THE RELATIONSHIP OF PRENATAL NICOTINE EXPOSURE AND NEWBORN HEART RATE: IS THERE A BASIS FOR SUDDEN INFANT DEATH?

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THE RELATIONSHIP OF PRENATAL NICOTINE EXPOSURE AND NEWBORN HEART RATE: IS THERE A BASIS FOR SUDDEN INFANT DEATH?

ABSTRACT

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Prenatal exposure to cigarette smoke increases the risk of Sudden Infant Death Syndrome (SIDS) by 2 to 4 fold. Although the etiology for this increased risk of SIDS is unknown, nicotine has been shown to cause adverse cardiovascular effects in animal models and adults. This study proposed a relationship between prenatal nicotine exposure and newborn heart rate during the immediate postnatal period of transition. Physiologically, transition is a time of cardiovascular adaptation for the newborn infant. Measuring the newborn heart rate during transition would allow the assessment of fetal drug exposure under the challenging, rather than basal, conditions associated with birth.

A total of 130 mother/infant couplets participated in this descriptive, correlational study. Placental cord blood was drawn at the time of delivery and analyzed for cotinine. Cotinine is the primary metabolite of nicotine and is considered to be the best available biomarker to quantify nicotine exposure. The heart rate of the newborn infants was

measured every one minute during the immediate postnatal period of transition, the first four hours of life.

Statistical analysis, using the Pearson Correlation to test the hypotheses, found statistically significant negative relationships between venous cord blood cotinine levels, maximum heart rate ($r = -.271$, $p = .002$), and variance of the heart rate ($r = -.206$, $p = .019$). The coefficient of determination ascertained that 7% of the heterogeneity in the maximum heart rate and 4% of the heterogeneity of the variance in the heart rate were a result of the linear relationship with the cord blood cotinine.

The findings of this study suggest that newborn infants with higher venous cord blood cotinine levels have a limited ability to maximize and vary their heart rate. Cardiac output in the infant is primarily dependent on heart rate. If the infant is unable to maximize cardiac output during times of stress, the infant is at an increased risk for morbidity and possible mortality.

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CHAPTER 1

INTRODUCTION

Sudden Infant Death Syndrome (SIDS) is the diagnosis given to a devastating phenomenon that is the leading cause of death in infants between the ages of one month and one year throughout the world. SIDS is defined by the National Institute of Child Health and Human Development (NICHD) as the sudden death of an infant less than one year of age which remains unexplained after a thorough case investigation that includes performing a complete autopsy, examining the death scene, and reviewing the clinical history. The diagnosis of SIDS is by necessity a diagnosis of exclusion of all other possible causes of death, and by the appearance of characteristic external, internal, and microscopic features which confirm the disease (Berry, 1992; Byard et al., 1996).

While the etiology of SIDS is unknown, general characteristics of infants at risk have been identified. The risk factors for SIDS include: prematurity, low birthweight, male gender, American Indian or African American descent, maternal infections during pregnancy, maternal drug abuse, and maternal smoking during pregnancy (Gibson, 1992; Hoffman & Hillman, 1992; Kohlendoefer et al., 1998; Shannon, 1992).

Of the known risk factors, maternal smoking during pregnancy has been repeatedly identified as a major element in SIDS. Current literature suggests that maternal smoking during pregnancy increases the risk of SIDS, two to four fold. Passive exposure to

environmental tobacco smoke (ETS) has also been attributed as increased risk factor for SIDS (Alm et al., 1998; Kohlendoefer et al., 1998).

Problem of Study

It is not known why prenatal exposure to nicotine increases the risk of SIDS. Research has demonstrated adverse hemodynamic effects of nicotine, such sudden death, in adults and in animal models (Benowitz, 1996; Slotkin, 1998). However, there is no published literature addressing the cardiovascular effects of nicotine in newborn infants.

The pilot for this study suggested that during transition, the first four hours of life, infants of mothers who smoked during their pregnancy had a statistically significant lower maximum heart rate (HR) and a lower variance of HR when compared to infants with no reported prenatal exposure to nicotine. The pilot findings revealed the HR variables on which to focus the primary study.

In order to evaluate the effects of prenatal nicotine exposure on newborn heart rate variables, quantitative measurements of nicotine exposure must be correlated with newborn HR variables such as the maximum HR and the variance of the HR. Therefore, this study was designed to address the question: Is there a relationship between a quantitative measure of nicotine exposure and newborn heart rate variables during the immediate postnatal period of transition?

Rationale for Study

SIDS is the diagnosis given to a phenomenon that is the leading cause of death in infants between the ages of one month and one year throughout the world. Reports of sudden and unexpected infant deaths have been recorded since biblical times. These unexplained deaths were attributed to "overlaying" or suffocation, and were considered an ecclesiastical crime prior to the 18th century (Busuttil, 1992; Goldberg, 1992; Limerick, 1992).

The incidence of SIDS ranges from 0.3-7.5 per 1000 live births. Although there has been a decline in the rate of SIDS over the past few years with the intervention of positioning the infant supine to sleep, SIDS still continues to be a leading cause of death in infants (SIDS Alliance, 1998).

Prior to 1972, the most widely accepted hypothesis for SIDS attributed death to an abrupt closure of the upper airway. This hypothesis was supported by inflammation and ulcerations of the vocal cords, petechiae on the lungs, and fluid in the air spaces of the lungs on infants who had died of SIDS. Although this hypothesis was supported by physical findings at autopsy, it did not explain why infants died during sleep, why most died between two weeks to three months of age, nor the increased prevalence during the winter months. It is now thought that the deaths are multi factorial in origin and related to a disturbance in autonomic function (Douglas et al., 1997; Naeye, 1980; Slotkin, 1998; Spiers & Guntheroth, 1997).

While the etiology of SIDS is unknown, general characteristics of infants at risk

have been identified. The risk factors for SIDS include: prematurity, low birth weight, male gender, American Indian or African American descent, maternal infections during pregnancy, maternal drug abuse and maternal smoking during pregnancy (Gibson, 1992; Hoffman & Hillman, 1992; Kohlendoefer et al., 1998; Shannon, 1992).

Of the known risk factors, maternal smoking has been repeatedly identified as a major element in SIDS (Alm et al., 1998; Cooke, 1998; Naeye, 1980; NICHD, 1997). Using data from large, epidemiologic case-control studies, researchers have retrospectively examined the risk factors for SIDS. Alm et al. (1998) and Mitchell et al. (1993) found that infants of mothers who smoked during pregnancy were four times more likely to die of SIDS than infants of mothers who do not smoke during the pregnancy. Blair et al. (1996) found that infants exposed prenatally to cigarette smoke were 2.9 times more likely to die from SIDS than infants not prenatally exposed to cigarette smoke. Kohlendorfer et al. (1998) found that maternal smoking during pregnancy increased the risk of SIDS 2.2 times. Other researchers assert that exposure to environmental tobacco smoke (ETS) also increases the risk of SIDS (Anderson & Cook, 1997; Bergman & Wiesner, 1976; Golding, 1997; Klonoff-Cohen & Edelstein et al., 1995; Mitchell et al., 1993).

The increased risk of SIDS secondary to nicotine exposure has been scientifically proven (Golding, 1997; Mitchell et al., 1993). What is not known is why infants with nicotine exposure die more readily of SIDS than infants without nicotine exposure. One method of evaluating the effects of prenatal nicotine exposure on the cardiovascular

function of the newborn would be to evaluate the heart rate of the newborn infant during the immediate postnatal period of transition and to correlate the heart rate with cotinine, the primary metabolite of nicotine. Physiologically, transition is a time of cardiovascular adaptation for the newborns (Blackburn & Loper, 1992). Measuring the newborn heart rate during transition would allow the assessment of fetal drug exposure under the challenging, rather than basal, conditions associated with birth. It is hypothesized that alterations in heart rate variables would be more readily apparent during this time frame since profound changes in the fetal myocardium and pulmonary systemic bed are necessary during this time for the transition to postnatal circulation in the newborn infant (Polin & Fox, 1997).

Burns and Grove (1997) contend that nursing is accountable to society to provide quality care, and to seek ways to improve the quality of nursing care. The primary goal of nursing research is to develop a scientific knowledge base for nursing care, standards, and practices. If a relationship between venous cord blood cotinine and newborn heart rates can be established, then the protocol for evaluating the heart rates of newborn infants exposed prenatally to nicotine will need to be modified. Current nursing standards as dictated by the American Academy of Pediatrics (1997) for all newborn infants state that "vital signs, including heart rate, respiratory rate, and temperature will be taken every 30 minutes until the neonate is stable for two hours" (p. 156). Subsequent nursing assessments are per the hospital routine which is generally every 6-12 hours. If newborn infants with prenatal nicotine exposure are shown to have altered heart rate patterns then

the frequency of nurses obtaining heart rate measurements will need to be modified. The current infrequent rate of heart rate measurement would not allow for the detection of an abnormal trend such a low heart rate, or arrhythmia.

The relevance of the study problem to nursing is that nurses are the primary care providers for the families before and after delivery. Nurses elicit the prenatal assessment of nicotine exposure and are in a position to counsel the families on the adverse effects of nicotine exposure to both the mother and the newborn infant. The Nursing Center for Tobacco Intervention (1999) contends that all pregnant smokers should be strongly encouraged to stop smoking throughout their pregnancy. All pregnant smokers should at least be offered a minimal intervention and whenever possible intensive counseling on the dangers of smoking.

After delivery the newborn infant is assessed regularly by the nurse and any abnormalities in the heart rate would be detected by the nurse. Schwartz et al. (1998) found that prolonged QT intervals in the first week of life were strongly associated with SIDS. QT intervals are directly related to the heart rate (Polin & Fox, 1997). If an abnormal pattern of heart rates is detected, at-risk infants could then be evaluated prior to discharge with an in-depth assessment of cardiovascular function. This evaluation may detect abnormalities that could predict the potential for sudden infant death and allow for implementation of preventive measures.

Theoretical Framework

The theoretical framework for this study was provided by Diffusion Theory.

A review of the published literature revealed that maternal substances are transferred across the placenta to the fetus by the process of diffusion (Polin & Fox, 1997). Pastrakuljic et al. (1998) and Sastry et al. (1998) found that nicotine readily diffuses across the placenta with less than 1% of nicotine being metabolized to cotinine in the placenta. Diffusion depends primarily upon lipid solubility, plasma protein binding, molecular size, concentration difference, and the surface area of the membrane separating the exchanging compartments (Guyton & Hall, 1996; Schroder, 1995).

All membranes consist of a double layer of lipid molecules in which proteins are embedded. The lipid layers prevent the movement of most molecules across the cell membrane, while the proteins provide selective transfer of certain substances. The lipid solubility of a substance determines how quickly the substance can move through the lipid bilayer. The lipid solubility of nicotine is high, and it diffuses rapidly across the placenta into the fetal circulation (Lambers & Clark, 1996).

Many drugs bind to the plasma proteins and are not readily released into the circulation. Nicotine has negligible plasma protein binding, less than 5%, and is readily available for tissue uptake (Svensson, 1987).

Substances with molecular weights < 500 readily pass the placental barrier (DeVane, 1995). The molecular weight of nicotine is 162.24 (Saini, 1998). At this low molecular weight, and with negligible plasma protein binding, nicotine quickly diffuses through the placental circulation to the fetal circulation.

The rate of diffusion is directly proportional to the concentration of the substance

inside and outside of the membrane (Guyton & Hall, 1996). Nicotine has been found in fetal cord serum in concentrations comparable to or exceeding maternal serum (Luck et al., 1985; Polin & Fox, 1997; Suzuki et al., 1974).

The surface area of the placenta also contributes to the amount of nicotine available to the fetus. Early in gestation the total surface area of the placenta is small, and gradually increases in size throughout gestation. The larger placental surface area found later in gestation allows for greater amounts of nicotine to be diffused to the fetus (Sastry, 1991).

