IRON AND ITS RELATIONSHIP TO NEURAL TUBE DEFECTS AMONG OFFSPRING OF TEXAS BORDER COUNTY WOMEN

THESIS

Presented to the Graduate School of
Southwest Texas State University
in Partial Fulfillment of
the Requirements

For the Degree

MASTER of SCIENCE IN HEALTH PROFESSIONS

By

Barbara D. Scaife B.S., M.S.

August, 2000

ACKNOWLEDGEMENTS

I would like to thank Dr. Charles Johnson for his counsel and encouragement during my time as part of the program of Health Research. My gratitude goes to Dr. Kate Hendricks, M.D. of the Texas Department of Health (TDH) for allowing me to use TDH data and for spending hours reading and advising me concerning my literature review, as well as on the remainder of my thesis. I wish to thank Dr. Lucina Suarez for compiling my data set and for advising me on statistical procedures. I am grateful to Dr. Jean Brender for guidance on my hypotheses, methods, results, and conclusion sections of my thesis, for the numerous times she read drafts of these sections and advised me, and for the weekly time that she met with me. I would also like to thank Dr. Russell Larsen of the Texas Neural Tube Defect Project at TDH for encouraging me and for offering many helpful suggestions. A thanks also goes to Dr. Richard DeMouy for reading my proposal and offering suggestions. I wish to thank my husband, Bill, for supporting me as I worked on my thesis and graduate work, in spite of the many meals he cooked and the lack of house cleaning that has occurred these past two and one-half years. Lastly, I thank my three children for enduring the inconveniences that my graduate study has caused them.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

IRON AND ITS RELATIONSHIP TO NEURAL TUBE DEFECTS AMONG OFFSPRING OF TEXAS BORDER COUNTY WOMEN

by

BARBARA D. SCAIFE, B.S., M.S.
Southwest Texas State University
August 2000

SUPERVISING PROFESSOR: Jean D. Brender

Neural tube defects (NTDs) are major malformations of the developing central nervous system. The most common types are anencephaly and spina bifida. In 1992, a small case-control study in Cameron County, TX by the Texas Department of Health found hematocrit values among women giving birth to babies with anencephaly and any NTDs to be significantly lower ($p < 0.01$) than among control-women. Those results led to a much larger case-control study designed to ascertain risk factors for NTDs.

This study explored whether an association existed between iron and NTDs in 148 cases and 158 controls. Dietary iron intake, blood hemoglobin and hematocrit values, and serum ferritin values were analyzed to see whether case and control values differed. No associations were found between case-women and control-women for dietary iron intake, hemoglobin, or hematocrit. Associations were found between ferritin and NTDs among women with a BMI less than 21.3 and among women less than 24 years of age.

CHAPTER I

INTRODUCTION

The purpose of this research was to identify potential associations between iron and neural tube defects. To identify potential associations, the following research questions were asked. First of all, do women who have babies with neural tube defects have a lower dietary iron intake than women who give birth to normal babies? Secondly, do women who have babies with neural tube defects have lower hemoglobin or hematocrit levels than women who give birth to normal babies? Lastly, do women who have babies with neural tube defects have lower serum ferritin levels than women who give birth to normal babies?

Definition, Embryology, and Classification of Neural Tube Defects

Neural tube defects (NTDs) are major malformations of the developing central nervous system. The most common types are anencephaly, in which a portion of the skull fails to close over a degenerated brain, and spina bifida, in which a portion of the neural tube fails to close along the vertebral column. A less common type of NTD, an encephalocele, is characterized by a sac containing meninges or other neural tissue protruding from an opening in the skull.

Basic embryology provides a foundation for understanding how neural tube defects are formed. An embryo develops from a fertilized ovum dividing into an eight-celled gametocyte to an embryo composed of three layers: the endoderm, the innermost layer; the mesoderm, the middle layer; and the ectoderm, the outermost layer. The ectodermal layer of the embryo gives rise to the nervous system. The ectoderm thickens and becomes the neural plate with a neural groove at approximately 18 days after conception. As the neural plate begins to fold, cells are dividing so that some parts of the neural plate extend toward each other like fingers. The fingers extending from each side of the neural plate interdigitate and fuse, forming the early brain and the upper portion of the neural tube (Moore and Persaud, 1993). Neurulation is the process of the neural plate folding and closing. Secondary neurulation occurs when mesodermal cells from the primitive streak form a tube (canalization) from the lower portion of the sacral spine to the coccyx (Elwood, Little, and Elwood, 1992).

Developmental events, such as neural plate formation, spinal cord cell differentiation, and neuronal migration occur when signaling molecules, also called growth factors, act in neural tube development. Examples of signaling molecules include members of the transforming growth factor family, including activin, and the fibroblast growth factor family (Sadler, 1995).

Moore and Persaud (1993) first suggested that the neural tube closes in a bidirectional manner, much like a zipper attached in the middle and zipping in both directions. A few years later, Golden and Chernoff's (1995) research supported multiple neural tube closure sites in humans. Their research suggested that neural tube defects

might occur either because individual zippers fail to close or because two or more zippers fail to meet (Elwood, Little, and Elwood, 1992).

Depending on the site, aberrant neural tube closure leads to different types of NTDs. A zipper failing to close in the cranial region is associated with anencephaly, a defect that occurs at 23 to 26 days of gestation. Anencephaly is characterized by severely deformed or underdeveloped skeletal brain regions, with a partial to complete absence of neural tissue. Spina bifida is a defect in which a zipper fails to close in the spinal region at 26 to 30 days of gestation. This defect may involve one or more unclosed vertebral arches or no closure of many vertebral arches (Elwood et al., 1992; Lemire, 1988).

CHAPTER II

LITERATURE REVIEW OF NEURAL TUBE DEFECTS

Epidemiology of Neural Tube Defects

NTD research utilizes epidemiologic techniques to learn about the differences in NTD occurrence over time and by race/ethnicity, location, and sex of the affected infant.

Geographic Variations in Neural Tube Defects

Worldwide NTD rates vary widely, ranging from combined anencephaly and spina bifida rates between 1974 to 1988 of 3.7/10,000 in Finland and 4.3/10,000 in France to 29.0/10,000 in Northern Ireland and 34.3/10,000 in Mexico (Congenital Malformations Worldwide, 1991). NTD rates in the United States since 1980 have ranged from 7.1/10,000 (1982-1985) in the western U.S. (Elwood et al., 1992) to 13.4/10,000 in the 1990s along the Texas-Mexico border when all gestational ages were included (Hendricks et al., 1999). Anencephaly rates in Texas were stable in the 1980s, with rates ranging from 3.8 - 4.3/10,000 total births (live births and still births) (Brender, Carmichael, Preece, Larimer, and Suarez, 1989). For 1989-1991, anencephaly rates in Harris County, TX followed the Texas trend with a rate of 3.8/10,000 ($\geq$ 20 weeks only). Spina bifida rates in Harris County were 2.0/10,000 (Canfield, Annegers, Brender, Cooper, and Greenberg, 1996).

Secular Trends in Neural Tube Defect Rates

Secular trends in NTD rates have varied in different areas. Rates in New England, New York state, and Ontario peaked in 1930-1934. A secondary peak, affecting only the lower socioeconomic classes, occurred in the early 1940s in Boston. Great Britain experienced high rates in the 1930s and early 1940s, as did Germany in the 1940s. A moderate peak occurred in the 1960s in the British Isles, Sweden, and New York State. Many theories have been given to explain the cause of these peaks, such as changes in the maternal population, exposure to an infectious disease epidemic, or exposure to conditions present during the mothers' childhood or puberty, such as poor nutritional status (Elwood et al., 1992).

During the 1970s, the birth prevalence of NTDs declined in some countries, partially due to antenatal diagnosis and therapeutic abortion. Antenatal diagnosis programs were associated with a decrease in NTD rates by 65-70% in England, Wales, Scotland, and Sweden. Alpha-fetoprotein screening was used widely in the United Kingdom, as was ultrasound screening in Sweden. Less widespread availability of antenatal procedures in the U.S. led to a 40% decrease in rates. Other possible reasons for a decline include improved nutrition of women during childhood and during the childbearing years, improved availability of foods throughout the year, increased nutrition education efforts, and increased intake of supplemental vitamins (Elwood et al., 1992).

Declines in country-specific rates were not consistent across all NTD subgroups. A declining trend has been noted in Scotland, Norway, Canada, Ireland, New Zealand and

the U.S. in anencephaly rates in females. In contrast, anencephaly rates in males and spina bifida rates have shown no trend (Elwood et al., 1992).

Risk Factors for Neural Tube Defects

Differences in risk for NTDs vary among different races and ethnicities. In the U.S., Hispanics have the highest risk of having an NTD-affected pregnancy, according to two recent studies. The birth prevalence of prenatally diagnosed NTD cases was estimated in California for 1990-1994. The Hispanic NTD rate was 11.2/10,000; followed by rates for nonHispanic Whites of 9.6/10,000; Blacks, 7.5/10,000; and Asians, 7.5/10,000 (Feuchtbaum et al., 1999). NTD rates (anencephaly, spina bifida, and encephalocele) along the Texas-Mexico border for 1993-1995 were 14.9/10,000 for Hispanics and 10.6/10,000 for Anglos. The Texas-Mexico border NTD rates were based on cases diagnosed both prenatally and at birth (Hendricks, Simpson, and Larsen, 1999).

Definite patterns emerge in NTD rates by sex. Female cases of anencephaly occur more frequently than male cases (Seller, 1987). Copp and Bernfield (1994) suggested that the female excess of anencephaly is caused by a subtle difference in neurulation between embryos of the two sexes. There is an excess of female thoracic spina bifida cases (Seller), but lumbar or sacral spina bifida cases involving improper canalization occur with equal or greater frequency in males compared with females (Copp and Bernfield).

Many risk factors are known for NTDs. One risk factor for birth defects in general, as well as NTDs, is insulin-dependent diabetes mellitus (IDDM). Compared with nondiabetic mothers, insulin-dependent diabetic women (nongestational) in a study in

Atlanta between 1968 and 1980 showed a fifteen-fold increased risk for major central nervous system abnormalities (RR=15.5, 95%CI = 3.3-73.8). Insulin-dependent gestational diabetic women had a three-fold increased risk (RR=3.0, 95% CI = 0.2-50.6), while gestational diabetic women not requiring insulin showed no increased risk for central nervous system defects compared with nondiabetic mothers (Becerra, Khoury, Cordero, and Erickson, 1990).

Another group with an increased NTD risk are obese women. Prior to conception, these women (BMI >29kg/m^2) have close to a two-fold increase in risk of having a baby with an NTD (Shaw, Velie and Schaffer, 1996; Waller et al., 1994). Waller et al. found that spina bifida occurred more frequently (OR 2.6, 95% CI = 1.5 - 4.5) in obese women (BMI >31 kg/ m^2) than other types of NTDs, after excluding diabetic women and adjusting for maternal age, race, education, and family income. Very obese women with BMI $\geq$ 38 had an even higher odds ratio of 3.0 (95% CI 1.2 - 7.7). Werler, Louik, Shapiro and Mitchell (1996) found that women weighing less than 70 kg experienced a 40% reduction in risk if they consumed at least 400 μg of folate per day. Heavier women did not experience any risk reduction due to folate consumption.

The association between anticonvulsant drugs and NTDs has been studied since 1980. Rosa (1991) summarized several studies, reporting 9 cases of spina bifida out of 612 births to mothers taking valproic acid. Spina bifida relative risk was eleven times greater in this study compared with a relative risk of two for births to epileptic mothers treated with drugs other than valproic acid or carbamazepine. Rosa also reported 9 cases of spina bifida out of 984 births to mothers treated by carbamazepine alone, yielding a relative risk of 6.8 (Rosa; Elwood et al., 1992).

Exposure to heat is associated with increased NTD risk. Maternal fever or exposure to hot tubs or saunas during the first trimester increased NTD risk two-to-three fold (Lynberg, Khoury, Lu, and Cocian, 1994; Milunsky et al., 1992).

The study of environmental and occupational factors is in its infancy. Researchers have suggested many occupational factors that might correlate with increased risk of neural tube defects. These factors associated with NTDs include maternal occupations, such as nursing; paternal occupations such as farmer, painter, or food and beverage processor; maternal occupational exposures to solvents, x-rays, or anesthetic gases; and paternal occupational exposures to solvents, pesticides, ionizing radiation, and mercury (Sever, 1995). Studies indicating associations between anencephaly and both pesticides and organic solvents are of particular interest (Brender and Suarez, 1990). Khattak et al. (1999) found a relative risk of 13.0 (95% CI, 1.8 - 99.5) in a prospective, observational matched-control study of 125 women exposed to organic solvents during the first trimester of pregnancy versus pregnant women exposed to nonteratogenic substances during the same gestational period. Neural tube defects were among the congenital malformations that resulted.

Environmental exposure levels are generally much lower than occupational exposures. Because of the low prevalence of exposure, greater power is needed in environmental studies to find associations between an exposure and an outcome. Environmental exposures suggested that might be associated with neural tube defects include vinyl chloride, environmental pollution, solvents or metals from hazardous waste sites, agricultural chemicals, nitrates in water, organic solvents, and by-products of water treatment procedures. Routes of exposure are from air and water sources (Sever, 1995).

Sever suggests additional research is needed to discover associations between specific causative agents and spina bifida or anencephaly. Sever believes in assessing spina bifida and anencephaly separately, as one NTD form may be more associated with environmental factors than the other.

