time points to investigate non-linear models. This study measured cerebral blood flow at rest, hence examining cerebral blood flow activation during and after stimulation may yield interesting results.

Although this study has failed to create a model of neurobehavioral development, perhaps future studies may have greater evidence to propose one. This may have been much too grand of a goal. The addition of heart rate as a factor in relation to blood flow and fetal movement may be

one approach to finding stronger grounds for a theoretical model. Furthermore, incorporating individual differences between siblings and twins may provide greater insight and support into what neurobehavioral trends actually mean for fetal development. Previous studies have shown that there are stark differences between gender and race; so exploring fetal movement and cerebral blood flow within those parameters may also yield interesting results. Also, focusing on other areas of the Circle of Willis may be helpful in understanding the direction and location of blood flow throughout the cerebral cortex. Combining length of movement time and type of fetal movement may help researchers get a better understanding as well as a better-established definition of what is fetal movement.

**Conclusions**

This study provides a valuable perspective into fetal neurobehavioral development. Although this study was unable to find a relationship between cerebral blood flow and fetal movement, the findings suggested that the window near the 28$^{th}$ week of gestation might be an important transition period for cerebral blood flow, and different types of fetal movement. There seems to be a wealth of empirical evidence that can be tapped into for future studies. As prenatal research continues to remain dependent on technology to provide a window into the womb, there is still much to be explored and understood. There is much potential and strength in this field that may prompt new discoveries and directions in how we understand the earliest phases of life and development.

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Table 1

Descriptive Statistics for Variables The variables should appear in the table

| | M (SD) | Min-Max |
|---|---|---|
| lmca | 0.38 (0.08) | 0.17-0.62 |
| rmca | 0.39 (0.09) | 0.17-0.64 |
| is | 1.90 (1.09) | 0.00-3.53 |
| ic | 1.67 (1.04) | 0.00-3.37 |
| epch | 1.54 (1.03) | 0.00-3.33 |
| epi | 0.92 (0.86) | 0.00-2.64 |

Note: lmca= CBFV of left medial cerebral artery, rmca= CBFV of right medial cerebral artery, is= Frequency of isolated spikes, ic= Frequency of isolated clusters, epch= Frequency of epochs, epi= Frequency of episodes, M=mean, SD= standard deviation

Table 2

Correlations between All Variables Entered at Gestational Age Category

Correlations for Gestational Age Category 1

| | 2) rmca | 3) is | 4) ic | 5) epoch | 6) episode |
|---|---|---|---|---|---|
| 1) lmca | .450 (p=.00)** | .138 (p=.24) | .134 (p=.25) | .049 (p=.67) | -.082( p=.48) |
| 2) rmca | | -.019 (p=.87) | .029 (p=.80) | -.060 (p=.61) | -.011 (p=.92) |
| 3) is | | | .802 (p=.00)** | .789(p=.00)** | .399 (p=.00)** |
| 4) ic | | | | .810(p=.00)** | .578 (p=.00)** |
| 5) epoch | | | | | .551 (p=.00)** |
| 6) episode | | | | | |

Correlations for Gestational Age Category 2

| | 2) rmca | 3) is | 4) ic | 5) epoch | 6) episode |
|---|---|---|---|---|---|
| 7) lmca | .416 (p=.00)** | -.023(p=.83) | .008 (p=.84) | -.089 (p=.41) | .008 ( p=.94) |
| 8) rmca | | -.068 (p=.52) | -.045 (p=.67) | -.040 (p=.70) | -.032 (p=.77) |
| 9) is | | | .537 (p=.00)** | .684(p=.00)** | .385 (p=.00)** |
| 10) ic | | | | .766(p=.00)** | .537 (p=.00)** |
| 11) epoch | | | | | .635(p=.00)** |
| 12) episode | | | | | |

Correlations for Gestational Age Category 3

| | 2) rmca | 3) is | 4) ic | 5) epoch | 6) episode |
|---|---|---|---|---|---|
| 13) lmca | .275 (p=.03)** | .176 (p=.16) | .147 (p=.23) | .197 (p=.11) | .011 ( p=.93) |
| 14) rmca | | .122 (p=.321) | .044 (p=.72) | .077 (p=.53) | .007 (p=.95) |
| 15) is | | | .800 (p=.00)** | .846 (p=.00)** | .475 (p=.00)** |
| 16) ic | | | | .831 (p=.00)** | .575 (p=.00)** |
| 17) epoch | | | | | .632 (p=.00)** |
| 18) episode | | | | | |