The properties of diffusion with regard to characteristics of nicotine are summarized in Table 1. As seen from the characteristics of nicotine, it easily diffuses from the maternal circulation to the fetal circulation, making the substance readily available to the fetus.

Table 1. Properties of Diffusion and Characteristics of Nicotine

Diffusion Properties	Nicotine Characteristics
Lipid Solubility	High, rapidly diffused
Plasma Protein Binding	< 5% (very low)
Molecular Size	162.24 (small)
Concentration Difference	Variable, dependent on maternal intake
Surface Area	Large placental surface area later in pregnancy

Diffusion Theory was appropriate for use in this study because diffusion is the mechanism of substance transfer across the placenta to the fetus. The purpose of this study was to examine the effects of prenatal nicotine exposure, using venous cord blood serum cotinine levels, on newborn heart rate variables. The fetus can only receive the cotinine by diffusion from the maternal circulation, and once the cotinine is in the fetal circulation it must diffuse into either the CNS or the myocardium in order to produce effects on the newborn heart rate.

Assumptions

The following statements are assumptions which provided the foundation for the study.

1. Diffusion is the mechanism of transfer between the maternal and fetal circulations (Blackburn & Loper, 1992; Polin & Fox, 1997).
2. Nicotine easily diffuses between the maternal and fetal circulations (Luck et al., 1985; Polin & Fox, 1997; Suzuki et al., 1974).
3. Nicotine causes cardiovascular effects (Slotkin, 1998; Tolson et al., 1995).

Hypotheses

The following two associative hypotheses predicted the outcome of this study.

1. There is a relationship between venous cord blood cotinine and the maximum HR of term, appropriate for gestational age (AGA), well newborn infants during the immediate postnatal period of transition.
2. There is a relationship between venous cord blood cotinine and the variance of the

HR in term, AGA, well newborn infants during the immediate postnatal period of transition.

Definition of Terms

The following terms were conceptually and operationally defined for the purpose of this study.

1. Venous cord blood cotinine

   Conceptual Definition: Cotinine is a byproduct of the metabolism of nicotine (Benowitz, 1997), and is measured in a sample of blood drawn from the placental umbilical vein at the time of delivery.

   Operational Definition: The actual amount of cotinine measured, ng/ml, from a sample of blood which is drawn from the placental umbilical vein at the time of delivery.

2. Term, AGA, well newborn infants

   Conceptual Definition: Infants who are born at term, are a normal size, and have no obvious health problems (Blackburn & Loper, 1992).

   Operational Definition:

   a. Infants who are born between 37-42 weeks of gestation (Blackburn & Loper, 1992).

   b. The parameters of weight, head circumference, and length are greater than the 10^{th} percentile and less than the 90^{th} percentile (Blackburn & Loper, 1992).

c. Infants who have no detectable health problems at delivery as determined by nursing and medical assessment.

d. Infants who have apgars of ≥seven at one minute of age, and ≥eight at five minutes of age (Blackburn & Loper, 1992).

3. Transition

Conceptual Definition: The first few hours of life after delivery (Blackburn & Loper, 1992).

Operational Definition: The first four hours of life after delivery during which the infants receive close observation, a physical assessment, heart rate, respiratory rate, and temperature every 30 minutes.

4. Heart rate variables

Conceptual Definition:

a. Maximum heart rate: The highest heart rate attained by the infant during transition.

b. The variance of the heart rate is a measurement of the ability of the infant to vary the heart.

Operational Definitions:

a. Maximum heart rate: The highest heart rate recorded by the Corometrics cardiac monitor during transition.

b. The variance of the heart rate is the mean of the sum of squares

(Burns & Grove, 1997), and is one of the most useful indices of variability (Pedhazur & Schmelkin, 1991).

5. Prenatal nicotine exposure:

Conceptual Definition: The type of maternal exposure to nicotine during the pregnancy.

Operational Definition:

1. Active exposure: Smokes tobacco or using nicotine replacement therapy during the pregnancy (Benowitz, 1997).
2. Passive exposure: Exposure to environmental tobacco smoke (ETS) during the pregnancy (Benowitz, 1997).
3. No reported exposure: The mother denies both active and passive exposure to tobacco smoke during the pregnancy.

Limitations

The inclusive criteria for study entry was purposefully narrow in an attempt to control for confounding variables, and while theoretical limitations were not a concern, there are methodological limitations. The methodological limitations of a nonprobability, convenience sample of newborn infants restricted the population to which the findings could be generalized.

The cotinine level may not be an accurate reflection of the nicotine exposure during pregnancy. During labor and delivery women do not smoke nor are they exposed to cigarette smoke. The physical constraints of the process of labor and delivery in

combination with the half life of nicotine and cotinine may result in lower levels of analytes. Although the value may be lower, the cotinine level will still be an indicator of prenatal nicotine exposure.

Maternally consumed substances such as caffeine and illegal drugs can cause alterations in the newborn infant's heart rate and are potential confounding variables. Most mothers do not drink coffee or sodas during labor, hence this will help control for neonatal effects from caffeine. Substances such as cocaine and amphetamines are not as easily excluded. The Labor and Delivery staff has a high level of suspicion for maternal drug use and readily obtains maternal drug screens. A positive maternal drug screen for any illegal substance resulted in exclusion of the infant data from the study. Although there was careful verbal screening prior to study entry, drug-exposed infants may have inadvertently been admitted to the study.

The ethnicity of the population at the study site was predominately Caucasian, Hispanic, and African-American. Although the African-American population is represented at the institution, it has traditionally been difficult to get an adequate representation within the sample. Fear of being involved in research was the predominant factor for the low recruitment of African-American subjects in the pilot study. This low level of recruitment is a primary concern since African-Americans are already at a higher risk for SIDS.

Summary

SIDS is a tragic event that profoundly affects society from both a personal and a

holistic perspective. If the proposed study shows a relationship between prenatal nicotine exposure and altered heart rate variables in newborn infants, then a potential nursing method of screening and selection of high risk babies for more intensive SIDS prevention therapy can be developed. These findings may also provide a basis for future research directed towards determination of causality.

CHAPTER TWO

REVIEW OF LITERATURE

This chapter provided an overview of the incidence of smoking during pregnancy, pharmacokinetics of nicotine, functional cardiac development in the fetus and newborn, cardiac regulation of the newborn, and the effects of hypoxia on the newborn. The nicotine induced effects on the central nervous system and myocardium of the newborn, and the effects of passive exposure to environmental tobacco smoke are discussed and summarized.

Incidence of Smoking During Pregnancy

The American Lung Association (1999) reported that in 1996, 400,000 or 13.6% of all women giving birth smoked during pregnancy. The statistics provided by the Centers for Disease Control (CDC) in 1999 were somewhat higher and stated that overall, 28% of women reported smoking during their most recent pregnancy. The highest rate of smoking was reported in teenagers aged 15 to 19 years (CDC, 1999). Of the ethnic groups, American Indians have the highest rate of smoking during pregnancy followed by Caucasian mothers. Hispanic and Asian mothers have the lowest incidence of smoking during pregnancy (American Lung Association, 1999).

Adverse Pregnancy Outcomes Associated with Smoking

Tobacco smoking is associated with adverse pregnancy outcomes for both the

mother and the fetus. Adverse maternal outcomes may include spontaneous abortions, placenta previa, premature delivery, and stillbirths. Adverse fetal outcomes may include low birthweight, respiratory distress syndrome, and SIDS (CDC, 1999; World Health Organization, 1999).

Low birthweight is defined as a birthweight of less than 2500 gram at term gestation, and has been associated with prenatal nicotine exposure (Blackburn & Loper, 1993; CDC, 1999). Several components in cigarette smoke, in particular nicotine, are thought to induce placental insufficiency which reduces fetal growth. On the average, infants of mothers who smoke are 200 grams lighter than infants of mothers who do not smoke (CDC, 1999). Eskenazi et al. (1995) and Perkins et al. (1997) evaluated the relationship of birthweight and maternal serum cotinine. Both authors found a significant ($p < .01$), albeit weak, correlation, $r = -.0.19$ and $r = -0.17$ respectively, and concluded that birthweight decreased 1 ng/ml for every 1 ng/ml increase in the maternal cotinine level.

The effects of ETS on newborn birthweight has also been evaluated. Rebagliato et al. (1998) evaluated the effects of ETS on birthweight using a cohort of pregnant women ($n = 710$). Approximately 89% of the women stated that they were exposed to tobacco smoke during their pregnancy. Nicotine exposure was evaluated using maternal salivary cotinine measurements. The authors found that women with cotinine levels greater than 1.7 ng/ml had a 87.3 gram reduction in newborn birthweight when compared to women with cotinine levels less than 0.5 ng/ml.

The adverse effects of prenatal nicotine exposure are well recognized. Nurses that provide care to women and families of childbearing age are in an ideal position to provide counseling on the adverse effects of prenatal nicotine exposure.

Pharmacokinetics of Nicotine

While it is known that exposure to cigarette smoke increases the risk of SIDS, the pathogenic mechanisms of this increased risk are unknown. Cigarette smoking in adults has been associated with an increased risk of acute cardiac events such as myocardial infarction, sudden death, and stroke (Benowitz & Gourlay, 1997). One of the mechanisms that appears to precipitate the cardiac events in adults is the adverse hemodynamic effects of nicotine.

When the nicotine is taken into the body via the lungs, it enters the bloodstream by diffusion across the capillary membrane lining of the lung. Metabolism of nicotine is predominately by the liver, although 5-10% is excreted unchanged into the urine. The primary metabolite of nicotine is cotinine, with 70-92% of nicotine converted to cotinine. The remainder of the nicotine is converted to other metabolites, and excretion of the metabolites is through the urine (Benowitz, 1996; Benowitz & Jacob,1994).

Nicotine is also a naturally occurring substance found in tomatoes, eggplant, potatoes, cauliflower, and black teas. The amount of nicotine obtained from food products is variable, and even a diet rich in nicotine containing foods would be expected to yield a urine cotinine of less than 0.7ng/ml (Benowitz, 1996).

Quantitative analyses of nicotine metabolites, particularly cotinine, are considered

to be the gold standard for quantifying systemic exposure to tobacco products in smokers and non-smokers. The half life of nicotine is short, approximately 2-3 hours, while the half-life of cotinine is 16-24 hours (Benowitz, 1996; Perkins et al., 1991; Watts et al., 1990).

Cotinine is stable, and measurements of cotinine have been obtained from blood, amniotic fluid, pericardial fluid, saliva, urine, breast milk, and semen with consistent results (Benowitz, 1996; Idle, 1990; Jordanov, 1990; Milerad & Sundell, 1993; Tappin et al., 1995). Based upon stability, ease of measurement, and long half-life, cotinine is currently considered to be the best available biomarker for ETS and tobacco exposure (Benowitz, 1996; Benowitz, 1983).

Functional Development of the Heart

In order to understand the effects of nicotine on the newborn heart, it is first necessary to have an understanding of the basic embryology and functioning of the human heart. The heart is the first organ to become functional in the developing fetus, and provides the necessary circulatory system for embryogenesis and subsequent fetal growth. The vast majority of the knowledge known about the development of the heart has been derived from animal models (Polin & Fox, 1997).

Development of the heart begins around the third week after conception (Moore & Persaud, 1993). The heart develops from paired splanchnic mesodermal primordia located on the sides of the primitive foregut. The paired tubular primordial migrate to the midline, and subsequently fuse to form a single, straight heart tube

(Rosenquist, 1970).

Cellular differentiation, development of contractile proteins such as myosin and toponin, and development of the myofibril precede initiation of the heart beat (Patten, 1956). The heart begins to contract during the third week of life, with the contractions originating in the myogenic muscle layers of the developing heart (Moore & Persaud, 1993). By the fifth week of life the sinoatrial (SA) node develops, and begins to act as the pacemaker of the heart (Moore & Persaud, 1993).