Nutritional Factors and Neural Tube Defects

Researchers have studied nutritional relationships with NTDs for many years. The most promising nutrient studied has been folic acid. In an unrandomized study, Smithells et al. (1981) studied women who had experienced previous NTD pregnancies. The study indicated a significant protective effect among women who were supplemented with a multivitamin containing 0.36 mg folic acid versus unsupplemented women. The role of folic acid in preventing NTDs was established in the multicenter, multicountry Medical Research Council Vitamin Study, a randomized double-blind prevention trial. This trial found a 72% reduction in the incidence of NTDs associated with taking 4.0 mg folic acid daily in women with previous NTD pregnancies (MRC Vitamin Study Research Group, 1991). A prospective cohort study of over 22,000 women seeking amniocentesis or α-fetoprotein testing found significantly decreased NTD prevalence rates in women taking a multivitamin supplement containing folic acid during the first six weeks of pregnancy (prevalence = 0.9/1000) compared with women not consuming multivitamins during this period (prevalence = 3.5/1000 (Milunsky et al., 1989). Czeizel and Dudas (1992) found a significantly decreased incidence of NTDs in women without prior NTD pregnancies who took 0.8 mg of folic acid periconceptionally. Werler, Shapiro, and Mitchell (1993) also found a 60% reduction in

NTDs in women without prior NTD pregnancies who took folic acid daily (most commonly 0.4 mg folic acid) during the first trimester. A significant trend for proportionally reduced risk for NTDs with increasing dietary levels of folate was also found. Because these studies demonstrated the protective value of folic acid for preventing NTDs, the current recommendation is for all women who might become pregnant to take 400 μg folic acid daily. Women with previous NTD pregnancies should consume 4,000 μg folic acid daily. The folic acid should be taken at least one month before pregnancy and throughout the first trimester (American Academy of Pediatrics, 1999). Daly, Kirke, Molloy, Weir, and Scott (1995) have estimated that following these guidelines would result in a 48% decrease in the NTD rate via a 66% decrease in the folate responsive NTDs.

Other dietary factors have also been studied. A case-control study by Golding (1982) discovered that mothers giving birth to anencephalic babies were more likely to have consumed tea prepared using soft water than control-mothers. These case-mothers also had low blood hemoglobin levels. Hemoglobin levels reflect iron status and intestinal absorption of iron is decreased by tea consumption. Golding reasoned that tea drinking along with marginal iron status might reduce the absorption of iron sufficiently to create an environment without sufficient nutrients for normal fetal development.

An indicator of iron status, hematocrit, was used in an investigation of NTDs performed by the Texas Department of Health (TDH) in 1990-1991. A cluster of anencephalic births in Brownsville, Texas was investigated when three anencephalic babies were born during a 36-hour period. Further investigation revealed six anencephalic births had occurred in one Brownsville hospital during a six-week period

from March 27 through May 7, 1991. TDH conducted a case-control study on 28 cases in which babies with NTDs were born to women who conceived from January 1, 1989 through January 31, 1991. The rate of anencephalics in Cameron County during 1990-1991, 19/10,000, was double the rate in the same area during 1986-1989. The study concluded that hematocrit values among women giving birth to babies with anencephaly and any neural tube defects were significantly lower ($p<.01$) than control-women values. As noted in other studies, an association of lower socioeconomic status in cases versus controls was also confirmed (TDH report, 1992).

Etiology of Neural Tube Defects

The cause of NTDs is unknown, but the etiology of NTDs is believed to be multifactorial (Myrianthopoulos and Melnick, 1987). Dietary excesses or deficiencies may contribute to the development of NTDs in susceptible individuals in the presence of other genetic, physical, or chemical factors. Studies comparing serum levels of folic acid between affected and unaffected pregnancies show inconsistent results with, at best, small differences between the two groups (Elwood et al., 1992). Studies comparing red cell folate levels show similar results (Daly et al., 1995). The small difference in blood levels between the affected and unaffected groups may be partially explained by the narrow window effect. Normal red cell folate blood levels are greater than 95 ng/ml, but the difference between normal and abnormal values can constitute a narrow range. As a result of this "narrow window," a very large study with much statistical power would be necessary to detect a difference between serum values in affected and unaffected mothers (Neural Tube Defects, 1994).

Metabolic defects in metabolism are another possible cause of NTDs. A metabolic defect in methionine synthase metabolism might cause deficiencies in folate or vitamin B_{12} resulting in NTDs (Kirke et al., 1993). Kirke et al. found that plasma folate and vitamin B_{12} are independent risk factors for NTDs involving the enzyme methionine synthase either in the mother and/or the embryo. Mills et al. (1995) found elevated levels of homocysteine during pregnancy in mothers whose pregnancies resulted in babies with NTDs compared to B_{12}-matched mothers with normal children. These researchers suggested a metabolic defect in homocysteine metabolism, in which homocysteine is converted to methionine by methionine synthase, using vitamin B_{12} as a cofactor. Another cause of elevated homocysteine levels is defective methylene-tetrahydrofolate reductase (MTHFR) activity, necessary for the conversion of 5-methyltetrahydrofolate to tetrahydrofolate or deficient vitamin B_{12} needed as a cofactor in the reaction. Bailey and Gregory (1999) suggested that genotype might alter folate requirements. Their studies measuring MTHFR activity on lymphocytes showed modulated activity based on MTHFR C677T genotype, with the highest activity in normal individuals (C/C), less activity in heterozygous individuals (C/T), and the least activity in homozygous individuals (T/T).

It is generally accepted that periconceptional intake of folic acid could prevent many NTDs, as several studies have shown. A randomized, double-blind study at 33 centers in seven countries established that 4.0 mg supplemental doses of folic acid given prior to and through the first trimester of pregnancy to women with previous NTD pregnancies reduced the NTD recurrence risk by 72% (MRC Vitamin Study Research Group, 1991). In an observational study, prevention of NTDs in mothers with no prior NTD

pregnancies was reduced 60% by women consuming a 0.4 mg supplement of folic acid beginning prior to pregnancy and taken through the first trimester (Werler et al., 1993). This level of folic acid reduces the level of all NTDs (both occurrent and recurrent) by 48% (Daly et al., 1995).

CHAPTER III

LITERATURE REVIEW OF IRON AND ITS RELATIONSHIP TO NEURAL TUBE DEFECTS

Iron is a nutrient essential for life due to its use in processes such as oxygen transport, electron transfer, and DNA synthesis. Iron is an integral part of hemoglobin as well as nonheme proteins. It is present in the body as either ferrous iron (Fe^{2+}), the reduced form, or ferric iron (Fe^{3+}), the oxidized form (Ponka, Beaumont and Richardson, 1998). The conversion of ferrous iron to ferric iron is catalyzed by ceruloplasmin, an extracellular ferroxidase (Bezkorovainy, 1989). The abundance of iron in nature and its ability to change between the reduced and oxidized form under favorable conditions make iron useful in many biochemical reactions. Ferric iron may be hydrolyzed to an insoluble form. Despite its utility, the presence of unbound iron poses a significant hazard to the cell. In its unbound state, iron produces cytotoxic oxygen free radicals (Ponka et al.). In contrast, protein bound iron is incapable of forming free radicals (Conrad, Umbreit, and Moore, 1999). In summary, the potential toxicity and solubility problems of iron-requiring organisms are solved by transferring iron through the blood and into the cell by a controlled, protein receptor-mediated system (Ponka et al.).

Iron is important to a person's nutritional status. Three factors determine iron nutritional status: the amount of iron consumed, the bioavailability of iron, and the amount of iron lost from the body. The Recommended Dietary Allowance (RDA) for

iron varies depending on sex and physiologic state. The RDA for women of childbearing age and lactating women is 15 mg of iron per day, increasing to 30 mg per day during pregnancy. The recommendation for males nineteen years of age and older is 10 mg per day (National Academy of Sciences, 1989). The bioavailability of iron is dependent upon whether the iron is or is not associated with heme and upon other nutrients present in food that may either inhibit or promote iron absorption. About 5-35% of heme iron, present in foods of animal origin, is absorbed as opposed to 2-20% of nonheme iron, present in foods of vegetable origin. About 10% of dietary iron is present in the heme form, yet it accounts for up to one-third of the iron absorbed (Bjorn-Rasmussen, Hallberg, Isaksson, and Arvidsson, 1974). Iron is lost from the body through bleeding, menstruation, and through feces (Cohen and Braunstein, 1996).

The following paragraphs provide some facts about iron metabolism, including its digestion, absorption, storage, and recycling, as well as a short overview of growth factors in embryologic development.

Iron metabolism involves several processes, including iron digestion and absorption. Iron digestion begins in the stomach when hydrochloric acid and gastric pepsin denature protein that is bound to iron and reduce some of the iron to the ferrous form. The majority of iron absorption occurs in the duodenum and upper jejunum (Conrad et al, 1999).

All the details of iron absorption are unknown at this time, but a consensus exists about some information. Ascorbate is the usual reducing agent in the intestinal lumen. Iron chelators sequester iron, resulting in iron solubilization. Nonheme iron absorption begins when a protein binds iron, forming a complex that attaches to a specific transport

molecule on the luminal side of the enterocyte (intestinal mucosal cell). The iron moves into the enterocyte where a transferrin-like protein attaches to it. The iron then either becomes part of ferritin or it moves to the basolateral surface (side opposite the luminal side) of the mucosal cell (Beard and Dawson, 1997) where it is oxidized and moved to the outside of the cell by a mechanism involving apotransferrin and a newly described protein called hephaestin (Conrad et al., 1999). The apotransferrin-iron complex is converted to ferrotransferrin, possibly by the enzyme ceruloplasmin (Beard & Dawson). The absorption of heme iron is less complicated than that of nonheme iron. Heme iron is moved into the enterocyte after binding to a receptor. Once in the cell, heme oxygenase degrades the complex to iron, carbon monoxide, and bilirubin. At this point, the iron becomes part of the iron pool inside the enterocyte where it is metabolized through the same pathways as nonheme iron (Beard & Dawson, 1997). Figure 1 depicts iron absorption by the enterocyte.

Iron is recycled within the human body by the endocytotic recycling pathway. Iron is obtained for metabolic activities from intestinal absorption, as a result of the breakdown of iron-containing compounds, and from iron stored primarily in hepatocytes (van Eijk and de Jong, 1992). Transferrin, a plasma glycoprotein, binds to iron, (ferro-transferrin) for transport between sites of iron absorption, use, and cellular storage. Eighty percent of the iron transported by transferrin is used in the bone marrow; the other 20% is used in the liver. About 60% to 70% of the total body iron is incorporated into erythrocytes as heme iron. Another 10% of iron is used in the liver to synthesize myoglobin, cytochromes, or cytoplasmic enzymes. The remaining 20-30% of iron is stored in ferritin or hemosiderin (Ponka et al., 1998). Figure 2 illustrates iron recycling.

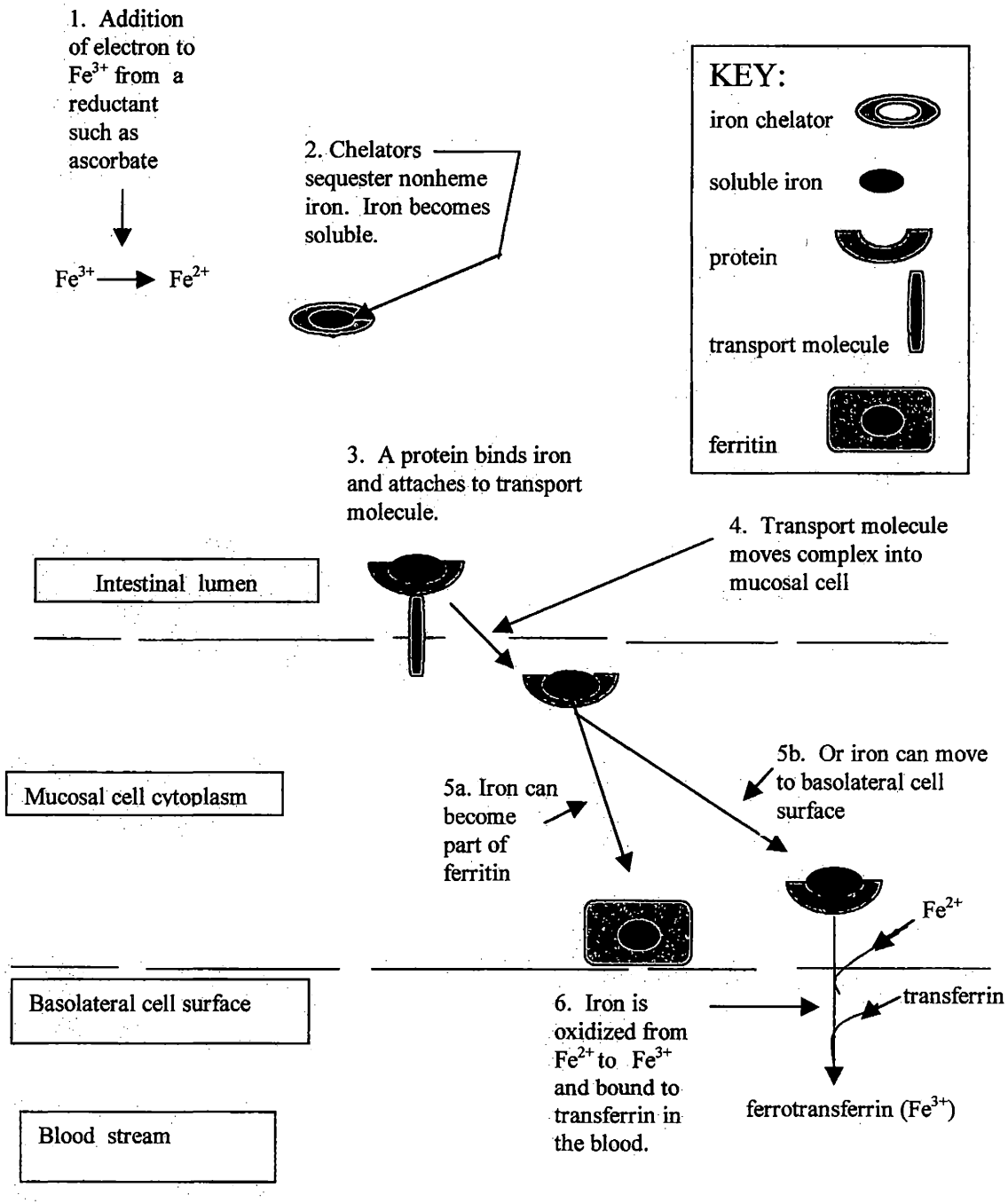

Figure 1. Absorption of iron by the intestinal cell.