**$p<.05$

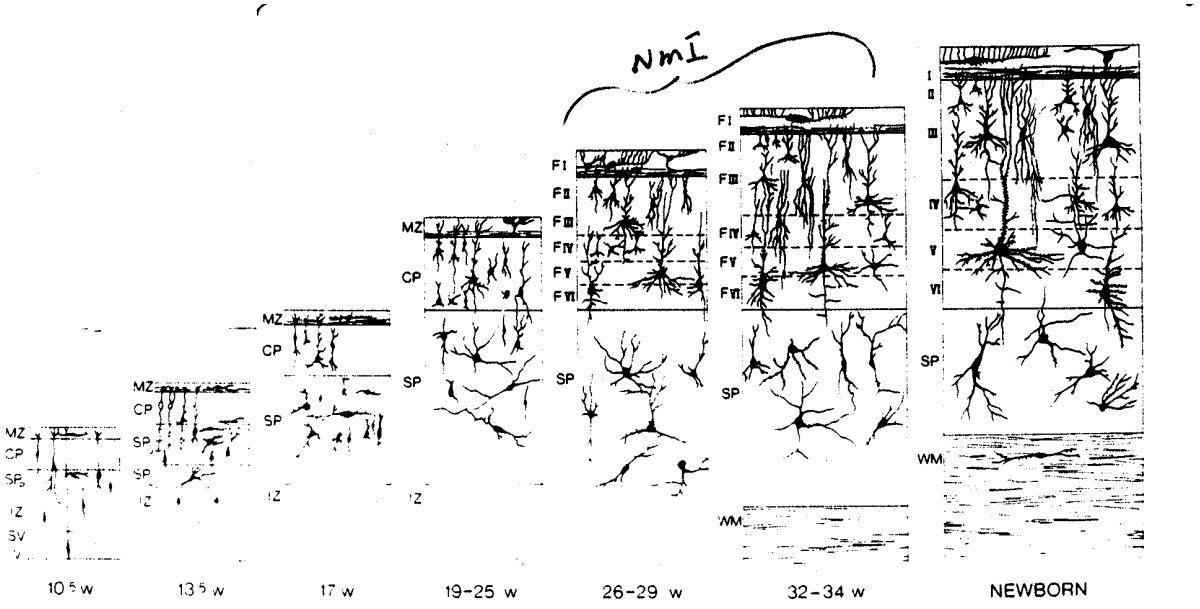
Note: lmca= CBFV of left medial cerebral artery, rmca= CBFV of right medial cerebral artery, is= Frequency of isolated spikes, ic= Frequency of isolated clusters, epoch= Frequency of epochs, episode= Frequency of episodes, Gestagecat= gestational age category

Table 3. Paired *t* test results comparing mean frequency of fetal movement types at each gestational age level

| | *t*-test | *p*-value |
|---|---|---|
| Isolated Spikes and Episodes | | |
| G1 | -8.106 | .000** |
| G2 | -10.151 | .000** |
| G3 | -6.705 | .000** |
| Isolated Spikes and Epochs | | |
| G1 | -7.294 | .000** |
| G2 | -5.779 | .000** |
| G3 | -4.644 | .000** |
| Isolated Spikes and Isolated Clusters | | |
| G1 | -5.312 | .000** |
| G2 | -4.354 | .000** |
| G3 | -3.266 | .002** |
| Epochs and Episodes | | |
| G1 | -5.896 | .000** |
| G2 | -9.270 | .000** |
| G3 | -6.597 | .000** |

*$p<.05$

*Note.* G1= 20-28 gestational weeks, G2=28-32 gestational weeks, G3=32-40 gestational weeks,




Scheme of prenatal neuronal development in the prefrontal cortex. (From Mrzljak et al., 1988)

*Figure 1.* Theoretical model that illustrates the scheme of prenatal neuronal development in the prefrontal cortex. During neuromotor integration (26-29 gw), the first dendritic spines are observed on upper and lower dendrites of fetal pyramidal neurons of layer FV. (Mrzljak et al., 1988)