Regulation of the Heart Rate

Regulation of the Fetal Heart Rate

The mechanisms of the regulation of the fetal heart rate are not precisely known. Clark et al. (1986) asserts that the development of the SA node as the pacemaker, the metabolic needs of the developing fetus, and the increase in systemic arterial pressure most likely provides the basis for the variation in the fetal heart rate.

The pattern of heart rate change in the developing fetus has been found to be consistent and is based upon gestational age. The initial heart rate (HR) is approximately 100 beats per minute, but by the fifth to eighth week the HR is 160, and at the eight to tenth week the HR has increased to 170. At the fifteenth week of the gestation, the HR gradually begins to slow to approximately 150 beats per minute, and continues at that rate until delivery (Polin & Fox, 1997).

Regulation of the Newborn Heart Rate

Regulation of the heart rate of the newborn infant is thought to be a combination

of autonomic control and sympathetic innervation of the heart. Autonomic control and sympathetic innervation of the heart begins early in gestation, but is incomplete at term gestation (Chow et al., 1993; Friedman et al., 1968; Lebowitz et al., 1972; Pappano, 1977).

Animal studies have shown that the peripheral and cardiac sympathetic nerve processes mature postnatal. Ursell et al. (1990) and Gauthier et al. (1975), using a canine model, found a mature pattern of sympathetic innervation by approximately two months of age.

Prior to the development of a fully functional sympathetic innervation of the heart, circulating catecholamines assist in cardioregulatory function. Padbury and Martinez (1988) suggest that there is a unique, early neonatal dependence on circulating catecholamines for maintenance of physiologic homeostasis. The circulating catecholamines, epinephrine and norepinephrine, are produced by the adrenal medulla and the para-aortic chromaffin tissue. Beta and alpha adrenergic receptors are located on the myocardial cell membrane surfaces and mediate the sympathetic output to the heart (Polin & Fox, 1997).

Response of the Infant to Hypoxia

During times of stress, the levels of blood oxygen may drop. This lower level of blood oxygen is termed hypoxia. The effects of hypoxia on the infant are different than those seen in the older child. Initially, the infant develops tachycardia in an attempt to increase cardiac output (Robinson, 1996). Blood is diverted away from organs with less

metabolic needs such as the intestines, skin and spleen, and redistributed to the heart, brain, and liver. The presence of fetal rather than adult hemoglobin allows the infant to have a greater blood oxygen content and an enhanced delivery of oxygen when the capillary oxygen levels are low (Polin & Fox, 1997).

While the infant does have the ability to respond to hypoxia, this ability is limited since tachycardia is the primary mode of increasing cardiac output. The ability to increase the heart rate is primarily dependent upon circulating catecholamines. Normally, the maturation of cardiac innervation coincides with a decline in the autonomic response in order to assure continuous protection of the infant from hypoxia (Polin & Fox, 1997).

Slotkin (1997) implanted nicotine infusion pumps into pregnant rats, and infused nicotine at levels that simulated smokers plasma levels. One to two days after delivery, newborn rats with and without prenatal nicotine exposure were exposed to 5% oxygen. The control animals exhibited an initial tachycardia and a slight decline in heart rate. The animals with prenatal nicotine exposure demonstrated no initial tachycardia, and a precipitous decline in heart rate. Slotkin (1998) demonstrated in rats that nicotine caused alterations in the adrenal medulla which in turn inhibited the release of catecholamines during hypoxia.

One of the primary responsibilities of the nurse is to monitor and evaluate the newborn infant for hypoxia. Manifestations such as cyanosis and tachycardia are readily apparent, and should be rapidly detected and corrected in order to avoid subsequent adverse problems

Nicotine Induced Central Nervous System Effects

Nicotine in the blood stream readily diffuses across the blood-brain barrier (Riah et al.,1998). Slotkin (1998) contends that nicotine targets specific neurotransmitter receptors in the brain, and causes a premature stimulation of the receptors that control the timing of cell replication and differentiation. This premature receptor stimulation by nicotine elicits abnormal cell proliferation and premature differentiation which can lead to an eventual decrease in the number of cells and alteration in synaptic activity.

Normally, the maturation of cardiac innervation coincides with a decline in the autonomic response in order to assure continuous protection of the infant from hypoxia. However, Slotkin (1998) contends that nicotine induces terminal differentiation in the chromaffin cells prior to the development of fully functional innervation. Slotkin et al. (1997) found that rat pups exposed prenatally to nicotine had a defect in adrenal cell function, and were unable to appropriately release adrenomedullary catecholamines during hypoxia. This defect in adrenal cell function could leave the infant with a premature loss of autonomic response and a limited ability to respond to stress. Animal models suggest that Slotkin's theory is correct. Newborn rats with prenatal exposure to nicotine have an impaired ability to self resuscitate during repeated episodes of hypoxia (Fewell & Smith,1998).

Nicotine-Induced Myocardial Effects

Researchers have also hypothesized that nicotine may have a direct effect on cardiac function. Effects on sinoatrial reactivity, a reduction in the β-adrenergic receptors

in the myocardium with subsequent reduction in the cardiac response to adrenergic stimulation, measurable nuclear DNA oxidative damage in cardiac, lung, and liver tissue, and transient inhibition of cardiac DNA synthesis are some of the myocardial effects that have been found in animal models with prenatal exposure to nicotine (Howard & Briggs, 1998; Navarro et al., 1990; Slotkin et al., 1997; Tolson et al.,1995). Mendelowitz (1998) and Neff et al. (1998) found that nicotine has an excitatory effect on cardiac vagal neurons, and may increase the vagal effect on the heart with subsequent slowing of the heart rate. Slotkin et al. (1999) demonstrated in rats, that prenatal nicotine exposure enhances cardiac muscarinic cholinergic receptors. This type of receptor is responsible for inhibiting autonomic actions.

Nicotine Induced Cardiac Effects in the Fetus

Although there are no published studies regarding the relationship of prenatal nicotine exposure and the effects on newborn heart rates during transition, there are studies that describe the effect of maternal nicotine exposure on fetal heart rates and cardiac function. The consistent finding after maternal smoking is fetal tachycardia for 20 to 30 minutes with little change in cardiac function. Researchers proposed that increased adrenergic discharge induced by the nicotine may result in vasoconstriction and reduced uterine perfusion leading to the fetal tachycardia (Pijpers et al., 1984; Quigley et al., 1979; Sorenson & Borlum, 1987). Kimya et al. (1998) suggested that chronic smoking causes an increase in vascular resistance of the placenta and umbilical artery. These findings of increased vascular resistance in the umbilical artery may be explained by the findings of

Ahlsten et al. (1986) and Ahlsten et al. (1990) who found reduced prostacyclin activity in the umbilical arteries of infants whose mothers smoked. Prostacyclin is a potent vasodilator.

The fetal effects of transdermal patches and nicotine gum have also been evaluated with varying results found. The findings of fetal tachycardia with the use of a transdermal patch are consistent with those seen with maternal smoking (Onchen et al., 1997; Wright et al., 1997) Interestingly, nicotine gum does not seem to produce the fetal tachycardia seen with smoking and the transdermal patch (Lindblad & Marsal, 1987; Onchen et al., 1997).

Postmortem Analyses of Nicotine Exposure

Exposure to nicotine shortly before death has been suggested by postmortem analyte determinations of nicotine and cotinine levels in victims of SIDS. Pericardial fluid, an ultrafiltrate of plasma, is protected from hemolysis after death making it an ideal fluid for postmortem analyte determinations (Rajs et al., 1997). Milerad et al. (1994) analyzed the pericardial fluid in 24 victims of SIDS. In 22 of the 24 SIDS victims, cotinine was present in the pericardial fluid at levels ranging from 3.5 ng/ml to 110 ng/ml. Of the 22 victims, 25% had cotinine levels greater than 20 ng/ml in the pericardial fluid. Rajs et al. (1997) also utilized pericardial fluid to quantify nicotine exposure prior to death in victims of SIDS ($\underline{n} = 67$). The researchers found cotinine in the pericardial fluid of all 67 victims at levels of 0.2 ng/ml to 152 ng/ml. Of the 67 victims, 25% had cotinine levels greater than 30 ng/ml.

Physiologic Effects Associated with Passive Exposure to ETS

Passive exposure to ETS has been shown to be a factor for heart disease in animal models, and a risk factor for increasing the risk of fatal and nonfatal cardiac events in humans (Glantz & Parmley, 1995). Increased aortic and pulmonary artery atherosclerosis, altered platelet function, and primary endothelial dysfunction in the aortic rings, coronary vessels, and vascular smooth muscle have been found in animal models to be physiologic effects from exposure to ETS (Hutchison et al., 1998; Jorge et al., 1995; Zhu et al., 1993). Glantz and Parmley (1995) contend that the physiologic effects seen with exposure to ETS are not a result of any single element in the smoke, but are a result of the many elements found within the smoke.

Prenatal and postnatal exposure to ETS has been found to increase the prevalence of asthma in children two months to five years of age (Gergen et al., 1998), and to increase the risk of low birth weight and preterm delivery in women greater than 30 years of age (Ahluwalia et al., 1997). An increased incidence of SIDS has also been associated with exposure to ETS (Alm et al., 1998; Blair et al., 1996; World Health Organization, 1999).

Interventions to Decrease Prenatal Nicotine Exposure

All pregnant smokers should be strongly encouraged by nursing to stop smoking, or to reduce their exposure to ETS. The interventions that have been designed to help pregnant smokers quit include smoking cessation advice, feedback, individual and group counseling (Moner, 1993). However, interventions for the reduction of smoking have been

met with mixed success. Wakefield and Jones (1998) assessed the effectiveness of a hospital based smoking intervention provided by nurse midwives. At the initial antenatal visit, women in the experimental group (n = 110) were given brief cessation advice, booklets, and a demonstration of the effects of smoking on the fetal heart rate. The control group (n = 110) received only cessation advice. Smoking status was verified with urine cotinine analysis. Smoking cessation was 6.4% in the intervention group and 1.8% in the control group by late pregnancy. However, at six months postpartum there was no difference in the groups. The authors concluded that minimal advice to quit smoking would most likely have the same effect as their intervention.

Wisborg et al. (1998) evaluated the effects of smoking cessation education provided by two groups of midwives, one of which had received special instruction in educating women to stop smoking. The control group (n = 2629) received care and instruction from the midwives who had not received special instruction. The intervention group (n = 527) received care from the midwives who had received special instruction in smoking cessation. No differences were found between the groups in smoking cessation when validated by salivary cotinine analysis during the pregnancy. The authors concluded that the integration of special instruction into antenatal care failed to affect maternal smoking habits.

Secker-Walker et al. (1998) randomly assigned pregnant smoking women to receive either structured advice and referral to behavior change counseling or brief advice and a booklet at their first antenatal visit with physicians. No difference was seen between

the two groups in smoking status as assessed by urinary cotinine analysis.

Summary

The available literature has demonstrated that both active and passive exposure to cigarette smoke produces cardiovascular effects in animal models and in humans, and that the effects are most likely a combination of central and local mediation. Autopsy results of victims of SIDS indicate that a significant portion of the infants were exposed to cigarette smoke prior to death. The neonatal dependence on tachycardia to increase cardiac output, the dependence on circulating catecholamines to mediate the increased heart rate, and the inability to appropriately release adrenomedullary catecholamines during hypoxia after exposure to nicotine suggest that these factors may be a potentially deadly combination for newborn infants.