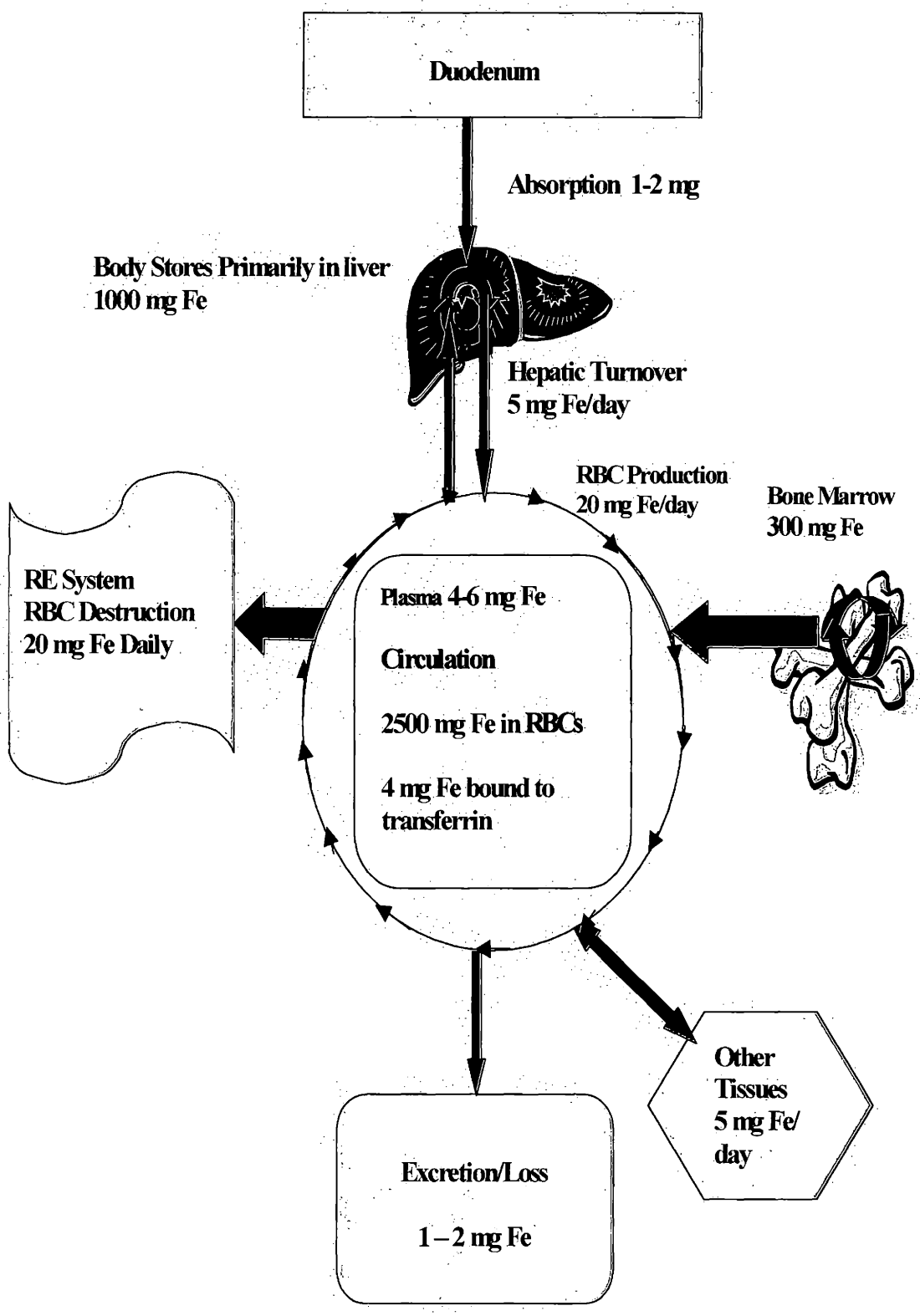

Figure 2. Iron Recycling in the Human Body (Adapted from van Eijk and de Jong, 1992).

Erythrocytes play a crucial role in iron recycling. Each erythrocyte circulates in the bloodstream for about 120 days, before the monocyte-macrophage (reticuloendothelial) system removes it. The heme moiety is released from hemoglobin and catabolized, releasing iron. The rate of iron release from senescent erythrocytes equals the rate of iron uptake for erythropoiesis (Ponka et al., 1998).

Iron storage proteins play a crucial role in iron recycling. Ferritin, involved in iron metabolism and recycling, is the primary storage form of iron. Human ferritin is composed of 24 subunits of either L-ferritin or H-ferritin that are combined in different proportions to produce the ferritin types found in different tissues and species (Bezkorovainy, 1989).

When cells contain excess iron, as in iron overload, hemosiderin, another storage form of iron, is seen. Hemosiderin is thought to be an insoluble degradation product of ferritin. It accumulates within lysosomes in certain disease states such as hereditary hemochromatosis (van Eijk and de Jong, 1992; Ponka et al., 1998).

Growth factors, some which use or contain iron, are vital for communication between cells during embryologic development. Growth factors stimulate cell proliferation, differentiation, locomotion, and death, and may be of either maternal or fetal origin. One type of growth factor is the polypeptide-signaling factor. The polypeptide-signaling factor is a long polypeptide in its precursor state, but is cleaved to a much shorter peptide in its active state. The polypeptide-signaling factors are transmembrane proteins that stimulate the desired biochemical reactions by binding to matching receptors located in cell membranes (Zhao, Weiss, and Stock, 1998).

Transfer of Iron to the Embryo or Fetus

Passage of iron to the human embryo takes place through the placenta. About eight percent of the mother's maternal body iron is transferred to the unborn child over the course of a 280-day pregnancy. The iron transfer occurs as the transferrin bound to iron circulates in the mother's blood and through the placenta. Many placental transferrin receptors are available to remove transferrin-bound iron from the mother's circulation. The iron detaches from the maternal transferrin and is transported to the capillary lumen where the iron is bound by fetal transferrin. The iron is transported to all the embryonic or fetal areas requiring iron (Bridges, 1990).

Iron and the Brain

Iron is present in varying amounts at different brain locations during different periods of life. In this section the roles and location of iron in the human brain will be discussed. The general order of the discussion will begin from the embryonic period and proceed to later prenatal and postnatal periods.

Iron has numerous roles in the body. Iron is involved in three types of reactions: oxygen transport and storage, electron transport, and oxidation-reduction reactions. Brain iron is used in the cytochrome oxidase system for the production of energy. NADPH reductase activity and the myelination of axons both require iron (Beard and Dawson, 1997). Fatty acid desaturase is necessary for lipid synthesis and lipid dehydrogenases are used for lipid degradation (Connor and Menzies, 1996). Iron is a cofactor for many enzymes used in neurotransmitter systems, such as ribonuclease

reductase that is essential for DNA synthesis; tyrosine hydroxylase that catalyzes the conversion of tyrosine to 3,4 dihydroxyphenylalanine (dopa); aconitase, an enzyme in the citric acid cycle; guanylate, an enzyme necessary for the synthesis of the purines, adenine and guanine; and tryptophan 5-monooxygenase that catalyzes the conversion of L-tryptophan to 5-OH L-tryptophan, a precursor to serotonin (Beard and Dawson; Qian and Wang, 1998).

Iron-requiring enzymes are present in different species. Table 1 lists iron-requiring enzymes present during gestation in at least some species, the gestational age during which the enzyme was present, and whether researchers have found the enzyme in the brain or spinal cord.

The iron content of the brain varies by region. Connor and Menzies (1995) studied the brains of adults at autopsy and found staining for iron in oligodendrocytes of both gray and white matter. Microglial cells were found in oligodendrocytes of the cerebral cortex and hippocampus, in iron-rich areas like the substantia nigra, deep cerebellar nuclei, and in astrocytes in the caudate nucleus. Studies of adult, human, post-mortem brain by Morris et al. (1992) found iron in the basal ganglia, especially in the globus pallidus and the substantia nigra zona reticula.

Roskams and Connor (1994), when studying newborn rats, found iron in the midbrain, cerebral cortex, and cerebellum-pons. The iron levels were highest at birth and decreased to their lowest levels between 15 and 21 days of age, but the decrease was less dramatic in the midbrain. Iron levels began to increase from postnatal day 17 and continued into old age.

Transferrin has been found in oligodendrocytes, astrocytes and neurons in studies on

Table 1.

Iron-Requiring Enzymes Present During Development

Enzyme	Species	Present During Embryogenesis?	Age/Stage/Period	In Brain / Spinal Cord?
[a]Aldehyde dehydrogenase	Human	No evidence	Fetal	No evidence
[b]Glutamate synthase	Human	No evidence	?	Yes
[c]Linoleoyl-CoA desaturase	Human	No evidence	Fetal	No evidence
[d]Myeloperoxidase	Human	Maybe	5-16 wks	No evidence
[e]p-hydroxyphenylpyruvate oxidase	Human	No evidence	Fetal	No evidence
[f]Superoxide dismutase	Human	No evidence	Fetal	Yes
[g]Xanthine oxidase/dehydrogenase	Human	Maybe	Developing tissues	Yes
[h]Tyrosine hydroxylase	Human	Yes	10.5-15.5 days	Yes
[i]Aconitase	Baboon	No evidence	140 days	No evidence
[j]Prostaglandin-G/H synthase	Pig	No evidence	Late gestation	Yes
[k]Uteroferrin	Pig	No evidence	Fetal	No evidence
[l]Lysyl hydroxylase	Chicken	Maybe	Embryonic	No evidence
[m]Procollagen proline 3-dioxygenase	Bird	Yes	Embryonic	Yes
[n]Catalase	Rat	No evidence	19 days	Yes
[o]Cytochrome c oxidase	Rat	Yes	9.5-12.5 days	Yes
[p]Gamma 9-desaturase	Rat	No evidence	Fetal	Yes
[q]NAD(P)H quinone oxidoreductase	Rat	No evidence	Fetal	No evidence
[r]Nitric oxide synthase	Rat	No evidence (Organogenesis past neurulation)	13 days	Yes
[s]Peptidyl proline hydroxylase	Rat	No evidence	Fetal	Yes
[t]Phenylalanine hydroxylase	Rat	No evidence (Organogenesis past neurulation)	11-20 days	No evidence
[u]Aldehyde oxidase	Mouse	No evidence	16 days	No evidence
[v]Guanylate cyclase	Moth	Maybe	Embryonic	Yes

[a]Yoshida, A. et al., 1990. [b]Cruz, C. et. al., 1991. [c]Rodriguez, A. et al., 1998. [d]Slayton et al., 1998. [e]Coufalik, A. and Monder, C., 1978. [f]Takashima, S. et al., 1990. [g]Saksela, M., et al, 1998c. [h]Katz, D.M., 1991. [i]Morton, R.L. et al., 1998. [j]Norton, J.L. et al., 1996. [k]Michel, F.J., Fliss, M.F., Bazer, F.W., and Simmen, R.C., 1992. [l]Puistola, U. and Anttinen, H., 1982. [m]Kalcheim, C. and Leviel, V., 1988. [n]Mover and Ar, 1997. [o]Miki, A. et al., 1988. [p]Schaeren-Wiemers, N. et al., 1995. [q]Sherratt, A.J., Banet, D.E., and Prough, R.A., 1990. [r]Santacana, M. et al., 1998. [s]Zimmerberg, J., Greengard, O., and Knox, W.E., 1975. [t]Yeoh, G.C. et al., 1988. [u]Ventura, S.M. and Dachtler, S.L., 1980. [v]Wright, J.W., et al., 1998.

human brain. At 18 weeks of gestation, transferrin was present in neurons in the Purkinje cells and in neural and glial cells of the pontine reticular formation. The pontine nuclei first contained transferrin at 22 weeks gestation. Glial cells were transferrin-positive at 24 weeks gestation in the cerebellar white matter and the pontine nuclei. Transferrin appeared in the reticular formation at 18 weeks gestation (Ozawa and Takashima, 1998).