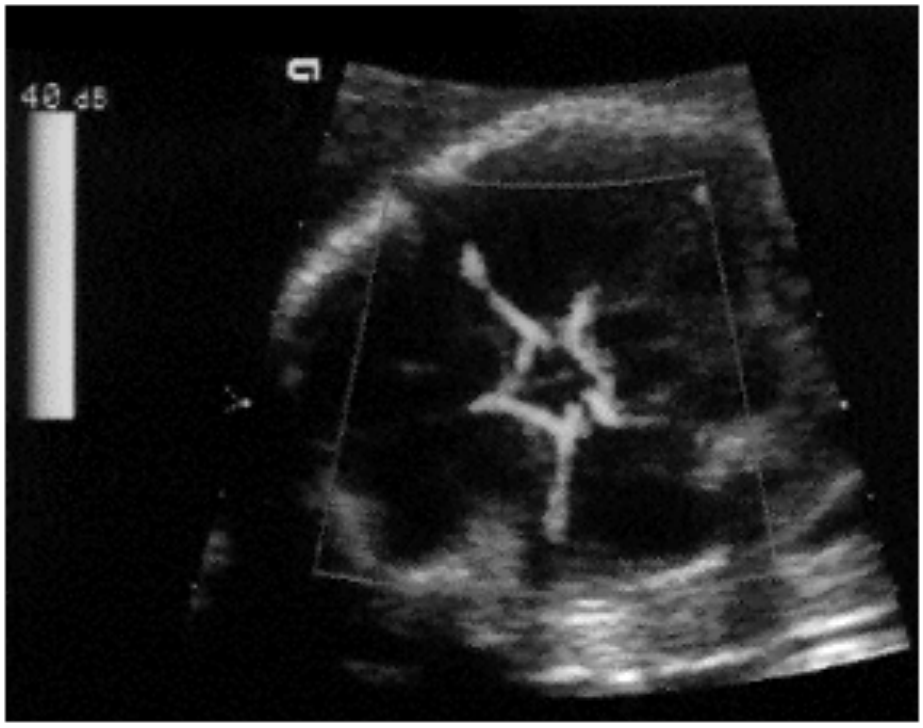



*Figure 2.* Color Doppler Energy (CDE) image of the Circle of Willis in a 28 week-old fetus.