One of the fundamental roles of nursing is the dissemination of knowledge. The harmful effects of prenatal nicotine exposure are documented in the literature, as well as the limited effects of interventions directed towards smoking cessation. Wakefield and Jones (1998) and the World Health Organization (1999) conclude that brief interventions such as minimal advice may be just as effective as an active intervention. Nurses that provide care to women of childbearing age are in a perfect position to provide education on the dangers associated with both active and passive prenatal nicotine exposure .

CHAPTER THREE

PROCEDURE FOR COLLECTION AND TREATMENT OF DATA

The study was conducted using a nonprobability, descriptive, correlational design after the research prospectus was approved by Texas Woman's University (Appendix A). The purpose of this design was to examine the relationship between venous cord blood cotinine levels and newborn heart rate (HR) variables (maximum HR and variance). This design was appropriate since there was no manipulation of variables, no control group, no random selection of subjects, nor was there an attempt to establish causality (Burns & Grove, 1997).

Since the sample population was not randomly selected nor was there random assignment to groups, the population needed to be homogeneous in regards to entry criteria. The homogeneous sample population, narrow inclusion and exclusion criteria allowed equivalence and reduced the impact of extraneous variables on the study findings (Burns & Grove, 1997). Inclusion and exclusion criteria with rationales are detailed in Appendix B.

Setting

The setting for the study was a 200 bed community based hospital in south Texas. There are 100-120 newborn deliveries per month at the hospital, and of these deliveries approximately 30% of the mothers smoke or are exposed to ETS. The ethnicity of

mothers delivering at the facility was approximately 70% Caucasian, 27% Hispanic, 2% African American, and 1% Asian.

Approximately 85% of the study population had private insurance coverage, with the remainder of the population covered by Medicaid. The majority of the mothers had received prenatal care, with less than 2% not receiving prenatal care.

Population and Sample

The target population was term, AGA, well newborn infants of mothers with uncomplicated pregnancies and deliveries. This target population of well newborns was chosen to avoid extraneous variables that can be associated with infants who have known prenatal complications or health problems after birth.

The sample population was a convenience sample of infants born at a suburban hospital in south Texas. Subjects were solicited by the investigator from women who were admitted to Labor and Delivery in labor, and who met the Inclusion Criteria.
The Inclusion Criteria was not exclusive of gender or race. Since the sample population was a nonrandom, convenience sample, it was appropriate to use all the infants that met the inclusive criteria. Inclusion and Exclusion Criteria for the study are detailed in Appendix B.

The sample size was estimated based upon recommendations by Cohen (1988) for differences between correlation coefficients. Using an effect size of 0.40 with a two tail test, an alpha of 0.05, and a power of 0.85, a minimum of 115 infants were necessary. The sample included all infants who met the inclusion criteria and whose parents gave

consent for study.

Based upon the number of deliveries per month (100-120) at the study site, it was estimated that a sufficient number of infants will meet the inclusion criteria within a six month time frame. The subjects were recruited and the data collected during the time that the researcher was working at the study site. In general, the researcher was at the study site five to seven days per week, and on call 24 hours per day every other day. This type of work schedule allowed the researcher up to 120 hours per week for subject recruitment and data collection.

Protection of Human Subjects

Permission to conduct this study was obtained from the Human Subjects Review Committee (HSRC) at the study site (Appendix C). The study was classified as exempt from HSRC review at Texas Woman's University since the research protocol was reviewed and approved by the IRB at the study site, and by the IRB at the Centers for Disease Control.

The parents were provided with essential information for informed consent prior to signing the consent form (Appendix C). The four elements of informed consent as described by Burns & Grove (1997) were followed for the study. These four elements included: a) disclosure of essential information, b) comprehension, c) competency, and d) voluntarism. Since the study was of minimal risk to the infant, the written consent of only one parent was required (Burns & Grove, 1997), although both parents were allowed to sign if they wish.

The comprehension of the parents concerning the study was ensured by using lay terminology during the discussion and by wording the consent form at a 9th grade level of education. The parents comprehension was assessed by asking questions concerning the purpose of the study, the risks of the study, the length of the study, confidentiality of the findings, withdrawal from the study, and benefits from the study. The competency of the parents to give consent was determined by the researcher during the discussion of the study. If parents were unable to comprehend the study, they were not asked to allow their infant to participate in the study. Voluntary consent was obtained from all prospective parents of infants who met the inclusion criteria for study entry.

Instruments

There were three instruments utilized for this study. The three instruments included a Maternal/Infant Demographic Data Form, the Corometrics Solar 7000N cardiac monitor, and liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry.

Maternal/Infant Demographic Data Form

The Maternal/Infant Demographic Data Form (Appendix D) included maternal data such as age, parity, ethnicity, medical, obstetric history, and history of nicotine exposure. The infant data that was collected included gender, ethnicity, weight, length, head circumference, apgars, and gestational age. The maternal and infant demographic data were compiled to provide an overview of the sample population.

Corometrics Solar 7000N Cardiac Monitor

The newborn heart rate data were recorded using the Corometrics Solar 7000N cardiac monitor. The Corometrics Solar 7000N met the federal standards for infant cardiac monitoring. All of the data storage is in digital, time-indexed format. The memory technology is non-volatile, solid state flash-card memory. This type of memory prevents loss of data if the power is interrupted. Heart rates were obtained every one minute from the Corometrics monitor during the transition period in the nursery after delivery. The procedure for data collection is detailed in Appendix E.

Gift and Soeker (1988) describe five sources of error or imprecision when using physiologic measurement; environment, user, subject, machine, and interpretation error. To avoid environmental error, heart rates will only be obtained in the transitional nursery. User error will be avoided by having only the researcher setup the monitor and place the electrodes on each infant. Machine error can be random or systematic. Random error is beyond control, but systematic error can be controlled by calibration. The cardiac monitors are evaluated weekly by the biomedical department at the hospital.

The accuracy of the Corometrics Solar 7000N was determined prior to initiation of the study. Verification of the accuracy and precision of the equipment used for measuring physiologic variables is imperative (Szaflarski & Slaughter, 1996). The use of methods comparison studies is an accepted manner of determining accuracy, but many researchers mistakenly use correlation coefficient (r) and linear regression for statistical analysis which can result in incorrect data interpretation (Bland & Altman, 1986; Szaflarski & Slaughter,

1996).

Bland and Altman (1986) outlined a statistical method for examining the nature and extent of agreement between a gold standard method of measurement and the clinical method of measurement. The method described by Bland and Altman provides estimates of systematic and random error, allows assessment of the agreement between methods, and provides visual verification of outliers and differences between methods.

In order to establish the accuracy of the Corometrics Solar 7000, a methods comparison analysis using one randomly selected, term newborn infant was performed using the Corometrics monitor and the Quinton Q750B 12 lead EKG. The 12 lead sequential system is considered the current gold standard for recording cardiac measurements (Herman et al., 1991; Schneiderman et al., 1989). Synchronous recordings of the heart rate were obtained for one hour with recording intervals set for one minute.

Examination of the paired data with a scatter plot (Figure 1) revealed good agreement and minimal variability, with the majority of the values clustered around the line of equality. Examination of the Bland-Altman plot (Figure 2) revealed the majority of the observations clustered around the mean. There appeared to be a relationship between the difference and the mean of the heart rate data, although, there were a few outliers noted. The mean difference of -0.5333 was close to zero, so there was little evidence of overall bias. A bias of zero would indicate perfect agreement between the methods. Calculation of the actual precision, or the "95% limits of agreement" from the mean difference and the S.D. of the differences, revealed that the true value of precision for the Corometrics

monitor lies between -7.3 and +6.2, 95% of the time, and are within ± 2 S D.

Figure 1. Paired Data Scatter Plot.

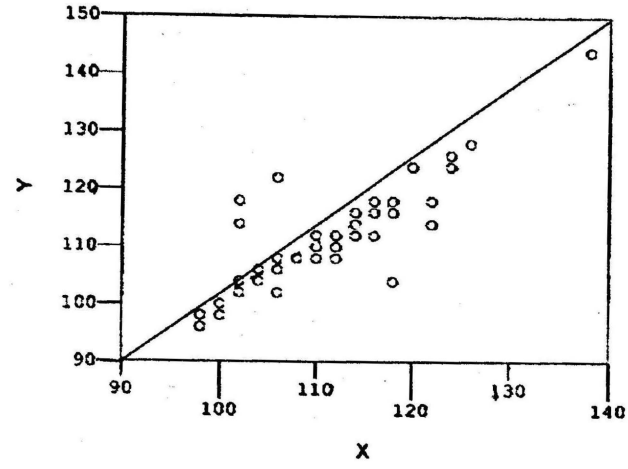
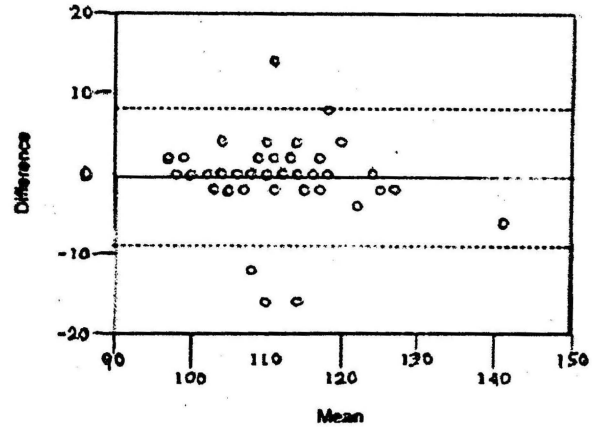


Figure 2. Bland-Altman Plot.



In summary, the data analysis revealed that there was good agreement with low bias, and good repeatability between the Corometrics cardiac monitor and a12-lead EKG. The calculation of precision using the 95% limits of agreement (- 7.3 to + 6.2) were within ± 2 SD, and this magnitude was considered sufficient to allow the use of the Corometrics cardiac monitor for physiologic studies.

Liquid Chromatography Mass Spectrometry

The desired method of cotinine measurement for this study was liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry (LCMS) as described by Bernert et al. (1997). This method provides excellent sensitivity with a low limit of detection (0.05 ng/ml) and high selectivity. The analytical validity of the LCMS was determined by comparison with gas chromatography mass spectrometry (GCMS). There was a strong correlation (r = .991) between LCMS and GCMS. The assay precision was determined using both bench and blind quality controls. The accuracy of the lab analyses was evaluated by randomly sending two samples from the same infant for analysis.

Venous Cord Blood Cotinine Analysis

Physiologic Basis for Choice of Sampling Site

The attainment of accurate cotinine levels was integral to the study. Factors such as source of sample and sampling site have been shown to alter the accuracy of the results of the analyte analysis. Cotinine has been shown to be easily measured in a variety of body

fluids (Watts et al., 1990). However, when quantitative assessment of tobacco exposure is desired, blood is the recommended analyte of choice (Watts et al., 1990; Benowitz, 1983).

It has been shown that the site of blood sampling, arterial or venous, can profoundly alter the interpretations of analyses. Gourlay & Benowitz (1997), Chiou (1989), and Robinson et al. (1992) contend that venous blood is not a reliable route for sampling. Venous blood is the returned blood from tissues and organs, and should not be used to represent the drug in the central blood pool. Since it is arterial blood that carries the drug and metabolites to various parts of the body, it is logical to use arterial data for pharmacodynamic modeling or analysis. Gourlay and Benowitz (1997) found that the mean, peak arterial plasma concentrations of nicotine after smoking or administration of nasal spray were twofold greater than that of venous plasma.

The vast majority of newborn blood cotinine estimations in the literature have been done with no distinction between arterial and venous blood sampling leaving the reader to assume that the analyte concentration was a mixture of arterial and venous blood. Based upon the findings of Gourlay and Benowitz (1997), this would not be an accurate reflection of cotinine levels. In order to accurately measure the level of fetal exposure, a single sampling site would need to be utilized, preferably one that would mimic the arterial circulation of the adult.