Transferrin is present in the hindgut of midgestation mouse embryos. At 10.5 days gestation, transferrin stained strongly in unaffected *curly tail* mice, a strain that is used as a model for neural tube defects. In contrast, little to no transferrin was seen in the hindgut epithelium of mice affected by neural tube defects (affected *curly tail* mice). Neither affected nor unaffected *curly tail* mice exhibited transferrin mRNA in their hindgut epithelium, suggesting that the transferrin seen in unaffected *curly tail* mice was not synthesized in the hindgut region. The distribution of the transferrin receptor was the same in both groups of *curly tail* mice. The difference in transferrin expression between affected and unaffected *curly tail* mice may be due to inhibition of transferrin binding in affected curly tail mice. Another possibility is that the low level of transferrin in affected *curly tail* mice may be the normal situation (Hoyle, Henderson, Matthews, and Copp, 1996). *Curly tail* mice, as a model for NTDs, exhibit delayed closure of the posterior neuropore because of an imbalance in cell proliferation in the posterior neuropore region (Copp et al., 1988). Unaffected *curly tail* mice may up-regulate transferrin binding in order to increase transferrin level in the hindgut epithelium, thereby compensating for the inadequate cell proliferation (Hoyle et al.).

The transferrin receptor is found in endothelial cells, neurons, and oligodendrocytes of the brain in various species. The transferrin receptor density is normally two to three times higher in the cerebellum of the rat than in the cerebral cortex. Studies on myelin-deficient rats, whose oligodendrocytes fail to mature while other cell types are normal, have shown a decrease in the transferrin receptor of 56% in the cerebrum and of 70% in the cerebellum compared with control rats. Based on these results, mature oligodendrocytes are hypothesized to be responsible for a large portion of the transferrin receptor expression in the brain (Roskams and Connor, 1992). A study by Moos, Oates, and Morgan (1998) using rats indicated that the transferrin receptor on neuronal cells was almost nonexistent during embryonic and early postnatal life, but during later postnatal life, the receptors increased in number, reaching a plateau from postnatal day 21 through adulthood.

Ferritin was studied in the pons and cerebellum of fetuses to adults. Oligodendrocytes were positive for ferritin, but neurons did not contain ferritin. Ferritin appeared in oligodendrocytes at 21 weeks gestation in the reticular formation of the pons and at 25 weeks gestation in pontine nuclei. Increases in ferritin occurred from 33 weeks gestation to infancy (Ozawa, Nishida, Mito, and Takashima, 1994a). Ferritin was first seen in the cerebrum at 25 weeks gestation. Fully developed normal adult brains rarely have ferritin positive microglia, but diseased brains acquire more ferritin-containing glial cells (Ozawa, Nishida, Mito, and Takashima, 1994b).

Brain ferritin is crucial for normal development of the brain. Oligodendrocytes contain H-ferritin (heavy) during early brain development and is comparable to high iron usage and low iron storage. H-ferritin on immature oligodendrocytes is accompanied by

transferrin accumulation and mRNA expression. The L subunit promotes the mineralization of iron into ferritin (Levi et al., 1992). Adult human brain ferritin contains the H subunit in twice the quantity of the L subunit brain as a reflection of the need for iron in brain for metabolic processes. However, the ratio of the H:L subunits varies according to the area of the brain (Connor et al., 1995), cell type (Connor and Menzies, 1996), age, and disease status (Connor et al., 1995).

A cell type's ratio of H:L subunits is related to its function. Neurons contain mostly H-ferritin indicating that they require much iron, but are unable to store it in large quantities. Microglia, scavenger cells needing relatively little iron for metabolic purposes, (Connor and Menzies, 1996) contain more L-ferritin than H-ferritin (Connor and Menzies, 1995). Oligodendrocytes are the only brain cell type to contain a mixture of H-ferritin and L-ferritin, implying that oligodendrocytes metabolically use large amounts of iron as well as contain large amounts of intracellular iron (Connor and Menzies, 1996).

The behaviors of iron, transferrin, and transferrin receptors change when rats are fed an iron-deficient diet. Moos et al. (1998) found that when the availability of iron was reduced, transferrin receptors were expressed on cells in both young and older rats. In another study, fourteen days on an iron deficient diet caused brain iron levels in rats to decrease by 50% in the microsomal fractions and 30% in the cytosol compared to control rats. In response, brain transferrin levels in the cytosol almost doubled. Brain ferritin levels became lower than controls, but then remained consistent (Chen, Connor, and Beard, 1995).

Iron and Neural Tube Defects

Two studies have suggested that insufficient iron is associated with NTDs. Golding linked dietary factors and iron status to neural tube defects in 1982. He suggested that tea consumption in women with poor iron status could reduce iron absorption sufficiently to produce an environment with insufficient nutrients for normal embryo development. An unpublished case-control study performed by TDH in response to the 1990-1991 Cameron County anencephaly cluster also suggested an iron connection. In this study, hematocrit values of case-women were significantly lower ($p<0.01$) than those of control-women (TDH report, 1992).

It is not immediately apparent how iron is related to neural tube defects. However, it is known that the developing fetus and placenta need iron (Bridges, 1990). A number of iron-containing enzymes are known to be present during development (Table 1). Additionally, some of these appear to be in the right place (brain and spinal cord) at the right time (during neurulation). It is also known that the developing placenta and embryo require about 5 mg of iron per day (van Eijk and de Jong, 1992). One of the listed enzymes might be present in inadequate amounts during embryogenesis if the iron status of the mother is compromised.

CHAPTER IV

METHODS

Study Design Definitions

A case-woman was a woman recognized as a result of the Neural Tube Defect (NTD) Project's surveillance system as having given birth to or having terminated an NTD-affected baby, fetus, or embryo in one of the 14 counties involved in the study on or after June 1, 1995. These counties included Cameron, Hidalgo, Starr, Webb, Zapata, Maverick, Kinney, Val Verde, Terrell, Brewster, Presidio, Jeff Davis, Hudspeth, and El Paso. The case-woman must have lived in one of the 14 counties at the time of delivery or termination and throughout the study. The woman also must have signed a consent form before participating in any part of the study.

A control-woman was a woman chosen through a random selection process by the Neural Tube Defect Project as having given birth to a live baby of any gestational age without a noticeable or prenatally diagnosed abnormality present at birth making medical intervention necessary. The woman must have given birth in a hospital or birthing center in one of the 14 counties of the study region on or after January 1, 1995. The control-woman also must have resided in the 14 county study area at the time of birth and throughout the study. The woman must have signed a consent form before participating in any part of the study.

Study Design

Two control-women were chosen for each case-woman in the study. The number of control-women chosen for a given year was two times the number of women meeting the case-woman definition from two years earlier, i.e. the number of control-women in 1995 was two times the number of women meeting the case-woman definition for 1993.

Control-women were chosen from 17 strata within the 14 county region. Fifteen of the strata corresponded to the 15 largest obstetric hospitals in the four larger counties: Cameron, El Paso, Hidalgo, and Webb. The 16th stratum corresponded to one small hospital and 37 birthing centers in the four larger counties. The 17th stratum corresponded to the five hospitals and three birthing centers in the 10 remaining border counties. The number of control-women chosen from each of the 17 strata was based on the proportion of live births occurring in that stratum two years previously compared to the number of control-women needed. Potential control-women in each stratum were assigned numbers from one to the number of annual births in each stratum. Control-women were chosen using a random number table, selecting numbers between one and the annual number of births in each stratum. Due to the low numbers of women giving birth in strata 16 and 17, the numbers assigned to control-women in these strata were consecutive numbers arranged by the alphabetic order of the facility name.

Each potential control-woman who matched definition requirements of a control-woman was approached in regard to participation in the study. A potential control-woman not matching the control-woman requirements was not asked to participate in the study. Potential control-women who refused to participate were not replaced.

Participation Rates

The Texas Neural Tube Defect Project utilized three teams for data collection. The teams were based in El Paso, Laredo, and Harlingen. The total number of cases and controls included women who refused to participate, who had moved, or who could not be found by the data collection team. The El Paso team enrolled 55% of cases identified and 50% of controls identified; the Laredo team enrolled 83% of cases identified and 34% of controls identified; and the Harlingen team enrolled 71% of cases identified and 69% of controls identified. Table 2 lists additional participation rate details.

Table 2.

Participation Rates of Cases and Controls

Team	Cases			Controls		
	Enrolled	Total	Percent	Enrolled	Total	Percent
El Paso	26	47	55	51	102	50
Laredo	38	46	83	28	83	34
Harlingen	92	129	71	99	143	69
Total	156	222	70	178	328	54

Data Collection

The Texas Neural Tube Defect Project collected data through an extensive mother questionnaire, a food frequency questionnaire, and laboratory blood tests. Data from the mother questionnaire were entered into a FoxPro database. To ensure data entry accuracy, the mother questionnaire was double entered into the database, each time by

different people, and the differences reconciled. The food frequency questionnaire was entered into Food Intake Assessment System (FIAS) software developed for the NTD project by the University of Texas Health Science Center – Houston and the USDA Human Nutrition Information Service. Laboratory data were entered into EpiInfo. Analyses for this thesis included 148 cases and 158 controls.

TDH staff edited data by looking for outliers, logical inconsistencies, and duplicate records. Inconsistencies were resolved by referring to the original raw questionnaire.

Data Analysis

The data obtained from TDH conformed to certain criteria. Data did not contain any identifying information. Data analyses were limited to Hispanic women because of the small numbers of other racial and ethnic groups represented in the data, making adjustment for ethnicity and race very difficult. Aggregation of some variables, such as mother's age and child's sex, were performed. All data were analyzed using SPSS, and in some cases followed by Computer Programs for Epidemiologists (PEPI) version 3.00 (Abramson and Gahlinger, 1999) for calculations of odds ratios and confidence intervals. The data were analyzed to detect if a significant associations exists in iron levels between cases and controls. Laboratory values that were analyzed included hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and serum ferritin. Dietary iron intake as well as consumption of tea, orange juice, and supplements from a food frequency questionnaire were analyzed to detect if there were significant associations between cases and controls. The food frequency questionnaire asked "For each food

listed, indicate the average number of times per month, week, or day you have eaten the food between three months before conception and three months after conception?" Variables of age, body mass index, and pica were examined to detect if these variables were effect modifiers or confounders of the relationships between dietary iron intake, hemoglobin levels, hematocrit levels, and ferritin levels and risk for NTD-affected offspring. Pica may be caused by an instinctive compulsion to replace minerals missing from the diet. Information about pica was obtained by the question, "Between three months before conception and three months after conception, did you eat any items that are normally not considered food items such as starch, dirt, or clay?" Starch or clay can bind to iron in the gastrointestinal tract, preventing iron absorption.

Odds ratios were calculated using the CASECONT (Analysis of 2 x 2 tables) and MANTELX programs included in PEPI. The CASECONT program used the chi-square test to compute odds ratios. Confidence intervals (95%) were calculated in single tables by Cornfield's approximation described by Fleiss (1979). The confidence intervals between tables were calculated using the Cornfield-Gart procedure (Fleiss, 1979). The MANTELX program uses the extended Mantel-Haenszel test for trend that compares the values of each variable. The heterogeneity chi-square test is based on the uniformity of the trend in the different strata. This test is computed by summing the Mantel-Haenszel chi-square for trend in each stratum and subtracting the overall Mantel-Heanszel chi-square (Abramson and Gahlinger, 1999).

Mean levels for variables reflecting iron status were calculated, as well as the proportion of women with values above and below normal values. T-tests were used to determine if significant differences existed between cases and controls. Using values for

controls, levels of analytes were divided into quartiles. Both crude odds ratios and stratified odds ratios with 95% confidence intervals were compared between cases and controls, using the lowest quartile as the reference except for ferritin. The reference used for ferritin was the highest quartile because the normal range for ferritin includes values in the highest quartile. Also, a regression equation for predicting if a woman would have a baby with an NTD or a baby without this abnormality was estimated based on a specific analyte using logistic regression (Selvin, 1996). This procedure allows for control of several potentially confounding factors, such as BMI (body mass index) and maternal age, while looking at the relationship between maternal iron and NTDs, maternal hemoglobin and hematocrit and NTDs, and serum ferritin and NTDs.

The NTD Project has been evaluated and approved by the Texas Department of Health's Institutional Review Board (IRB) for the Protection of Human Subjects. Appendix A contains a copy of the most recent approval. The proposed data used in this analysis did not contain any identifiers, so there was no potential for violating any NTD project participant's privacy.

CHAPTER V

RESULTS

Descriptive Statistics

Case- and control-women in the Texas Neural Tube Defect Project (TNTDP) were similar with respect to their age, education, income, gravidity, and birthplace. First, descriptive statistics for age, country of birth, education, income, and gravidity will be discussed. Some differences existed for length of gestation and pregnancy outcome, both related to their case or control status. Laboratory measures were analyzed for significant differences between cases and controls, as well as differences in intake of dietary supplements, smoking, and alcohol use. Finally, results related to the three hypotheses will be discussed.

Case- and control-mothers were similar in age. Case-mothers in the TNTDP ranged in age from 14 to 44 years with a mean of 23.7 years. Control-mothers in the TNTDP ranged in age from 14 to 39 years with a mean of 24.1 years. Over 50% of both case- and control-mothers were 20 – 29 years of age with 30% or more of each group 20 – 24 years of age.

One-half of both case- and control-women were born in the U.S. The remainder were born in Mexico.

Case- and control-women had similar amounts of education. The education of case-mothers ranged from 0 to 16 years, while the education of control-mothers ranged from 0 to 18 years. Eight percent of case-mothers and 3% of control-mothers had five years or less of education; 26% of case-mothers and 20% of control-mothers received eight or fewer years of education. Fifty percent of both case- and control-mothers had less than 12 years of education. Fifty percent of both case- and control-women received at least 12 years of education.

Household incomes for case- and control-families were statistically similar. Household income was less than $10,000 per year in 48% of case-women and 41% of control-women. Twenty percent of both case- and control-households earned from $10,000 to $15,000 per year and 34% of case-households and 41% of control-households earned greater than $15,000.