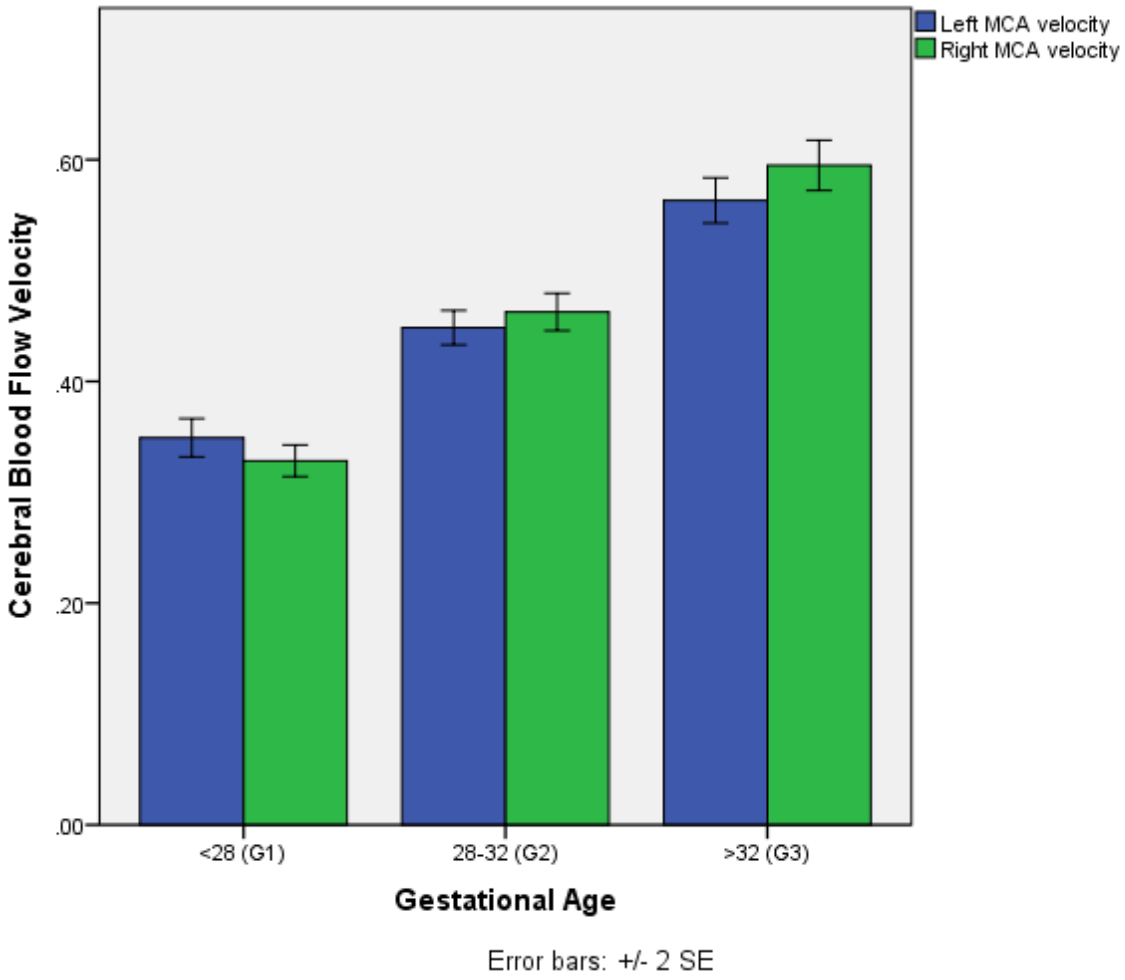



*Figure 3.* Mean Cerebral Blood Flow Velocities (CBFV) of the right and left medial cerebral artery as a function of gestational age levels. The left MCA velocity is significantly greater than right MCA during G1. There is no significant difference at G2 and G3.

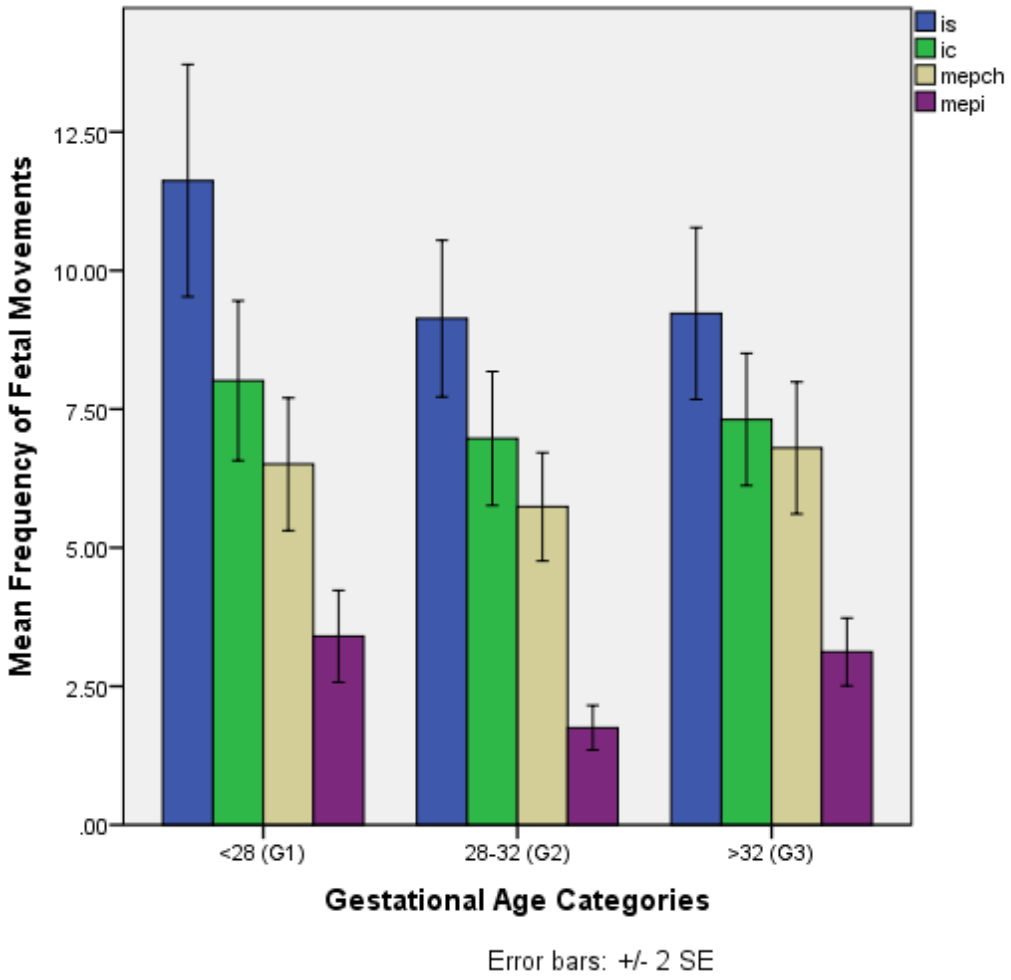



*Figure 4.* Mean Frequency of Fetal Movements as a function of gestational age levels. Mean frequencies for episodes (mepi) significantly decrease from G1 to G2, and significantly increase from G2 to G3. Isolated spikes (is), isolated clusters (ic), and episodes (mepch) do not significantly change.