Pattern of Fetal Blood Flow

The pattern of blood flow in the fetus is different from that of the newborn infant

or adult. What would be considered arterial circulation in the adult, is in reality venous circulation in the fetal state. Oxygen, nutrients, and drugs diffuse across the placenta and are delivered to the fetus via the umbilical vein while the dual umbilical arteries return deoxygenated blood and waste products back to the placenta (Moore & Persaud, 1993).

The umbilical vein provides branches to the left lobe of the liver. Approximately 50% of the umbilical venous blood enters the hepatic circulation, with the remainder of the venous blood bypassing the liver by shunting through the ductus venosus. The blood entering into the inferior vena cava from the ductus venosus tends to flow through the foramen ovale with subsequent distribution to the left atrium and ventricle, thus entering the ascending aorta to supply the brain, heart, and upper body. The shunting through the ductus venosus allows approximately half of all transported drugs to bypass the liver where it could potentially be metabolized, and to flow directly to the fetal heart and brain at conceivably higher concentrations (Rudolph, 1995).

In order to obtain an accurate estimate of fetal cotinine, blood samples were obtained from the placental umbilical vein at the time of delivery. The procedure for obtaining the venous cord blood samples is detailed in Appendix B. Venous cord blood cotinine levels were obtained on infants in both groups in order to objectively assess nicotine exposure. Cotinine levels were obtained from infants of reported nonsmoking mothers as well as from reported smoking mothers since passive smoke is transferred to the fetus (Lambers & Clark, 1996), and the unreliability of self-reports and questionnaires concerning tobacco use has been well documented (Ford et al., 1997; Perez-Stable et al.,

1995; Walsh et al., 1996).

Pilot Study

Prior to the main data gathering event, a pilot study was performed. The purpose of this pilot study was to evaluate the effectiveness of the research methodology and design, and to refine the steps of the research process (Burns & Grove, 1997).

The hypothesis for the pilot study was that there is a difference in transitional heart rate variables in newborn infants with and without prenatal exposure to nicotine. Since nothing is known concerning the effects of prenatal nicotine exposure on newborn transitional heart rate variables a nonprobability, comparative, descriptive research design was utilized. An experimental design which requires control, manipulation, and randomization would be extremely difficult and impractical, not to mention the ethical concerns of requiring the experimental group to smoke during pregnancy.

Since the determination of a difference between two independent groups was the purpose of the pilot study, sample size was calculated based on the recommendations of Cohen (1988) when using the t-test for independent measures for inferential analysis. Using an effect size of 0.50 with a two tail test, an alpha of 0.05, and a power of 0.80, two groups of 64 infants each were calculated to be necessary for a proposed study. A pilot study generally requires a minimum of 10% of the calculated sample size, which would be six infants in each group. In this case 30% of the estimated sample size was utilized since subjective rather than objective methods of assessing nicotine exposure were utilized. Ideally, serum cotinine levels, the primary metabolite of nicotine, are considered the gold

standard for assessing nicotine exposure.

Pilot Study Findings

A brief overview of the pilot study findings are as follows. The sample population was composed of term, AGA, well newborn infants delivered at a suburban hospital by mothers who had no known health problems, and uncomplicated pregnancies and deliveries. Narrow inclusive and exclusive criteria allowed for a homogeneous sample population, and reduced the impact of extraneous variables.

Group assignment was based upon maternal responses and behaviors. Infants whose mothers denied active or passive prenatal exposure to nicotine were placed in the non-exposed group, while infants whose mothers acceded to active or passive prenatal exposure where placed in the exposed group. Maternal behaviors such as going out to smoke, or smelling like smoke, even with denial of exposure, resulted in the infants being excluded from the non-exposed group. Table 2 details the demographics of the pilot study subjects.

Table 2. Demographics of Pilot Subjects With and Without Prenatal Nicotine Exposure.

Variable	Exposed (n = 19)	Non-Exposed (n = 19)
Mean Birthweight	3349 grams	3516 grams
Maternal Age	24 years	27 years
Ethnicity:		
Caucasian	n = 17 (45%)	n = 17 (45%)
Hispanic	n = 1 (2.5%)	n = 2 (5%)
African-American	n = 1 (2.5%)	n = 0

The heart rate (HR) of the infants was monitored every one minute during the transition period after delivery using the Corometric Solar 7000 cardiac monitor. The accuracy of the Corometric monitor (95% CI ± 2SD) had been determined prior to study initiation by using the guidelines suggested by Bland and Altman (1986).

Transitional heart rate variables of newborn infants with and without prenatal nicotine exposure were assessed in the pilot. Inferential analysis using the t-test for independent measures found statistical significance ($p < .05$) in maximum heart rates, range of the heart rates, and variance of the heart rates in the prenatally exposed group of infants when compared to the non-exposed infants (Table 3) .

Table 3. Inferential Analysis of the Transitional Heart Rate Variables.

Variable	Significance
Mean Heart Rate	0.062
Minimum Heart Rate	0.331
Maximum Heart Rate	0.016 *
Range	0.027 *
Variance	0.004 *
Median	0.122

* $p < 0.05$

The results of this pilot study suggest that newborns with prenatal nicotine exposure are unable to increase and vary their heart rates to the levels of non-exposed infants. Based upon the findings of the pilot study, subsequent research will focus on determining if there is a relationship between serum cotinine levels, and the maximum HR, range of the HR, and variance in the HR of the newborn infant.

Refinements Derived from the Pilot

The pilot study did allow for refinement in the technique for collection of the cord blood and for reduction of hemolysis of the blood serum samples. Initially, a five ml syringe was used to aspirate the blood from the placental umbilical vein since the researcher was unsure of the amount of blood that could be obtained. Subsequent trials using 10ml and 20ml syringes rapidly determined that a 20ml syringe could be used to obtain a large volume of cord blood without difficulty.

Hemolysis of the blood serum was noted on early samples. Initially, the cord blood

was injected into the glass laboratory tubes by allowing the vacuum inside the tubes to aspirate the blood sample into the tube. After discussion with the laboratory manager, the researcher began removing the caps from the glass tubes prior to injecting the blood. This resolved the problem with hemolysis of the serum samples.

Subject recruitment was an unanticipated problem that arose during the pilot study. Recruitment of subjects from the African-American population was found to be difficult. Only one African-American mother would consent to the pilot study, six other African-American women declined. Even the use of African-American nurses to accompany the researcher and help explain the study made no difference in the recruitment of African-American subjects. The typical response from the African-American family was that they "did not want their baby involved in research."

In summary, the pilot study revealed problems with the placental cord blood collection techniques, and difficulty in recruiting African-American subjects. The findings from the pilot study were used to modify the blood collection procedure, and improve the quality of the blood samples obtained for the proposed study. Knowing the difficulty in recruiting adequate numbers of African-American subjects will allow the researcher to prolong the time frame for data collection in the proposed study if necessary in order to obtain a greater number of African-American subjects.

Data Collection

A complete step by step protocol for the conduction of the study can be found in Appendix E. This protocol details the procedures of the study after IRB approval from the study site has been obtained (Appendix C).

Once it has been determined that a woman admitted to the Labor and Delivery unit meets the inclusion criteria, the mother and family will be approached for study participation. Information concerning the study will be given in the manner detailed under the "Protection of Human Subjects" section. Once informed consent for study participation has been obtained from the parents, demographic data will be collected on both the mother and the infant, and recorded on the Maternal/Infant Demographic Data Form (Appendix D) before and after delivery. The demographic data recorded will include nominal, ordinal, ratio, and interval level data.

The ratio/interval level data will include:

1. Maternal age.
2. Number of pregnancies, number of miscarriages/abortions, and number of living children.
3. Number of cigarettes smoked per day.
4. Infant birth weight, length, and head circumference.
5. Number of minutes of heart rate recording
6. Mean, median, minimum, maximum, range, and variance of each infant's

heart rate data.

The nominal/ordinal level data will include:

1. Prenatal exposure to cigarette smoke: active, passive, and no reported.
2. Infant gender.
3. Maternal and infant ethnicity
4. Prenatal maternal medical history
5. Prenatal labs (PNL) including blood type, serology, Hepatitis B status, Group B Strep culture results, and antibody status.
6. Last menstrual period (LMP) and expected date of confinement (EDC).
7. Labor and delivery history including anesthesia, mode of delivery, and apgar scores.

Data Analysis

Data analysis was done using the SPSS 9.0 PC version statistical package. For the demographic date obtained on the mothers and infants, statistics were calculated including frequencies, percentages, and measures of central tendency. The demographic data analysis allowed an overview of the sample population characteristics. Variability (range, variance, S.D., SEM) and measures of central tendency (mean, median, minimum, maximum) were calculated from the heart rates of each infant.

The two hypotheses were predicting a relationship between two parameters, venous cord blood cotinine and a newborn heart rate variable (maximum heart rate or variance). Statistical analysis using the Pearson correlation measured the degree and

direction of linear relationships between the cotinine level and the maximum heart rate and variance of the newborn heart rate. The assumptions underlying the Pearson correlation were met by determining linearity with examination of a scatterplot and by using mutually exclusive data from each infant.

The strength of the relationship was measured by the coefficient of determination (r^2). The coefficient of determination assessed the proportion of variability in one variable that can be determined from the relationship with the other variable.

Summary

The study proposed was of a nonprobability, descriptive, correlational design. Pearson Correlations were conducted to evaluate the relationship between venous cord blood cotinine levels and a newborn heart rate variable (maximum or variance). The use of narrow inclusive and exclusive criteria allowed for the control of extraneous variables, but reduced the generalizability of the study findings.

CHAPTER 4

ANALYSIS OF DATA

This study was designed to examine the relationship between venous cord blood cotinine levels and transitional heart rate variables in newborn infants. Using venous cord blood cotinine levels and transitional newborn heart rates as the research variables, this study examined variables in a situation that had already occurred, prenatal exposure to nicotine. This chapter provides a description of the maternal and infant sample based upon the demographic data, determination of the validity of the cotinine analysis, and the results of the statistical analysis of the heart rate data and the cotinine levels.

Description of the Sample

For the study, 146 subjects were solicited with 134 agreeing to participate. Reasons for maternal refusal included not wanting to know about problems with their baby, and not wanting to have their babies involved in research.

Of the 134 mothers who agreed to allow their infants to participate, four mothers were excluded because of positive drug screens for cocaine and marijuana. The remaining130 mother/infant couplets constituted the study sample.

Description of the Maternal Sample

The maternal age of the sample population ranged from 16 years to 41 years, with a mean maternal age of 27 ± 5.6 (SD). Of the maternal sample, 98% received prenatal care.

The ethnic composition of the study sample was 85% Caucasian ($n = 111$), 11%

Hispanic (n = 14), 3% African American (n = 4), and 1% Asian (n = 1). The mean maternal age based upon ethnicity is detailed in Table 4.

Table 4. Average Maternal Age Based Upon Ethnicity

Ethnicity	n	Age	Range of Age
Caucasian	111	27 ± 0.6	16-41
Hispanic	14	28 ± 1.3	19-36
African American	4	27 ± 3.4	17-32
Asian	1	33	

Age = Mean years ± SEM. n = Number of women in each ethnic group.

The mode of delivery was predominantly vaginal (n = 91), with the remainder of the deliveries by c-section (n = 39). All of the women in the sample population had epidural anesthesia for delivery.

Veracity of the Maternal Statements

As part of the routine admission process to the hospital, women were asked if they smoked or if they were exposed to environmental tobacco smoke during their pregnancy. For those women that met the inclusion criteria and agreed to participate in the study, the notation on the demographic data sheet was either no exposure, passive exposure, or active exposure. The three categories of exposure, no exposure, passive, and active, would correspond to cotinine levels of less than 0.05 ng/ml, 0.05-6.0 ng/ml, and greater than 6.0 ng/ml respectively (Benowitz, 1996; Bernert, 1997). There were 88 women who stated they had no prenatal exposure, 17 who stated they had passive exposure, and 25 who reported that they actively smoked during their pregnancy.