The gravidity of case- and control-women was similar. The gravidity of case-women ranged from one to nine, while control-women's gravidity ranged from one to eight. The mean for gravidity of the cases and controls was 2.5 and 2.4, respectively. Gestation for cases ranged from 10 to 42 weeks, while gestation for controls ranged from 28 to 42 weeks. Thirty-eight percent of case-women had gestations of less than 28 weeks. The pregnancy outcomes for case -women included the following: live birth, 48%; stillbirth, 18%; induced abortion, 32%; and miscarriage, 2%. All control-women gave birth to live babies.

Laboratory Test Analysis

Laboratory values that reflected iron status were analyzed according to case or control status. No significant differences were found between the mean values of cases and controls for hemoglobin, hematocrit, MCV, MCH, MCHC, and serum ferritin. However, significant differences were found when below normal values for MCV and MCH were compared between case- and control-women ($p < 0.05$). More case- than control-women had below normal values for MCV and MCH.

Table 3 shows similarity for percent normal values, percent below normal values, and percent above normal values for all other laboratory measures in cases and controls.

Table 3

Percent of Normal, Below Normal, and Above Normal Lab Values for Iron Related Measures

Name of Measure (Normal Range)	Cases or Controls	Mean	Normal Value (%)	Below Normal (%)	Above Normal (%)
Hemoglobin (12.0 – 15.6 g/dL)	Cases	12.7	77.4	22.6	0
	Controls	12.8	82.2	17.8	0
Hematocrit (35.0 – 46.0 %)	Cases	37.9	83.1	16.1	0.8
	Controls	38.3	88.1	11.9	0
MCV ($80 – 100\ \mu m^3$)	Cases	86.7	87.6	12.4[b]	0
	Controls	87.0	92.2	7.8[b]	0
MCH (27.0 – 33.0 PG)	Cases	29.0	81.5	17.7[c]	0.8
	Controls	29.1	82.2	14.4[c]	3.4
MCHC (32.0 – 36.0 g/dL)	Cases	33.2	91.9	8.1	0
	Controls	33.4	97.5	2.5	0
Serum Ferritin (12 – 156 ng/mL)[a]	Cases	35.8	76.9	20.7	2.5
	Controls	42.2	80.7	16.8	2.5

[a]Normal range for females

[b]Significant difference between the percent of cases and controls with below normal MCV ($p < 0.05$)

[c]Significant difference between the percent of cases and controls with below normal MCH ($p < 0.05$)

Health Related Behaviors

Table 4 shows the number and percentage of cases and controls that used nutritional supplements, tobacco, and alcohol during the preconception period. Numbers of cases and controls using supplements, tobacco, and alcohol were similar between cases and controls. A seven percent difference or less existed between cases and controls in all categories.

Table 4

Health Related Behaviors

Criteria		Yes	No	Multivitamin	Prenatal Vitamin	Single Folic Acid Vitamin
		# (%)	# (%)	# (%)	# (%)	# (%)
Supplement use +1 month?[a]	Cases	23 (12.9)	129 (87.1)	5 (3.4)	14 (9.5)	
	Controls	19 (14.5)	135 (85.5)	4 (2.5)	19 (12.0)	
Supplement use +2 month?[b]	Cases	55 (37.2)	93 (62.8)	3 (2.0)	51 (34.5)	1 (0.7)
	Controls	57 (36.6)	100 (63.4)	4 (2.5)	53 (33.5)	1 (0.6)
Any supplement use?	Cases	82 (55.4)	66 (44.6)			
	Controls	90 (57.0)	68 (43.0)			
Any folic acid use?[c]	Cases	8 (5.4)	140 (94.6)			
	Controls	5 (3.2)	153 (96.8)			
Any smoking?[c]	Cases[d]	29 (19.6)	119 (80.4)			
	Controls[e]	20 (12.7)	138 (87.3)			
Any alcohol use?[c]	Cases	45 (30.4)	103 (69.6)			
	Controls	44 (27.8)	114 (72.2)			

Note: There are no significant differences between cases and controls within any criterion.

[a]Supplement use one month after conception

[b]Supplement use second month after conception

[c]During preconception

[d]Cases (N=148) 119 (80.4%) Nonsmokers; 4 (2.7%) < 1 cigarette/day; 14(9.5%) < ½ pack/day; 11 (7.4%) half pack or more/day

[e]Controls (N=158) 138 (87.3%) Nonsmokers; 3 (1.9%)< 1 cigarette/day; 13 (8.2%)< ½ pack/day; 4 (2.5%) half pack or more/day

Dietary Iron Intake and Related Factors Between Case- and Control-Women

In considering whether an association exists between iron and NTD, differences in dietary iron levels between cases and controls are of interest, as well as any measures that might affect iron levels. Table 5 indicates similarity between dietary iron intakes for cases and controls. Study mothers with BMIs less than 21.3 had no increased risk of NTDs. (Appendix B). However, when dietary iron levels are examined by body mass index (BMI = weight in kg / height in m^2), women with BMIs less than 21.3 and with dietary iron levels less than 25.0 mg had 1.64 times the risk of NTDs compared to women with similar BMIs who consumed 25.0 mg or more of dietary iron (Table 6).[1] Women with BMIs greater than or equal to 21.3 had no increased risk of NTDs with low dietary iron intake.

Table 5

Association Between Dietary Iron Levels and NTDs

Dietary Iron[a] (mg)	Cases N (%)	Controls N (%)	Stratum-Specific Odds Ratio	95% CI[b]
< 25.0	78 (54.2)	77 (49.7)	1.20	0.74 – 1.94
≥ 25.0	66 (45.8)	78 (50.3)		

[a]Based on the comparison group distribution (50th percentile)

[b]Cornfield approximation (Fleiss, 1979)

[1]BMIs between 19 and 25 are typical of persons in a normal weight range. Mildly overweight persons are 20 percent to 40 percent above their normal weight and have BMIs between 27 and 30.

Table 6

Association Between Dietary Iron Levels and NTDs by Body Mass Index

Body Mass Index[a]	Dietary Iron[b] (mg)	Cases N (%)	Controls N (%)	Stratum-Specific Odds Ratio	95% CI[c]	Mantel – Haenszel Odds Ratio[d]
< 21.3	< 25.0	15 (51.7)	15 (39.5)	1.64	0.55 – 4.91	1.14 (0.72 – 1.80)[e]
	≥ 25.0	14 (48.3)	23 (60.5)			
≥ 21.3	< 25.0	59 (53.2)	61 (52.6)	1.02	0.59 – 1.78	
	≥ 25.0	52 (46.8)	55 (47.4)			

[a]Based on the comparison group distribution (lowest quartile, sum of other 3 quartiles)

[b]Based on the comparison group distribution (50th percentile)

[c]Cornfield's approximation (Fleiss, 1979)

[d]Method of Mantel and Haenszel (Fleiss, 1981:174)

[e]Cornfield-Gart method (Fleiss, 1979)

The heterogeneity test for the two odds ratios was not significant. Data in the table in Appendix C shows no increased risk for NTDs in the second and third quartile of BMI when compared to the lowest BMI quartile. The odds ratio of women in the highest BMI quartile indicates a risk 1.76 times the risk of women with BMIs in the lowest quartile. Appendix D shows no significant association between maternal age and NTDs. The association between iron and NTDs was also modified by maternal age, although not significantly. Table 7 shows that women who were less than 24 years of age and consumed less than 25.0 mg dietary iron per day had 1.71 times the risk of NTDs compared to women with dietary iron intakes of 25.0 mg or more.

Table 7

Association Between Dietary Iron Levels and NTDs by Maternal Age

Maternal Age[a] (yrs)	Dietary Iron[a] (mg)	Cases N	Cases (%)	Controls N	Controls (%)	Stratum-Specific Odds Ratio	95% CI[b]
< 24	< 25.0	44	(55.7)	33	(42.3)	1.71	0.87 – 3.39
	≥ 25.0	35	(44.3)	45	(57.7)		
≥ 24	< 25.0	34	(52.3)	44	(57.1)	0.82	0.40 – 1.69
	≥ 25.0	31	(47.7)	33	(42.9)		

[a]Based on the comparison group distribution (50th percentile)

[b]Cornfield's approximation (Fleiss, 1979)

Women who were 24 years or older and who consumed less than 25.0 mg of dietary iron had 0.82 times the risk of NTDs compared to women with higher iron intakes. Both the odds ratios and the heterogeneity test of odds ratios were not statistically significant.

The dietary consumption of orange juice was also examined because the ascorbic acid in orange juice facilitates the absorption of nonheme iron, the form of iron from nonmeat sources (Duyff, 1996). Low orange juice consumption in servings per month had a nonsignificant protective effect on risk for NTDs (Table 8). When stratified by age, no association was noted between orange juice consumption and NTDs among mothers less than 24 years of age. However, as shown in Table 9, mothers ages 24 and higher who consumed less than five servings of orange juice per month experienced a significant protective effect for having a NTD-affected pregnancy. These mothers had 0.42 times the risk of NTDs as mothers who consumed five or more servings of orange juice per month. This protective effect was unexpected. Since I have tested over twenty hypotheses in my analyses, it is possible this result might be significant by random chance.

However, the heterogeneity test was also significant at p = 0.050. This heterogeneity test has low power, so a significant result is considered reliable (Abramson & Gahlinger, 1999).

Because tea and coffee are known to decrease the absorption of iron (Duyff, 1996), the association between the combination of hot tea and iced tea consumption and the combination of teas and coffee were examined. No significant associations were found between NTDs and tea consumption, as shown in Table 10. Tea consumption was also examined for effect modification by maternal age. Table 11 shows that no significant differences in NTD risk were found. Table 12 indicates that when coffee and tea consumption are combined, no significant association was found. Maternal age (Table 13) was also not an effect modifier for the association between the tea and coffee consumption and NTDs.

Table 8

Association Between Orange Juice Consumption and NTDs

Orange Juice Consumption[a, b]	Cases N	(%)	Controls N	(%)	Odds Ratio	95% CI[c]
< 5	52	(36.1)	69	(44.5)	0.70	0.43 – 1.15
≥ 5	92	(63.9)	86	(55.5)		

[a]Based on the comparison group distribution (50th percentile)

[b]Servings per month

[c]Cornfield's approximation (Fleiss, 1979)

Table 9

Association Between Orange Juice Consumption and NTDs by Maternal Age

Maternal Age[a]	Orange Juice Consumption[a, b]	Cases N (%)	Controls N (%)	Stratum-Specific Odds Ratio	95% CI[c]	Mantel-Haenszel Odds Ratio[d]
< 24	< 5	33 (41.8)	31 (39.7)	1.09	0.55 – 2.16	0.71 (0.44 – 1.12)[e]
	≥ 5	46 (58.2)	47 (60.3)			
≥ 24	< 5	19 (29.2)	38 (49.4)	0.42	0.20 – 0.90	
	≥ 5	46 (70.8)	39 (50.6)			

[a]Based on the comparison group distribution (50th percentile)

[b]Servings per month

[c]Cornfield approximation (Fleiss, 1979)

[d]Method of Mantel and Haenszel (Fleiss, 1981:174)

[e]Cornfield-Gart method (Fleiss, 1979)

Table 10

Association Between Tea Consumption and NTDs

Tea Consumption[a, b]	Cases N	(%)	Controls N	(%)	Odds Ratio	95% CI[c]
0 - 2	68	(47.6)	62	(40.0)	1.36	0.84 – 2.21
3 - 56	75	(52.4)	93	(60.0)		

[a]Based on the comparison group distribution (50th percentile)

[b]Servings per month

[c]Cornfield approximation (Fleiss, 1979)

Table 11

Association Between Tea Consumption and NTDs by Maternal Age

Maternal Age[a](yrs)	Tea Consumption[a,b]	Cases N	(%)	Controls N	(%)	Odds Ratio	95% CI[c]
< 24	0 - 2	42	(53.8)	37	(47.4)	1.29	0.66 – 2.55
	3 - 56	36	(46.2)	41	(52.6)		
≥ 24	0 - 2	26	(40.0)	25	(32.5)	1.39	0.66 – 2.93
	3 - 56	39	(60.0)	52	(67.5)		

[a]Based on the comparison group distribution (50th percentile)

[b]Servings per month

[c]Cornfield approximation (Fleiss, 1979)

Table 12

Association Between Tea/Coffee Consumption and NTDs

Tea/Coffee Consumption[a]	Cases N	(%)	Controls N	(%)	Odds Ratio	95% CI[c]
0 - 10[b]	83	(58.0)	77	(49.7)	1.40	0.86 – 2.27
11 - 140	60	(42.0)	78	(50.3)		

[a]Based on the comparison group distribution (50th percentile)

[b]Servings per month

[c]Cornfield's approximation (Fleiss, 1979)

Table 13

Association Between Tea/Coffee Consumption and NTDs by Maternal Age

Maternal Age[a] (yrs)	Tea Consumption[a]	Cases N	Cases (%)	Controls N	Controls (%)	Odds Ratio	95% CI[c]	Mantel-Haenszel Odds Ratio[d]
< 24	0 - 10[b]	51	(65.4)	46	(59.0)	1.31	0.65 – 2.65	1.37 (0.86 – 2.19)[e]
	11 - 140	27	(34.6)	32	(41.0)			
≥ 24	0 - 10	32	(49.2)	31	(40.3)	1.44	0.70 – 2.96	
	11 - 140	33	(50.8)	46	(59.7)			

[a]Based on the comparison group distribution (50th percentile)

[b]Servings per month

[c]Cornfield's approximation (Fleiss, 1979)

[d]Mantel-Haenszel test (Fleiss, 1981)

[e]Cornfield-Gart method (Fleiss, 1979)

Blood Hemoglobin and Hematocrit Values Between Case- and Control-Women

Research question 2 looked at the association of hemoglobin and hematocrit with NTDs. Hemoglobin and hematocrit may indicate low blood iron levels (Brown, 1993). The distribution of hemoglobin levels at the 50th percentile (based on the comparison group distribution) was not significantly different between cases and controls (Table 14). Analysis of hematocrit yielded similar results (table not shown)(OR = 1.34 95% CI = 0.78 – 2.30). Maternal age and BMI were examined as effect modifiers of the association of hemoglobin and hematocrit with NTDs. Tables 15 – 18 indicate there were no significant associations between hemoglobin and NTDs or hematocrit and NTDs when stratifying and controlling for maternal age or BMI.