Based upon the cotinine levels, 68 women had no exposure, 39 women had passive exposure, and 23 had active exposure to nicotine during their pregnancy. There was a statistically significant correlation between the veracity of the maternal statement of prenatal nicotine exposure and the venous cord blood cotinine level, $r = .403$, $p = .001$. Table 5 shows a cross tabulation of the maternal statements and the cord blood cotinine levels.

Table 5. Maternal Statements of Prenatal Nicotine Exposure Compared to Cord Blood Cotinine Levels.

Type of Exposure	Cord Blood Cotinine Level			Total
	< 0.05 ng/ml	0.05-6.0 ng/ml	> 6.0 ng/ml	
No Reported Exposure	63	25	0	88
Passive Exposure	4	12	1	17
Active Exposure	1	2	22	25
Total	68	39	23	130

Description of the Newborn Sample

The newborn sample was composed of 61 females and 69 males, 47% and 53% respectively of the study population. Demographics based upon gender and ethnicity of the newborn sample are detailed in Table 6.

Table 6. Gender and Ethnicity of Newborn Sample Population

Boy (n = 69)				Girl (n = 61)			
Cauc.	Hisp.	A.A	Asian	Cauc.	Hisp.	A.A	Asian
58	8	2	1	53	6	2	0

Gender = Number of subjects. Cauc. = Caucasian. A.A. = African American. Hisp. = Hispanic.

The mean birthweight of the newborn study population was 3503 grams ± 39.9 (SEM), with a range of 2555 - 4000 grams. Based upon gender, the average birth weight for males was 3525 grams ± 51.8 (SEM), and 3481 grams ± 61.3 (SEM) for females.

There was a statistically significant, albeit weak, negative correlation between the venous cord blood cotinine level and the birthweight of the newborn population, $r = -.183$, $p = .045$. Birthweight tended to be lower for infants born to mothers with higher cotinine levels. However, the coefficient of determination (r^2) was 0.033, with only 3.3% of the variance in birthweight resulting from the linear relationship with the cord blood cotinine.

Findings

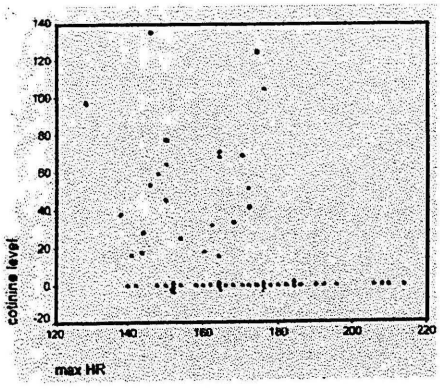
Placental cord blood was drawn at the time of delivery, and analyzed for cotinine. The venous cord blood cotinine levels ranged from 0 ng/ml to 135 ng/ml. Cotinine levels as high as 287 ng/ml were found, however these levels were discarded because of positive maternal drug screens. The reliability of the cotinine analysis was determined by sending two samples from two randomly chosen infants. The samples had identical values of 0 ng/ml and 1.6 ng/ml respectively.

The heart rates of the newborn infants were monitored every one minute for the

first four hours of life. The mean heart rate of the sample population was 129 ± 1.04 (SEM), with a range of 70 to 214. The mean maximum heart rate of the sample population was 167 ± 1.36 (SEM), with a range of 128-214.

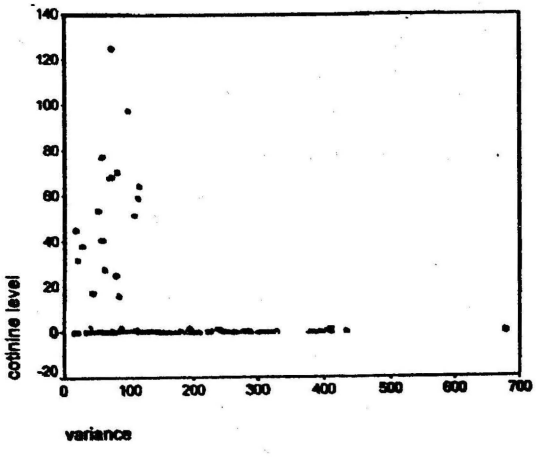
The first hypothesis stated that there is a relationship between venous cord blood cotinine and the maximum HR of term, appropriate for gestational age, well newborn infants during the immediate postnatal period of transition. In order to test the the first hypothesis of this study, the maximum newborn heart rates were correlated with cord blood cotinine levels. Results of the Pearson Correlation revealed that there was a statistically significant negative correlation between the maximum heart rates and the cotinine levels, $r = -.271$, $p = .002$. Infants with increased nicotine exposure demonstrated a lower maximum HR. The coefficient of determination (r^2) was .07, which signifies that 7% of the variance in the maximum HR was associated with the cotinine levels. Figure 3 depicts a bivariate scatterplot of the relationship between the maximum heart rates and the cotinine levels.

Figure 3. Bivariate Scatterplot of the Relationship Between Maximum Heart Rate and Cord Blood Cotinine



The second hypothesis of the study states that there is a relationship between venous cord blood cotinine and the variance of the HR in term, AGA, well newborn infants during the immediate postnatal period of transition. The mean variance of the heart rate of the sample population was 160 ± 9.79 (SEM), with a range of 17 to 677. There was a statistically significant negative correlation between the variance of the heart rates and the cotinine levels, $r = -.206$, $p = .019$. Infants with increased prenatal nicotine exposure experienced lower HR variance. The coefficient of determination (r^2) was .04, signifying that 4% of the variance of the HR is associated with the cotinine levels. Figure 4 depicts a bivariate scatterplot of the relationship between the heart rate variance and the cord blood cotinine.

Figure 4. Bivariate Scatterplot of the Relationship Between Heart Rate Variance and Cord Blood Cotinine.



Summary of Findings

A total of 130 mother/infant couplets participated in this descriptive, correlational study to determine if there was a relationship between prenatal nicotine exposure and newborn heart rate variables during the immediate postnatal period of transition. The maternal sample population was composed primarily of Caucasian women in their late 20's. The newborn sample population was of predominately Caucasian ethnicity with an even distribution of male and female subjects.

Placental cord blood was drawn at the time of delivery and analyzed for cotinine. The maternal statements of prenatal nicotine exposure correlated significantly with the cord blood cotinine levels, signifying that the mothers were truthful concerning their exposure to nicotine during their pregnancy.

The heart rate of the newborn infant was measured every one minute during transition. The maximum heart rate and the variance of the heart rate were then correlated with the cord blood cotinine level. Inferential analysis, using the Pearson Correlation, found that there were statistically significant, albeit weak, negative relationships between the venous cord blood cotinine levels, the maximum heart rate, and the variance of the heart rate. The coefficient of determination (r^2) ascertained that 7% of the heterogeneity in the maximum heart rate and 4% of the heterogeneity of the variance in the heart rate were a result of the linear relationship with the cord blood cotinine.

CHAPTER 5

SUMMARY OF THE STUDY

The etiology of the increased risk for SIDS in infants with prenatal nicotine exposure is unknown. Because nicotine has been shown to cause adverse cardiovascular effects in adults, animal models, and in the fetus, this study was designed to determine if there was a relationship between prenatal nicotine exposure, the maximum heart rate, and the variance of the heart rate in term, well, appropriate for gestational age, newborn infants during the immediate postnatal period of transition. Transition was chosen as the time frame for this study in order to assess the effects of fetal drug exposure after the challenging, rather than basal, conditions associated with birth. The theoretical framework utilized for this study was Diffusion Theory since diffusion is the mode of substance transfer between the maternal and fetal circulations.

This chapter includes a summary of the study and discussion of the study findings. Conclusions, implications for practice, and recommendations for future research are also discussed.

Summary

A descriptive, correlational design was used to determine if there was a relationship between prenatal nicotine exposure and newborn heart rate variables during transition. The setting for the study was a community based hospital in south Texas.

Subjects were recruited from mothers who were admitted to the hospital in labor. A narrow inclusion criteria allowed for a homogeneous sample population of term, well infants born to mothers with no known health problems, and an uncomplicated pregnancy, labor and delivery. A convenience sample of 130 mothers and their newborn infants constituted the sample population. In order to quantify the prenatal nicotine exposure, a sample of placental cord blood was drawn at the time of delivery to be analyzed for cotinine.

Prior to the study initiation, the accuracy of the cardiac monitor had been determined using the guidelines of Bland and Altman (1986). The cardiac monitor was found to have almost perfect agreement with a 12-lead EKG, and was deemed suitable for use in the study. After delivery, the infant was placed on the cardiac monitor and the heart rate was monitored and recorded every one minute during transition, the first four hours of life. The relationship of the cord blood cotinine, the maximum heart rate, and the variance of the heart rate were determined using a Pearson Correlation.

Discussion of the Findings

The non-directional, associative hypotheses that guided this study were:

1. There is a relationship between venous cord blood cotinine and the maximum HR of term, appropriate for gestational age (AGA), well newborn infants during the immediate postnatal period of transition.
2. There is a relationship between venous cord blood cotinine and the variance of the HR in term, AGA, well newborn infants during the immediate postnatal period of

transition.

Both hypotheses were supported by the findings of this study. A statistically significant negative, or inverse, relationship was demonstrated between cord blood cotinine, the maximum heart rate, and the variance of the heart rate in newborn infants during transition. The findings of this study suggest that newborn infants with higher venous cord blood cotinine levels have a limited ability to maximize and vary their heart rate. Cardiac output in the infant is primarily dependent upon heart rate. If the infant is unable to maximize cardiac output during times of stress, the infant is at an increased risk for morbidity and possible mortality.

Validation of Diffusion Theory

The logic of using Diffusion Theory as the theoretical framework for this study was validated by the evidence of fetal nicotine exposure as quantified by the cord blood cotinine levels. The maternal statements of prenatal nicotine exposure were significantly correlated with the infant's cord blood cotinine levels. The only means by which the fetus could have acquired the prenatal nicotine was by placental diffusion from the maternal circulation.

The Relationship Between Cord Blood Cotinine, Maximum HR, and Variance of the HR

There is no published literature discussing the inverse relationship between cord blood cotinine, maximum heart rate, and variance of the heart rate in newborn infants. However, animals models have demonstrated that nicotine modifies central structures related to control of the heart rate, and that animals with prenatal nicotine exposure have a

limited ability to self resuscitate.

The study infants demonstrated a lower maximum heart and decreased variability of the heart rate with higher levels of cotinine. The findings of this study are similar to those seen when animals with prenatal exposure to nicotine are exposed to hypoxic conditions. Because of the ethical issues involved, there are no human studies that replicate these findings. Hence, extrapolation from animals models is the best available answer.

The findings of lower maximum heart rates and decreased heart rate variance after delivery in the newborn infants with higher levels of cord blood cotinine are consistent with the findings of Fewell and Smith (1998) and Slotkin (1997). Rats with prenatal nicotine exposure experienced a precipitous decline in heart rate when exposed to hypoxia, and had a limited ability to self resuscitate. The challenging experience of birth can be considered a hypoxic event for the newborn, and a limited ability to self resuscitate may be expressed as an inability to maximize and vary the heart rate.

The lower maximum heart rates are also consistent with the findings of Mendelowitz (1998), Neff et al. (1998), and Slotkin (1999) who examined the effects of nicotine on specific brainstem neurons that control heart rate, and found that nicotine directly activates vagal cardio-inhibitory neurons. While it is impossible to examine brainstem neurons in newborn infants, the study findings suggest that higher levels of nicotine do appear to inhibit the heart rate in newborn infants.

The lower maximum heart rate seen with increased cotinine levels could also be

secondary to a lower circulating catecholamine level. Maturation of the sympathetic innervation of the heart is a postnatal process (Friedman et al.,1968). The importance of circulating catecholamines for maintenance of physiologic homeostasis during the first two months of life was described by Padbury and Martinez (1988). Slotkin (1997) demonstrated in rats that prenatal nicotine exposure causes a defect in adrenal cell function. While the adrenal cells are able to produce the catecholamines, there was an inability to release adrenomedullary catecholamines during hypoxia. If Slotkin's Theory of Altered Adrenomedullary Function is true in newborn infants, lower levels of circulating catecholamines could mean that during periods of stress infants would lack the ability to maximize their HR response.

The findings of fetal tachycardia associated with maternal smoking were not replicated in this study, nor were they expected to be found. The fetal tachycardia noted by Pijpers (1984), Sorenson and Borlum (1987) was limited to 20 to 30 minutes after the maternal smoking and resolved spontaneously. The time frame for the tachycardia would have been hours past since most women are in labor for hours and do not smoke during labor nor do they normally reapply transdermal nicotine patches.

Rajs et al. (1997) and Milerad et al. (1994) found elevated levels of cotinine in the pericardial fluid of victims of SIDS. In approximately 25% of the cases, both authors found levels greater than 20 ng/ml. In this study 15% (n = 19) of the infants had cotinine levels greater than 20 ng/ml with a range of 25-135. Of these 19 infants, 89% (n = 17) had cotinine levels greater than 30 ng/ml. These results are very disturbing in light of the

pericardial fluid findings of cotinine in victims of SIDS by Rajs et al. (1997) and Milerad et al. (1994).

The Relationship of Prenatal Nicotine Exposure and Birthweight

The CDC (1999) contends that one of the adverse fetal effects associated with prenatal nicotine exposure is low birthweight. While this study only evaluated infants that were appropriate for gestational age, not low birthweight infants, a negative, or inverse relationship between birthweight and prenatal nicotine exposure was found. The CDC (1999) states that infants of mothers who smoke are approximately 200 grams lighter than infants of mothers who do not smoke. This study found that infants with cotinine levels greater than 6.0 ng/ml, which is consistent with active smoking (Benowitz, 1996), were 83 grams lighter than those with cotinine levels of less than 0.05 ng/ml. However, in this study only 23 infants had cotinine levels greater than 6.0 ng/ml. The discrepancy in results may be attributed to the small sample size.

The effects of ETS on newborn birthweight in this study were greater than that found in the literature. Rebagliato et al. (1995) determined that infants with cotinine levels greater than 1.7 ng/ml were 87.3 grams lighter than infants with cotinine levels less than 0.5 ng/ml. This study found that infants with cotinine levels greater than 1.7 ng/ml were 118 grams lighter than infants with cotinine levels less than 0.5 ng/ml. The discrepancy in birthweight is difficult to explain. Rebagliato's study was conducted in Spain with a sample size of 710 women, while this study was conducted in the United States with a sample size of 130 women. The average birthweight of infants born in Spain is not known.

The discrepancy in findings may be explained simply by sample size and cultural differences.

However, the overall reduction in birthweight associated with cotinine levels was consistent with that found in the literature. Eskenazi et al. (1995) and Perkins et al. (1997) found that birthweight decreased 1 gram for every 1 ng/ml increase in serum cotinine. The findings from this study are quite similar and suggested that birthweight decreased 0.9 gram for every increase of 1 ng/ml in serum cotinine.

Veracity of Maternal Statements of Prenatal Nicotine Exposure

Much to the surprise of the researcher, the maternal statements regarding prenatal nicotine exposure correlated with the cord blood cotinine levels. The predominant view in the literature was that active smokers were unlikely to be honest concerning their tobacco use, and the unreliability of self-reports and questionnaires concerning tobacco use was well documented (Ford et al., 1997; Perez-Stable et al., 1995; Walsh et al., 1996). However, a subsequent review of the literature found that Peacock et al. (1998) and Klebanoff et al. (1998), using self report and maternal serum cotinine, demonstrated that pregnant women do accurately report their smoking status.

Interestingly, there was a 44% increase in the number of women who had cotinine levels in the passive exposure range compared their verbal statement of exposure (39 vs 17). This increase in cotinine may have been attributed to consumption of dietary cotinine (tomatoes, potatoes, cauliflower, black tea), although this aspect of nicotine exposure was not discussed with the study subjects by the researcher. Another explanation

is that the women in the study sample may have underestimated their exposure to second hand smoke. Benowitz (1996) contends that 75% or more of the nicotine that is emitted from a cigarette is emitted into the air as sidestream smoke. Nicotine from ETS also deposits on room surfaces such as walls and carpets, and on clothing of smokers. Cotinine levels of 0.1 ng/ml to 0.3 ng/ml have been found in the urine of people exposed to environmental ETS (Benowitz, 1996). This amount of emitted nicotine from side stream smoke and environmental areas could contribute substantially to ETS and passively expose women to far greater levels of nicotine than originally thought. Because of these factors women may tend to underestimate their exposure to second hand smoke.

Conclusion and Implications

The following conclusions derived from this research are limited to term, well newborn infants born to predominately Caucasian mothers with no known health problems, and an uncomplicated labor and delivery.

1. Newborn infants in transition who have had prenatal nicotine exposure have a lower maximum heart rate. This lower maximum heart rate could potentially limit their ability to increase their cardiac output during times of stress.
2. Newborn infants in transition who have had prenatal nicotine exposure have a lower variance of heart rate. This lower variance of heart rate could potentially limit their ability to alter their cardiac output during times of stress.
3. Prenatal exposure to nicotine was associated with a lower birthweight in newborn infants.

4. Maternal statements of prenatal nicotine exposure from mothers who actively smoke are reflective of actual smoking activity.

The results of this study provide data that as the cord blood cotinine level increases the maximum heart rate decreases and the variance of the heart rate declines. The following nursing implications are drawn from the findings of this study.

1. The findings of this study suggest that newborn infants with higher venous cord blood cotinine levels have a limited ability to maximize and vary their heart rate. Cardiac output in the infant is primarily dependent upon heart rate. If the infant is unable to maximize cardiac output during times of stress, the infant is at an increased risk for morbidity and possible mortality. Nurses need to stress the adverse effects of nicotine on the infant heart rate to the mother and family.

2. Nurses should develop and implement strategies to encourage pregnant women not to smoke and to avoid passive smoke exposure. Current strategies in smoking cessation are not significantly successful. Nurses are viewed as sources of information by family, friends, and clients. The dissemination of knowledge is a duty of the nurse. Nurses, particularly those that care for women of childbearing age, are in the position to counsel on the negative effects of prenatal exposure to nicotine. These negative effects include alterations in the infant's ability to maximize and vary the heart rate, and a lower birthweight. In order to be effective, this counseling must be presented in a non-judgmental and supportive manner. The Nursing Center for Tobacco Intervention (1999) suggest that nurses provide at the

very least a minimal intervention which is comprised of the following steps:

a. Ask every pregnant woman about smoking.

b. Advise every pregnant woman to quit smoking early since this benefits the mother and fetus the most.

c. Assist every pregnant woman by providing motivational messages such as "quitting smoking is the most important gift that you can give to your baby."

d. Arrange for follow-up to assess the mother's progress.

3. Newborn assessment guidelines need to be reassessed The current AAP guidelines for monitoring the heart rate of newborn infants should be modified for those newborn infants with prenatal nicotine exposure. The current infrequent nursing measurements do not allow for detection of a trend, and needs to be revised with more frequent heart rate measurements. If an abnormally low heart rate pattern is detected, an EKG should be performed in light of the findings of Schwartz et al. (1998).

4. Women may under estimate their exposure to passive smoke. Nursing should stress that exposure to passive smoke and environmental nicotine may result in higher levels of nicotine exposure for the fetus.

5. Women of childbearing age or family members who smoke and desire to quit need appropriate referrals to primary care providers and community agencies that can assist them in this endeavor.

Recommendations for Further Study

The results of the study form the basis for the following recommendations:

1. This study should be replicated using an experimental design with random sampling, an increased sample size, and an increased multi cultural sample to increase the generalizability of the findings beyond the study sample.
2. While there was a significant relationship between cord blood cotinine, the maximum heart rate, and variance of the heart, only 4 to 7% of the heterogeneity in the heart rate variables was related to prenatal nicotine exposure. Some other factor, or factors must also be contributing to the alterations in heart rate seen with the higher levels of cotinine. Slotkin's Theory of Altered Adrenomedullary Function should be tested by correlating catecholamines and cotinine at delivery with the maximum heart rate and variance of the heart rate during transition.
3. A prospective, experimental study should be designed to investigate the evolution of the maximum heart rate and the variance of the heart rate in newborns with and without prenatal nicotine exposure.
4. An experimental study should be designed to evaluate infants that are admitted to the hospital with apparent life threatening events (ALTE). Cotinine levels should be obtained on admission and correlated with the heart rate and heart rate variability.

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APPENDIX A

Prospectus Approval

[redacted]

TEXAS WOMAN'S
UNIVERSITY
DENTON/DALLAS/HOUSTON

[redacted]
THE GRADUATE SCHOOL
P.O. Box 425649
Denton, TX 76204-5649
Phone: 940/898-3400
Fax: 940/898-3412

June 15, 1999

Ms. Janice M. Wheeler
San Marin Apt. NR. 605
8181 El Mundo
Houston, Tx 77054

Dear Ms. Wheeler:

I have received and approved the prospectus entitled **"The Relationship of Prenatal Nicotine Exposure and Newborn Heart Rate: Is There a Basis for SIDS?"** for your *Dissertation* research project.

Best wishes to you in the research and writing of your project.

Sincerely yours,

Leslie M Thompson

Leslie M. Thompson
Associate Vice President for Research and
Dean of the Graduate School

LMT/sgm

cc Dr. Anne Young, Nursing-Houston
Dr. Carolyn Gunning, Nursing

APPENDIX B

Inclusion and Exclusion Criteria

Inclusion Criteria and Rationale for Study Entry

Inclusion criteria	Rationale
37-41 weeks gestation	Infants at this gestation are considered term
2500-4000 grams birth weight	Infants of this weight range are considered appropriate for gestational age
Apgar score of 7 or > at one minute	Apgar score of ≥7 is considered normal at 1 minute
Apgar score of 8 or > at five minutes	Apgar score of ≥8 is considered normal at 5 minute
Stable in room air, no respiratory distress	Infants with respiratory distress are tachycardic as a compensatory mechanism for hypoxia
Normothermic: 97.6-99.6 rectal temp	Cold or febrile infants are tachycardic
Bottle feeding	Breast feeding infants are excluded secondary to transfer of nicotine through the breast milk

Exclusion Criteria and Rationale

Exclusion Criteria	Rationale
Maternal history of substance use/abuse, or positive drug screen	May cause alterations in the heart rate of the infant
Complicated pregnancy: Abnormal ultrasounds, serious infections	May adversely affect the fetus
Serious maternal health problems: Cardiac, renal, metabolic	May adversely affect the fetus
Obvious physical, genetic abnormalities	May have underlying cardiac disease
Congenital heart disease	May cause alterations in heart rate
Breast feeding	Nicotine is transmitted in the milk
Deterioration of physical condition during transition	May cause alterations in heart rate

APPENDIX C

IRB Approval and Consent Forms

COLUMBIA Kingwood
Medical Center

22999 U.S. Hwy 59
Kingwood, Texas 77339
(281) 359-7500

HSRC APPROVAL FORM

Name of Investigator (s): Jan Wheeler RNC,NNP,MSN

[redacted]

Address: 8181 El Mundo, Apt. 605

Houston, Texas 77054

Dear: Jan Wheeler

Your study entitled: Venous Cord Blood Levels and Neonatal Heart Rates:

Is There a Correlation with Sudden Infant Death?