Table 14

Association Between Hemoglobin and NTDs

Hemoglobin[a] (mg/dL)	Cases N	(%)	Controls N	(%)	Odds Ratio	95% CI[b]
< 12.8	66	(53.2)	57	(48.3)	1.22	0.71 – 2.08
≥ 12.8	58	(46.8)	61	(51.7)		

[a]Based on the comparison group distribution (50th percentile)

[b]Cornfield's approximation (Fleiss, 1979)

Table 15

Association Between Hemoglobin and NTDs by Maternal Age

Maternal Age[a] (yrs)	Hemoglobin[a] (g/dL)	Cases N	(%)	Controls N	(%)	Odds Ratio[b]	95% CI[c]
< 24	< 12.8	39	(54.9)	30	(48.4)	1.30	0.62 – 2.73
	≥ 12.8	32	(45.1)	32	(51.6)		
≥ 24	< 12.8	27	(50.9)	27	(48.2)	1.12	0.49 – 2.54
	≥ 12.8	26	(49.1)	29	(51.8)		

[a]Based on the comparison group distribution (50th percentile)

[b]Overall odds ratio, (adjusting for maternal age) = 1.21, 95% CI = 0.73 – 2.01

[c]Cornfield's approximation (Fleiss, 1979)

Table 16

Association Between Hematocrit and NTDs by Maternal Age

Maternal Age[a] (yrs)	Hematocrit[a] (%)	Cases N	Cases (%)	Controls N	Controls (%)	Odds Ratio[b]	95% CI[c]
< 24	< 38.5	40	(56.3)	33	(53.2)	1.13	0.54 – 2.38
	≥ 38.5	31	(43.7)	29	(46.8)		
≥ 24	< 38.5	31	(58.5)	26	(46.4)	1.63	0.71 – 3.73
	≥ 38.5	22	(41.5)	30	(53.6)		

[a]Based on the comparison group distribution (50th percentile)

[b]Overall odds ratio, (adjusting for maternal age) = 1.33, 95% CI = 0.80 – 2.21

[c]Cornfield approximation (Fleiss, 1979)

Table 17

Association Between Hemoglobin and NTDs by BMI

BMI[a]	Hemoglobin[b] (g/dL)	Cases N	Cases (%)	Controls N	Controls (%)	Odds Ratio[c]	95% CI[d]
< 21.3	< 12.8	16	(55.2)	12	(42.9)	1.64	0.51 – 5.36
	≥ 12.8	13	(44.8)	16	(57.1)		
≥ 21.3	< 12.8	46	(50.5)	44	(49.4)	1.05	0.56 – 1.96
	≥ 12.8	45	(49.5)	45	(50.6)		

[a]Based on the comparison group distribution (lowest quartile, sum of other 3 quartiles)

[b]Based on the comparison group distribution (50th percentile)

[c]Overall odds ratio, (adjusting for BMI) = 1.16, 95% CI = 0.70 – 1.94

[d]Cornfield approximation (Fleiss, 1979)

Table 18

Association Between Hematocrit and NTDs by BMI

BMI[a]	Hematocrit[b] (%)	Cases N	Cases (%)	Controls N	Controls (%)	Odds Ratio[c]	95% CI[d]
< 21.3	< 38.5	16	(55.2)	14	(50.0)	1.23	0.38 – 3.98
	≥ 38.5	13	(44.8)	14	(50.0)		
≥ 21.3	< 38.5	51	(56.0)	44	(49.4)	1.30	0.70 – 2.45
	≥ 38.5	40	(44.0)	45	(50.6)		

[a]Based on the comparison group distribution (lowest quartile, sum of other 3 quartiles)

[b]Based on the comparison group distribution (50th percentile)

[c]Overall odds ratio, (adjusting for BMI) = 1.29, 95% CI = 0.77 – 2.14

[d]Cornfield approximation (Fleiss, 1979)

Serum Transferrin and Ferritin Levels Between Case- and Control-Women

Serum transferrin levels were not analyzed because there were only 17 serum transferrin values available for the women in this data set at the time of these analyses. Eight (47%) of the transferrin values were from case-women and nine (53%) were from control-women. One value was below the normal range for transferrin[2] (168) by 12%; one value was above the normal range (365) by 7%. Both values belonged to cases.

The association between serum ferritin and NTDs as indicated by the cumulative odds ratio (1.509, 95% CI 0.97 – 2.35) is close to significance (Table 19). Using

[2]Serum transferrin normal range is 188 – 341 mg/dL

quartile 4 (53 – 280 ng/ml)[3] as a reference, there is no increased N
with serum ferritin levels of 30 – 52 ng/ml of ferritin. However, v
ng/ml of ferritin have 1.5 times the NTD risk, and women with 0 -
times the NTD risk of women with ferritin values in quartile 4.

The results of stratifying by maternal age suggest that womer
age who have ferritin levels less than 30 have twice the risk of hav
compared with women of the same age with ferritin levels of 30 o
contrast, for women ages 24 and above, ferritin levels less than 30
woman having an NTD baby.

Women who had ferritin levels less than 30 ng/ml and BMIs l
almost six times the risk of having an NTD baby compared with w
BMIs with ferritin levels of 30 ng/ml or greater (Table 21) ($p < 0.$
BMIs of 21.3 and higher were not at increased risk for NTD birth
levels.

Among women who were less than 24 years old and who had
ferritin levels less than 30 ng/ml were associated with 5.66 times tl
affected with NTDs (Table 22) ($p < 0.05$). Women in the same

Table 19

Ferritin in Quartiles by Neural Tube Defect Cases and Controls

Ferritin[a] (ng/ml)	Cases N	(%)	Controls N	(%)	Odds Ratio	Cumulative Odds Ratio[d]	95% CI
0 - 15	34	(28.1)	25	(21.0)	1.700[b]	1.509	0.97 – 2.35
16 - 29	39	(32.2)	32	(26.9)	1.523		
30 - 52	24	(19.8)	32	(26.9)	0.938		
53 - 280	24	(19.8)	30	(25.2)	1.000[c]		
Total	121		119				

[a]Based on the comparison group distribution (quartiles)

[b]Chi-square for trend = 3.118 (1 d.f.), $p > 0.05$

[c]Referent category

[d]Procedure by Liu and Agresti, 1986

Table 20

Association Between Ferritin and NTDs by Maternal Age

Maternal Age[a] (yrs)	Ferritin[a] (ng/ml)	Cases N (%)	Controls N (%)	Stratum-Specific Odds Ratio	95% CI[b]	Mantel – Haenszel Odds Ratio[c]
< 24	< 30	49 (71.0)	33 (55.0)	2.00	0.91 – 4.43	1.60 (0.95 – 2.69)[d]
	≥ 30	20 (29.0)	27 (45.0)			
≥ 24	< 30	24 (46.2)	24 (40.7)	1.25	0.55 – 2.85	
	≥ 30	28 (53.8)	35 (59.3)			

[a]Based on the comparison group distribution (50th percentile)

[b]Cornfield approximation (Fleiss, 1979)

[c]Method of Mantel and Haenszel (Fleiss, 1981, 174)

[d]Cornfield-Gart method (Fleiss, 1979)

Table 21

Association Between Ferritin and NTDs by BMI

Body Mass Index[a]	Ferritin[b] (ng/ml)	Cases N (%)	Controls N (%)	Stratum-Specific Odds Ratio	95% CI[c]	Summary Odds Ratio[d]
< 21.3	< 30	21 (77.8)	10 (37.0)	5.95	1.56–23.90	1.63 (0.98 – 2.72)[e]
	≥ 30	6 (22.2)	17 (63.0)			
≥ 21.3	< 30	49 (54.4)	46 (50.5)	1.17	0.63– 2.19	
	≥ 30	41 (45.6)	45 (49.5)			

[a]Based on the comparison group distribution (lowest quartile, sum of upper 3 quartiles)

[b]Based on the comparison group distribution (50th percentile)

[c]Cornfield's approximation (Fleiss, 1979)

[d]Extended Mantel-Haenszel test (X^2)

[e]Cornfield-Gart method (Fleiss, 1979)

Table 22

Association Between Ferritin and NTDs by Maternal BMI and Maternal Age (< 24 yrs)

Body Mass Index[a]	Ferritin[b] (ng/ml)	Cases N (%)	Controls N (%)	Stratum-Specific Odds Ratio	95% CI[c]	Mantel-Haenszel Odds Ratio[d]
< 21.3	< 30	18 (78.3)	7 (38.9)	5.66	1.20 – 28.79	2.00 (0.97 – 4.11)[e]
	≥ 30	5 (21.7)	11 (61.1)			
≥ 21.3	< 30	30 (66.7)	25 (61.0)	1.28	0.48 – 3.40	
	≥ 30	15 (33.3)	16 (39.0)			

[a]Based on the comparison group distribution (lowest quartile, sum of other 3 quartiles)

[b]Based on the comparison group distribution (50th percentile)

[c]Cornfield's approximation (Fleiss, 1979)

[d]Calculated by method of Mantel and Haenszel (Fleiss, 1981:174)

[e]Cornfield-Gart method (Fleiss, 1979)

Women ages 24 years and older with a BMI less than 21.3 and ferritin levels less than 30ng/ml had six times the risk of NTDs as women of similar ages and BMIs with ferritin levels of 30 ng/ml or more (Table 23). Women of similar ages with BMIs 21.3 and higher did not have an increased risk of NTDs based on ferritin levels.

Table 24 indicates that among women less than 24 years of age and with BMIs less than 21.3, ferritin levels less than 30 ng/ml were associated with 5.66 times the risk for having an NTD baby compared with women with similar BMIs and ages, but with ferritin levels 30 ng/ml and more. Among women 24 years of age and higher with BMIs less than 21.3, ferritin levels less than 30 ng/ml were associated with six times the risk of NTDs compared with women with ferritin levels of 30 ng/ml or more. The Mantel-Haenszel odds ratio was significant for increased risk of having an NTD baby for women

Table 23

Association Between Ferritin and NTDs by Maternal BMI and Maternal Age (≥ 24 yrs)

Body Mass Index[a]	Ferritin[b] (ng/ml)	Cases N (%)	Controls N (%)	Stratum-Specific Odds Ratio	95% CI	Mantel-Haenszel Odds Ratio[e]
< 21.3	< 30	3 (75.0)	3 (33.3)	6.00	0.275 – 366.240[c]	1.20 (0.56 – 2.58)[f]
	≥ 30	1 (25.0)	6 (66.7)			
≥ 21.3	< 30	19 (42.2)	21 (42.0)	1.01	0.41 – 2.47[d]	
	≥ 30	26 (57.8)	29 (58.0)			

[a]Based on the comparison group distribution (lowest quartile, sum of other 3 quartiles)

[b]Based on the comparison group distribution (50th percentile)

[c]Fisher's Exact confidence interval (Abramson and Gahlinger, 1999)

[d]Cornfield's approximation (Fleiss, 1979)

[e]Calculated by method of Mantel and Haenszel (Fleiss, 1981:174)

[f]Cornfield-Gart method (Fleiss, 1979)

Table 24

Association Between Ferritin and NTDs by Maternal BMI (BMI < 21.3) and Maternal Age

Maternal Age[a](yrs)	Ferritin[a] (ng/ml)	Cases N (%)	Controls N (%)	Stratum-Specific Odds Ratio	95% CI	Mantel - Haenszel Odds Ratio[d]
< 24	< 30	18 (78.3)	7 (38.9)	5.66	1.20 – 28.79[b]	5.73 (1.69 – 19.37)[e]
	≥ 30	5 (21.7)	11 (61.1)			
≥ 24	< 30	3 (75.0)	3 (33.3)	6.00	0.275 – 366.240[c]	
	≥ 30	1 (25.0)	6 (66.7)			

[a]Based on the comparison group distribution (50th percentile)

[b]Cornfield's approximation (Fleiss, 1979)

[c]Fisher's Exact confidence interval (Abramson and Gahlinger, 1999)

[d]Method of Mantel and Haenszel (Fleiss, 1981:174)

[e]Cornfield-Gart method (Fleiss, 1979)

with BMIs less than 21.3 and with low ferritin levels regardless of age group. Women with BMIs of 21.3 and higher and ferritin levels below 30 ng/ml had no increased risk for NTDs regardless of age category compared with women with similar BMIs and ferritin levels of 30 ng/ml or higher (Table 25).