(The applicant must complete the top portion of the page)

has been reviewed by the Human Subjects Review Committee at Columbia Kingwood Medical Center and it appears to meet our requirements in regard to protection of the individual's rights.

Please be reminded that the Hospital and the Department of Health and Human Services regulations typically require that signatures indicating informed consent be obtained from all human subjects in your study. These are to be filed with the Human Subjects Review Committee Chairman. Any exception to this requirement is noted below. Furthermore, according to HHS regulations, **another review by the HSRC is required if your project changes or if it extends beyond one year from this date of approval.**

Any special provisions pertaining to your study are noted below:

2 The filing of signatures of the subjects with the Human Subjects Review Committee is not required.

____ Other:

____ No special provisions apply.

Sincerely,

Chandler Mann, M.D.
Human Subjects Review Committee Chairman

9/3/96 0845

Date

Columbia Kingwood Medical Center
Human Subjects Informed Consent

"Venous Cord Blood Cotinine Levels and Neonatal Heart Rates:
Is There a Correlation with Sudden Infant Death?"

The goal of this research is to identify which baby is at risk for crib death before they leave the hospital. I hereby allow Jan Wheeler RN to take a sample of blood from the placenta after delivery. I also allow Jan Wheeleer RN to monitor my baby's heart rate during the transition period after birth. Transition is the time after birth that the baby is in the nursery to get its first bath and feeding, and to be watched closely by the nurses. This time may last up to four hours after delivery.

I understand that babies who are exposed to cigarette smoke are at a higher risk for crib death. I understand that the reason for this higher risk of crib death is unknown, but that scientists think that nicotine may alter the heart function. I know that there are two purposes to this study. The first is to see if the heart rates of babies who are exposed to cigarette smoke before birth are different from babies who are not exposed to cigarette smoke before birth. The second purpose is to see if there is a relationship between the level of cotinine in the blood and the baby's heart rate after birth.

I know that the blood from the placenta will be analyzed for cotinine. Cotinine is made when the body breaks down the nicotine in cigarette smoke. Cotinine stays longer in the blood than nicotine, and is easy to measure. I know that my baby's heart rate will be checked in the nursery with the heart monitor during transition. The way of getting the blood from the placenta after birth, and for checking my baby's heart rate has been explained to me.

I know that I may feel nervous about letting my baby be in the study. I know that my baby being in the study is my choice. I know that I can stop my baby's heart rate monitor at anytime. I know that my baby being in the study will be stopped if my baby develops any problems after birth.

I know that this study may not help my baby. But, I know that the information from the study may help future babies. I know that the only possible risk to my baby could be some minor skin irritation from the heart monitor electrodes. I know that there is a risk for improper release of the information about my baby. However, my baby will not be known by name, only by a number. The information about my baby will be kept in a locked cabinet during the study. After the study the information will be destroyed.

I know that Columbia Kingwood Medical Center is not able to offer financial help, or to pay the cost of medical care in the unlikely chance of injury from this study. However, first aid will be given as needed.

I know the possible risks that can happen. An offer to answer all of my questions about the study has been made. I know that my refusing to let my baby be in the study will have no penalty or loss of benefits to which I am entitled. I know that I can remove my baby from the study at any time without penalty or loss of benefits to which I am otherwise entitled.

If I have any questions about the study, I can talk with Jan Wheeler during the day at Columbia Kingwood Medical Center. Jan can be reached by calling 281-348-1351. I can also call Dr.Chandler Mann if I have any questions about my rights or my baby's rights for being in this study. Dr. Mann can be reached at 281-348-1301.

Parent/Guardian signature Date

Witness Date

Columbia Kingwood Medical Center
Consentimiento Informado

"Niveles de Cotidina en Sangre Venosa del Cordón Umbilical y Ritmo Cardíaco Neonatal: ¿ Existe una Correlación con la Muerte Súbita del Recién Nacido ?"

La meta final de este projecto es identificar cual bebé esta a riesgo de muerte súbita del recién nacido (ó muerte de cuna) antes de que éste abandone el hospital. Por lo tanto, yo autorizo a Jan Wheeler RCN, NNPa obtener una muestra de sangre de la placenta en el momento del alumbramiento, y monitorizar el ritmo cardíaco del mismo durante el período de transición después dell nacimiento.Este período de transición es aquel momento después del nacimientodurante el cual el bebé recibe su primer baño y alimento mientras está en el nursery ; puede durar 4 horas posterior al parto.

Yo sé que la sangre obtenida de la placenta será analizada para niveles de cotidina; cotidina es un subproducto mayor de la nicotina. La nicotina es una substancia que se encuentra en el humo del cigarrillo. La cotidina permanece mas tiempo en la sangre que la nicotina, y es fácil de medir. Yo sé que el ritmo cardiaco de mi bebé será monitorizado en el nusery , usando el monitor cardíaco, durante las 4 horas posterior al parto , y que comprenden el período de transición.

Yo sé que los bebés nacidos de madres fumadoras, ó aquellas que han sido expuestas al humo del cigarrillo están a mayor riesgo de sufrir muerte súbita del recién nacido que aquellos infantes nacidos de madres que no fuman ó que no han estado expuestas al humo del cigarrillo. Yo sé que la razón de este aumento en dicho riesgo es desconocida, pero algunos científicos postulan que la nicotina puede alterar la función cardíaca. Yo sé que existen dos propósitos en este estudio. El primero es descubrir si existe una diferencia en el ritmo cardíaco entre aquellos bebés cuyas madres fuman ó han estado expuestas al humo del cigarrillo, y aquellos cuyas madres no fuman ó no han estado expuestas al humo del cigarrillo. El segundo propósito es analizar si los niveles elevados de cotidina causan cambios en el ritmo cardíaco del bebé.

El procedimiento para obtener la sangre de la placenta seguido el momento del nacimiento, y para monitorizar el ritmo cardíaco del bebé me ha sido explicado. Mis dudas han sido aclaradas por Jan Wheeler RNC, NNP. Yo sé que pudiera sentirme nervioso de permitir el que mi bebé participe en el estudio, pero esta es enteramente mi opción. Yo sé que la monitorización del ritmo cardíaco de mi bebé pudiera ser descontinuada en cualquier momento, y que la participación del mismo en el estudio pudiera ser suspendida si éste presentara fiebre, niveles disminuídos de azúcar en sangre, ó problemas respiratorios durante el período de transición.

Yo sé que pudiera no existir algún beneficio immediato en mi bebé, pero esta información pudiera en el futuro beneficiar a otros bebés. Yo sé que el único riesgo posible para mi bebé sería algún tipo de irritación de piel asociado al monitor cardíaco. Yo también sé que existe el riesgo potencial de divulgar información de mi bebé de una manera inapropiada. Sin embargo el investigador solamente identificará mi bebé por número y no por nombre. La información acerca de mi bebé permanecerá guardada en un armario con llave, y luego será destruída cuando el estudio haya sido completado.

Yo sé que en caso de un evento inesperado relacionado a algún tipo de accidente físico imprevisto, el hospital Columbia Kingwood Medical Center no será responsable de proveer alguna compensaión económica, ni de absorver el costo del tratamiento médico. Sin embargo, los primeros auxilios serán provistos según sean necesarios.

He sido informado de los posibles riesgos que pudieran ocurrir; un ofrecimiento para contestar todas mis preguntas ha sido hecho. Yo sé que el no permitir la participación de mi bebé en el estudio no tendrá penalidad alguna ó pérdida de beneficios a los cuales yo tengo derecho. Yo sé que en cualquier momento puedo descontinuar la participación de mi bebé en este estudio sin ninguna penalidad ó pérdida de beneficios a los cuales tengo derecho.

Si tengo preguntas acerca del estudio puedo hablar durante el día con Jan Wheeler en Columbia Medical Center llamando al 281-348-1351. También puedo llamar al Dr. Chandler Mann, Presidente del Comité de Revisión de los Sujetos Humanos, al 281-348-1301, si tuviera alguna pregunta sobre mis derechos ó los derechos de mi bebé por participar en este estudio.

Firma Padre / Guardián _____ Fecha _____ Testigo _____ Fecha _____

APPENDIX D

Maternal/Infant Demographic Data Form

Maternal/Infant Demographic Data **ID #:**_____

Maternal Data:

Age:_____ Race:_____ G/P/LC/AB:_____LMP:_____ EDC:_____

Nicotine Exposure:_____

Other substances:_____

Prenatal HX:_____

Maternal PNL:_____

Maternal PNM:_____

Mode of Delivery:_____Anesthesia:_____

Complications of Delivery:_____

Infant Data:

Date of Birth:_____Time of Birth:_____ Apgar Scores:_____ Ballard:_____

Gender:_____ Race:_____Birth weight:_____ Length:_____ FOC:_____

Date & Time of Study Initiation/Completion:_____

Complications after study:_____

Comments:_____

_____

APPENDIX E

Procedure for Data Collection

Procedure for Data Collection

The procedure for data collection will be as follows:

1. Permission will be obtained from the Columbia Kingwood Human Rights Review Committee

2. The researcher will introduce self to parents and explain the purpose and procedures of the study

3. After written consent is obtained from the parents, maternal information will be entered on the Maternal/Infant Data Collection Form

4. Venous cord blood cotinine levels will be obtained at delivery and immediately taken to the lab for send out to the speciality lab. The procedure for blood collection is as follows:

   A. A protective cover gown and gloves will be worn in accordance with OSHA standards for protection from biological substances

   B. Obtain the placenta after delivery of the infant

   C. Identify the umbilical vein from the umbilical arteries and trace the umbilical vein down to where it enters the placenta:
      a. The umbilical vein will be larger and have a distended appearance compared to the artery

   D. Using a 20cc syringe and a 18 gauge needle aspirate 15ml of blood from the vein:
      a. Insert the needle at a 45° angle with the bevel up directly into the vein approximately 1-2" above where the vein enters the placenta
      b. Begin aspirating the blood immediately after entering the vessel
      c. After the desired amount of blood is obtained, stop aspirating and remove the needle from the vein

   E. Remove the red top cap from a 15 ml silicone coated glass red top tube. Inject the venous blood into the glass red top tube

   G. Dispose of the syringe and needle in the needle box. DO NOT recap the needle prior to disposal

H. Label the tube of blood with the pt. study number

I. Ensure that the study number on the tube of blood correlates with the number on the Maternal/Infant Data Collection Form

J. Fill out the lab requisition sheet, ensure that the study number is correct and that the desired test is a serum cotinine level

K. Take the tube of blood and the requisition to the laboratory and give to the technician for send out to the reference lab

After delivery:

1. A rectal temperature will be taken on admission to the nursery by the nursery nurse. If the rectal temperature is < 97.6 or > 99.6 infants will be excluded.

2. If the infant is normothermic, the infants will be placed on the Corometrics cardiorespiratory monitor on admission to the nursery.

3. Turn on the Corometrics monitor by depressing the power button

4. Select Lead III from the Lead Selection screen:
   a. Ensure that the cardiac tracing is visible on the monitor screen and that the EKG sequence has a normal QRS complex

   b. If the QRS complex is not normal, change to Lead I or II

5. Go to the Timed Recording Screen under the main EKG screen:
   a. Set the monitor to record the heart rate in one minute intervals

   b. Verify that the monitor is recording the heart rate every one minute by pulling up the vital sign graph on the monitor screen after the infant has been on the monitor for five minutes

6. If there is any change in the infants physical condition, based on the exclusion criteria, during the study the infant will be removed from the study

7. Remove the electrodes from the infant at the end of the four hours

8. Ensure that the Maternal/Infant Data Collection Form is complete and that the ID number corresponds with the cotinine sample ID number

9. Thank the parents for allowing their infant to participate in the study

10. Download and print the data from the Corometric monitor

11. Enter the heart rates into the SPSS program. Calculate the descriptive statistics

APPENDIX F

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