Because ferritin is the cell's storage form of iron, low ferritin levels can result if inadequate iron is absorbed. Poor iron absorption may result from pica. Seven of 148 case-women and 1 of 158 control-women who engaged in pica during the perinatal period had 7.8 times the risk of having an NTD baby as women without pica (Table 26). This association narrowly missed statistical significance at the 0.05 level. The mean serum ferritin values for case-women and control-women with pica were 22.2 and 34.0 respectively. The overall mean serum ferritin values for case- and control-women were 35.8 and 42.2 respectively. Because pica is caused by an innate need to obtain minerals absent from the diet, iron supplementation may decrease the incidence of women exhibiting pica.

Table 25

Association Between Ferritin and NTDs by Maternal BMI (BMI ≥ 21.3) and Maternal Age

Maternal Age[a]	Ferritin[a] (ng/ml)	Cases N (%)	Controls N (%)	Stratum-Specific Odds Ratio	95% CI[b]	Mantel-Haenszel Odds Ratio[c]
< 24	< 30	30 (66.7)	25 (61.0)	1.28	0.48 – 3.40	1.13 (0.62 – 2.05)[d]
	≥ 30	15 (33.3)	16 (39.0)			
≥ 24	< 30	19 (42.2)	21 (42.0)	1.01	0.41 – 2.47	
	≥ 30	26 (57.8)	29 (58.0)			

[a]Based on the comparison group distribution (50th percentile)

[b]Cornfield's approximation (Fleiss, 1979)

[c]Method of Mantel and Haenszel (Fleiss, 1981:174)

[d]Cornfield-Gart method (Fleiss, 1979)

Table 26

Association Between Pica and NTDs

Pica	Cases N (%)	Controls N (%)	Odds Ratio	95% CI[b]
Yes	7 (4.7)	1 (0.6)	7.8	0.98 – 353.47
No	141 (95.3)	157 (99.4)		

[a]Based on the comparison group distribution (50th percentile)

[b]Fisher's Exact confidence interval (Abramson and Gahlinger, 1999)

Logistic Regression Models

Table 27 lists factors of three logistic regression models with coefficients, standard errors of the beta coefficients, odds ratios v intervals, the Wald statistics, and the p-values. The first model c ferritin, divided into a dichotomy at the 50th percentile (based on distribution). The beta coefficient for ferritin is 0.5033 with a sta The odds ratio for ferritin and its association with NTDs is 1.654 limits of 0.9915 and 2.7599. Table 19 shows a similar odds ratio quartile and the highest quartile of ferritin of 1.700. The model h 3.7146, which barely misses significance at the 0.05 level. The p correct for the model is 56.25%.

The second model includes ferritin divided at the 50th percen into quartiles, with the lowest quartile that includes women most section of the dichotomy and the remaining BMI quartiles in the s dichotomy. The ferritin statistics in this model are similar to the The BMI coefficient is – 0.0137 with a Wald statistic of 0.0019.

interaction between ferritin and BMI results in increased risk for women with low BMI and low ferritin levels. The Wald statistic of 5.7322 is significant at $p < 0.05$ and a 95% confidence interval of 1.3433 and 19.2812. Even though the prediction percent correct for the model is 56.17%, model 3 is the best model because it accounts for the significant interaction between BMI and low ferritin in relation to risk for NTD offspring.

Table 27

Multivariate Analysis of Neural Tube Defect Cases and Controls

Model #	Predictor Variable	Beta	S.E. (Beta)	Odds Ratio	95% Confidence Interval	Wald Statistic	p-value
1	Ferritin level[a]	.5033	.2612	1.6542	.9915, 2.7599	3.7146	> .05
2	Ferritin level[a]	.5006	.2640	1.6497	.9834, 2.7675	3.5970	> .05
	BMI level[b]	-.0137	.3128	.9864	.5343, 1.8210	.0019	> .05
3	Ferritin level[a]	.1563	.2979	1.1691	.6520, 2.0963	.2751	> .05
	BMI level[b]	-.9484	.5216	0.3874	.1394, 1.0768	3.3053	> .05
	Interaction between ferritin level[a] * BMI level[b]	1.6271	.6796	5.0892	1.3433, 19.2812	5.7322	< .05

[a]in halves, lower half of ferritin ≤ 30 ng/ml

[b]lowest quartile of BMI and 3 upper quartiles (lowest quartile of BMI < 21.3)

CHAPTER VI

DISCUSSION AND CONCLUSIONS

Strengths and Limitations

The investigators used a case-control study design for its strength in studying relatively rare outcomes such as NTDs. Yet, the study has limitations. The source of cases and controls for this study is hospitals and birthing centers in the fourteen Texas counties bordering Mexico. NTD cases occurring in this region on or after January 1, 1995 were invited to participate. The enrollment procedure suggested that two control-women would be chosen for each NTD case. However, control-mothers were more difficult to recruit than case-mothers, resulting in less than two controls for each case-woman. The participation rate for control-mothers was 54% compared to 70% for case-mothers. The data used for this thesis included 148 cases and 158 controls, with only 6.33% more controls than cases. The limited study area and the difficulty recruiting control-mothers has limited the study's power and may have caused some analyses to fail to reach significance.

Case- and control-mothers were invited to enter the study at the time of birth, miscarriage, or pregnancy termination. The ascertainment of exposures was limited by the accuracy of what the mothers remembered about their periconceptional period, three months before conception to three months after conception. Another study limitation

involved temporal sequence. Blood was drawn for laboratory analyses at the end of pregnancy. Consequently, laboratory values such as ferritin, hemoglobin, and hematocrit were not necessarily the same at the end of pregnancy as they were during organogenesis. However, this should have biased us toward the null. During pregnancy, women's dietary iron needs increase. Blood values of case-women who often had births less than full-term were compared to blood values of control-women who usually carried their babies full-term. Therefore, case-women should have had higher iron stores than control-women because of shorter gestations. However, a higher proportion of case-women had lower ferritin levels than control-women.

Findings and Relation to Previous Work

This study of the relationship between iron and NTDs will add further information to the issue that began when Golding (1982) noticed that mothers of anencephalic babies more frequently drank tea and had lower hemoglobin levels than mothers giving birth to babies unaffected by NTDs. A TDH study of a cluster of anencephalic births in Cameron County, Texas in 1990-1991 concluded that hematocrit values for women having babies with anencephaly (and giving birth to babies with any NTD) were significantly lower ($p < .01$) than those for control-women. This study failed to find any increased risk of NTDs associated with hemoglobin or hematocrit. However, this study did find a significant difference between the percentages of below normal values for MCH and MCV between case- and control-women

Increased risk for NTDs was found associated with dietary iron intake and maternal ferritin levels. Low dietary iron and low serum ferritin increased the risk for NTDs 1.7

times and two times respectively for mothers less than 24 years of age. Low serum ferritin increased the risk for NTDs approximately six times for mothers with BMIs less than 21.3. Age did not modify this risk significantly. Mothers with BMIs of 21.3 or more did not have increased risk for NTDs due to low ferritin levels regardless of age category.

The association between orange juice consumption and NTDs was significant when modified by maternal age. Mothers 24 years of age and older had 0.42 the risk of NTDs when they consumed less than five servings of orange juice per month. Since orange juice increases the absorption of iron from nonheme sources, one would expect an increased risk of NTDs associated with low orange juice consumption. However, many other food sources of iron along with other factors that affect absorption of iron from the diet may have affected NTD risk.

One interesting finding of increased risk for NTDs was associated with pica. Mothers who engaged in pica had nearly eight times the risk of NTDs compared to mothers without pica. Significance for this finding was narrowly missed, possibly due to a cell value of one because only one control-mother exhibited pica, but the size of the odds ratio must not be ignored. Additional evidence of the association between iron, NTDs, and pica was that the mean serum ferritin values for case-women and control-women with pica were lower than the mean ferritin values for all case- and control-women. Further research directed at the mechanism by which pica increases risk for NTDs may be fruitful.

This case-control study of NTDs has found evidence for increased risk for NTDs associated with low dietary iron and low serum ferritin levels among women less than 24

years of age, and/or with a BMI less than 21.3. While the association between low serum ferritin and NTDs is modified by both BMI and age, BMI is the more important modifier of NTD risk. Women with BMIs less than 21.3 are small women with low weight for height. Their body size is evidence for eating less food with less opportunity to store iron in their bodies than heavier women. Younger age also implies fewer years over which to increase body stores of iron. We didn't evaluate all nutrients, so women with BMIs less than 21.3 and/or who were less than 24 years of age may have been deficient in several nutrients.

Conclusion

In conclusion, this study adds important information about the association between iron and NTDs among women with low BMI (< 21.3) and to a lesser extent, women of young age (< 24 years). An association between pica and NTDs was also found. Iron supplementation taken by women of childbearing age may be a valuable addition to folic acid supplementation in the prevention of neural tube defects among women for whom folic acid supplementation alone is not effective.

Appendix A

TEXAS DEPARTMENT OF HEALTH
1100 West 49th Street
Austin, TX 78756

DEPARTMENT APPROVAL OF PROPOSED RESEARCH

Texas Neural Tube Defect Project
IRB #940008-99
Renewal

This proposal has been reviewed and approved by the IRB - Texas I
Institutional Review Committee for Protection of Human Subjects in Re:
Clinical Investigations, Demonstrations or Other Activities/Projects,
DHHS/PHS-NRA, Public Law 93-348, as enunciated through Code of
45 CFR 46, Protection of Human Subjects, and Revised as of March 18,

Approved Signature: TDH-IRB Chairperson or Acting Chairperson

Committee Member Signature

Committee Member Signature

Appendix B

Association Between Lowest Body Mass Index (BMI) Quartile and Summation of Highest Three Quartiles with NTDs

BMI[a]	Cases		Controls		Odds Ratio	95% CI[b]
	N	(%)	N	(%)		
< 21.3	32	(22.2)	39	(24.8)	0.86	0.49 – 1.53
≥ 21.3	112	(77.8)	118	(75.2)		

[a]Based on the comparison group distribution (lowest quartile, sum of other 3 quartiles)

[b]Cornfield approximation (Fleiss, 1979)

Appendix C

Association Between Body Mass Index (BMI) in Quartiles and NTDs

BMI[a]	Cases		Controls		Odds Ratios	95% CI[b]
	N	(%)	N	(%)		
14.3 – 21.3	32	(22.2)	39	(24.8)	1.000	
21.3 – 23.6	24	(16.7)	39	(24.8)	0.750	0.35 – 1.58
23.6 – 27.4	33	(22.9)	41	(26.2)	0.981	0.48 – 1.99
27.4 – 52.4	55	(38.2)	38	(24.2)	1.764	0.90 – 3.46

[a]Based on the comparison group distribution (quartiles)

[b]Cornfield approximation (Fleiss, 1979)

Appendix D

Association Between Maternal Age and NTDs

Maternal Age[a] (yrs)	Cases N	(%)	Controls N	(%)	Odds Ratio	95% CI[b]
< 24	81	(54.7)	79	(50.0)	1.21	0.75 – 1.94
≥ 24	67	(45.3)	79	(50.0)		

[a]Based on the comparison group distribution (50th percentile)

[b]Cornfield's approximation (Fleiss, 1979)

REFERENCES

Abramson, J.H. and Gahlinger, Paul M. (1999). Computer Programs for Epidemiologists (PEPI) version 3.00.

American Academy of Pediatrics. (1999). Folic acid for the prevention of neural tube defects. Pediatrics, 104, 325-327.

Bailey, Lynn B. and Gregory, Jesse F. III. (1999). Polymorphisms of methylenetetrahydrofolate reductase and other enzymes: Metabolic significance, risks and impact on folate requirement. J. Nutr.,129, 919-922.

Beard, John L. and Dawson, Harry D. (1997). Iron. In Boyd L. O'Dell, and Roger A. Sunde. (Eds.) Handbook of nutritionally essential mineral elements (pp. 275-334). New York: Marcel Dekker, Inc.

Becerra, Jose E., Khoury, Muin J., Cordero, Jose F., and Erickson, J. David (1990). Diabetes mellitus during pregnancy and the risks for specific birth defects: A population-based case-control study. Pediatrics, 85, 1-9.

Bezkorovainy, Anatoly. (1989). Biochemistry of nonheme iron in man. I. Iron proteins and cellular iron metabolism. Clin. Physiol. Biochem., 7, 1-17.

Bjorn-Rasmussen, E., Hallberg, L., Isaksson, B., Arvidsson, B. (1974). Food iron absorption in man. Application of the two-pool extrinsic tag method to measure heme and non-heme iron absorption. J Clin. Invest., 53:247-256.

Brender, Jean D., Carmichael, Leland, Preece, Mary Jo, Larimer, Gail C., and Suarez, Lucina. (1989). Epidemiology of anencephaly in Texas, 1981-1986. Tex. Med., 85: 33-35.

Brender, Jean D. and Suarez, Lucina. (1990). Paternal occupation and anencephaly. Am. J. Epidemiol., 131, 517-521.

Bridges, Kenneth R. (1990). Iron imbalance during pregnancy. In Bern, Murray M. and Frigolitto, Fredric D. (Eds.), Hematologic disorders in maternal-fetal medicine (pp. 113-128). New York: John Wiley & Sons.

Brown, Barbara A. (1993). Hematology Principles and Procedures. (6th Ed.). Philadelphia: Lea & Febiger.

Canfield, M.A., Annegers, J.F., Brender, J.D., Cooper, S.P., and Greenberg, F. (1996). Hispanic origin and neural tube defects in Houston/Harris County, Texas. I. Descriptive epidemiology. Am J. Epidemiol., 143, 1-11.

Chen, Qing, Connor, James R., and Beard, John L. (1995). Brain iron, transferrin and ferritin concentrations are altered in developing iron-deficient rats. J. Nutr. 125, 1529-1535.

Cohen, Ranan D. and Braunstein, Nadine, S. (1996). Vitasearch Reference Guide to Vitamins and Minerals. Unpublished manuscript.

Congenital malformations worldwide - A report from the International Clearinghouse for Birth Defects Monitoring Systems. (1991). New York: Elsevier Science Publishers.

Connor, James R. and Menzies, Sharon L. (1995). Cellular management of iron in the brain. J. of the Neurol. Sci., 134 (Suppl.), 33-44.

Connor, James R. and Menzies, Sharon L. (1996). Relationship of iron to oligodendrocytes and myelination. Glia, 17, 83-93.

Connor, James R., Snyder, Brian S., Arosio, Paolo, Loeffler, David A., and LeWitt, Peter. (1995). A quantitative analysis of isoferrritins in select regions of aged, parkinsonian, and alzheimer's diseased brains. J. Neurochem. 65, 717-724.

Conrad, M.E., Umbreit, J.N., and Moore, E.G. (1999). Iron absorption and transport. Am. J. Med. Sci., 318, 213-229.

Copp, Andrew J. and Bernfield, Merton. (1994). Etiology and pathogenesis of human neural tube defects: Insights from mouse models. Current Opinion in Pediatrics, 6, 624-631.

Czeizel, Andrew E. and Dudas, Istvan. (1992). Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. N.Eng. J.Med., 327, 1832-1835.

Daly, Leslie E., Kirke, Peadar N., Molloy, Anne, Weir, Donald G., and Scott, John M. (1995). Folate levels and neural tube defects. JAMA, 274, 1698-1702.

Duyff, Roberta L. (1996). The American Dietetic Association's Complete Food & Nutrition Guide. Minneapolis: Chronimed Publishing.

Elwood, J. Mark, Little, Julian, and Elwood, J. Harold. (1992). Epidemiology and control of neural tube defects Monographs in epidemiology and biostatistics. New York: Oxford University Press.

Feuchtbaum, L.B., Currier, R.J., Riggle, S., Roberson, M., Lorey, F.W., and Cunningham, G.C. (1999). Neural tube defect prevalence in California (1990-1994): eliciting patterns by type of defect and maternal race/ethnicity. Genet. Test, 3, 265-272.

Fleiss, J.L. (1979). Confidence intervals for the odds ratio in case-control studies: state of the art. J. Chron. Diseases, 32, 69-77.

Fleiss, J.L. (1981). Statistical methods for rates and proportions. 2nd ed. New York: John Wiley & Sons.

Golden, Jeffrey A. and Chernoff, Gerald F. (1995). Multiple sites of anterior neural tube closure in humans: Evidence from anterior neural tube defects (Anencephaly). Pediatrics, 95, 506-510.

Golding, Jean. (1982). Evidence for an environmental aetiology for anencephalus. In T.V.N.Persaud (Ed.) Central nervous system and craniofacial malformations (pp. 19-45). New York: Alan R. Liss, Inc.

Hendricks, Katherine A., Simpson, J. Scott, and Larsen, Russell D. (1999). Neural tube defects along the Texas-Mexico border, 1993-1995. Am. J. Epidemiol., 149, 1119-1127.

Hoyle, Christine, Henderson, Deborah J., Matthews, David J., and Copp, Andrew J. (1996). Transferrin and its receptor in the development of genetically determined neural tube defects in the mouse embryo. Develop. Dynamics 207, 35-46.

Khattak, S., K-Moghtader, G., McMartin, K., Barrera, M., Kennedy, D., and Koren, G. (1999). Pregnancy outcome following gestational exposure to organic solvents, A prospective controlled study. JAMA, 281, 1106-1109.

Kirke, P.N., Molloy, A.M., Daly, L.E., Burke, H., Weir, D.G. and Scott, J.M. (1993).
Maternal plasma folate and vitamin B_{12} are independent risk factors for neural tube defects. Quarterly Journal of Medicine, 86, 703-708.

Lemire, Ronald J. (1988). Neural tube defects. JAMA, 259, 558-562.

Levi, Sonia, Yewdall, Stephen J., Harrrison, Pauline M., Santambrogio, Paolo, Cozzi, Anna, Rovida, Ermanna, Albertini, Alberto, and Arosio, Paolo, (1992). Evidence that H- and L-chains have co-operative roles in the iron-uptake mechanism of human ferritin. Biochem J. 288, 591-596.

Lynberg, Michele C., Khoury, Muin J., Lu, Xinpei, and Cocian, Teofil. (1994). Maternal flu, fever, and the risk of neural tube defects: A population-based case-control study. Am. J. Epidemiol., 140, 244-255.

Mills, James L., McPartlin, Joseph M., Kirke, Peadar. N., Lee, Young J., Conley, Mary R., Weir, Donald G., and Scott, John M. (1995). Homocysteine metabolism in pregnancies complicated by neural-tube defects. Lancet, 345, 149-151.

Milunsky, Aubrey; Jick, Hershel; Jick, Susan; Bruell, Carol; MacLaughlin, Dean; Rothman, Kenneth; and Willett, Walter. (1989). Multivitamin/folic acid supplementation in early pregnancy reduces the prevalence of neural tube defects. JAMA, 262, 2847-2852.

Milunsky, Aubrey, Ulcickas, Marianne, Rothman, Kenneth J., Willett, Walter, Jick, Susan S., and Jick, Hershel. (1992). Maternal heat exposure and neural tube defects. JAMA,268, 882-885.

Moore, Keith L. and Persaud, T.V.N. (1993). The developing human, Clinically oriented embryology 5th edition. Philadelphia: W.B. Saunders Company.

Moos, Torben, Oates, Phillip S., and Morgan, Evan H. (1998). Expression of the neuronal transferrin receptor is age dependent and susceptible to iron deficiency. J. Comp. Neurol.398, 420-430.

Morris, Christopher M., Candy, John M., Keith, Alexander B., Oakley, Arthur E., Taylor, Geoffrey A., Pullen, Robert G.L., Bloxham, Clive. A., Gocht, Andreas, and Edwardson, James A. (1992). Brain iron homeostasis. J. of Inorg. Biochem., 47, 257-265.

MRC Vitamin Study Research Group (1991). Prevention of neural tube defects: Results of the Medical Research Council Vitamin Study. Lancet, 338, 131-137.

Myrianthopoulos, Ntinos C., and Melnick, Michael. (1987). Studies in neural tube defects: Epidemiologic and etiologic aspects. Amer. J. Med. Genetics, 26, 783-796.

National Academy of Sciences. (1989). Recommended Dietary Allowances. 10th Ed. National Academy Press: Washington, D.C.

Neural tube defects, Ciba Foundation Symposium 181. (1994). New York: John Wiley & Sons.

Ozawa, Hiroshi, Nishida, Akira, Mito, Takashi, and Takashima, Sachio. (1994a). Development of ferritin-containing cells in the pons and cerebellum of the human brain. Brain & Development 16, 92-95.

Ozawa, Hiroshi, Nishida, Akira, Mito, Takashi, and Takashima, Sachio. (1994b). Development of ferritin-postitive cells in cerebrum of human brain. Pediatr. Neurol. 10, 44-48.

Ozawa, Hiroshi and Takashima, Sachio. (1998). Immunocytochemical development of transferrin and ferritin immunoreactivity in the human pons and cerebellum. J. Child Neurol. 13, 59-63.

Ponka, Prem, Beaumont,Carole, and Richardson, Des R. (1998). Function and regulation of transferrin and ferritin. Seminars in Hematology 35, 35-54.

Qian, Zhong Ming and Wang, Qin. (1998). Expression of iron transport proteins and excessive iron accumulation in the brain in neurodegenerative disorders. Brain Res. Rev., 27, 257-267.

Rosa, F.W. (1991). Spina bifida in infants of women treated with carbamazepine during pregnancy. N. Eng. J. Med., 324, 674-677.

Roskams, A. Jane and Connor, James R. (1992). Transferrin receptor expression in myelin deficient *(md)* rats. J. Neuroscience Research 31, 421-427.

Roskams, A. Jane I. and Connor, James R. (1994). Iron, transferrin, and ferritin in the rat brain during development and aging. J. Neurochem. 63, 709-716.

Sadler, T.W. (1995). Langman's medical embryology 7th edition. Baltimore, MD:Williams & Wilkins.

Seller, Mary J. (1987). Neural tube defects and sex ratios. Am. J. of Med. Genetics, 26, 699-707.

Selvin, S. (1996). Statistical Analysis of Epidemiologic Data, 2nd edition. New York: Oxford University Press.

Sever, Lowell E. (1995). Looking for causes of neural tube defects: Where does the environment fit in? Environ Health Perspect., 103(Suppl 6), 165-171.

Shaw, Gary M., Velie, Ellen M., and Schaffer, Donna. (1996). Risk of neural tube defect-affected pregnancies among obese women. JAMA, 275, 1093-1096.

Smithells, R.W., Sheppard, S., Schorah, C.J., Seller, M.J., Nevin, N.C., Harris, R., Read, A.P., and Fielding, D.W. (1981). Apparent prevention of neural tube defects by periconceptional vitamin supplementation. Arch. Dis. Child, 56, 911-918.

TDH report, An investigation of a cluster of neural tube defects in Cameron County, Texas, July 1, 1992.

van Eijk, H.G., and de Jong, G. (1992). The physiology of iron, transferrin, and ferritin. Biological Trace Element Research, 35, 13-24.

Waller, Kim D., Mills, James L., Simpson, Joe L., Cunningham, George C., Conley, Mary R., Lassman, Melinda R., and Rhoads, George G. (1994). Are obese women at higher risk for producing malformed offspring? Am J Obstet Gynecol, 170, 541-548.

Werler, Martha M., Louik, Carol, Shapiro, Samuel, and Mitchell, Allen A. (1996). Prepregnant weight in relation to risk of neural tube defects. JAMA, 275, 1089-1092.

Werler, Martha M., Shapiro, Samuel, and Mitchell, Allen A. (1993). Periconceptional folic acid exposure and risk of occurrent neural tube defects. JAMA, 269, 1257-1261.

Zhao, Zhiyong, Weiss, Kenneth, and Stock, David W. (1998). Growth Factors and Development. In Ulijaszek, Stanley J., Johnston, Francis E., and Preece, Michael A. (Eds.), The Cambridge encyclopedia of human growth and development (pp. 154-156). Cambridge, UK: Cambridge University Press.

TABLE REFERENCES

[a]Yoshida, A., Shibuya, A., Dave, V., Nakayama, M., and Hayashi, A. (1990). Developmental changes of aldehyde dehydrogenase isozymes in human livers: mitochondrial ALDH2 isozyme is expressed in fetal livers. Experientia. [Abstract]. 46, 747.

[b]Cruz, C., Charli, J.L., Vargas, M.A., and Joseph-Bravo, P. (1991). Neuronal localization of pyroglutamate aminopeptidase II in primary cultures of fetal mouse brain. J.Neurochem. [Abstract]. 56, 1594.

[c]Rodriguez, A., Sarda, P., Nessmann, C., Boulot, P., Leger, C.L., and Descomps, B. (1998). Delta6- and delta5-desaturase activities in the human fetal liver: kinetic aspects. J. Lipid Res. [Abstract]. 39, 1925.

[d]Slayton, W.B., Juul, S.E., Calhoun, D.A., Li, Y., Braylan, R.C., and Christensen, R.D. (1998). Hematopoiesis in the liver and marrow of human fetuses at 5 to 16 weeks postconception: quantitative assessment of macrophage and neutrophil populations. Pediatr. Res. [Abstract]. 43, 774.

[e]Coufalik, A. and Monder, C. (1978). Perinatal development of the tyrosine oxidizing system. Biol. Neonate. [Abstract]. 34, 161.

[f]Takashima, S., Kuruta, H., Mito, T., Houdou, S., Konomi, H., Yao, R., and Onodera, K. (1990). Immunohistochemistry of superoxide dismutase-1 in developing human brain. Brain Dev. [Abstract]. 12, 211.

[g]Saksela, M., Lapatto, R., and Raivio, K.O. (1998). Xanthine oxidoreductase gene expression and enzyme activity in developing human tissues. Biol. Neonate [Abstract]. 74, 274.

[h]Katz, D.M. (1991). A catecholaminergic sensory neuron phenotype in cranial derivatives of the neural crest: regulation by cell aggregation and nerve growth factor. J. Neurosci. [Abstract]. 11, 3991.

[i]Morton, R.L., Ikle, D., and White, C.W. (1998). Loss of lung mitochondrial aconitase activity due to hyperoxia in bronchopulmonary dysplasia in primates. Am. J. Physiol. [Abstract]. 274, L127.

[j]Norton, J.L., Adamson, S.L., Bocking, A.D., and Han, V.K. (1996). Prostaglandin-H synthase-1 (PGHS-1) gene is expressed in specific neurons of the brain of the late gestation ovine fetus. Brain Res.Dev. [Abstract]. 95, 79.

[k]Michel, F.J., Fliss, M.F., Bazer, F.W., and Simmen, R.C. (1992). Characterization and developmental expression of binding sites for the transplacental iron transport protein, uteroferrin, in fetal hematopoietic tissues. Biol. Neonate. [Abstract]. 61, 82.

[l]Puistola, U. and Anttinen, H. (1982). Evidence for a relative excess of lysyl hydroxylase in chick embryo tendon and cartilage compared with bone and skin. Biochim. Biophys. Acta. [Abstract]. 717, 118.

[m]Kalcheim, C. and Leviel, V. (1988). Stimulation of collagen production in vitro by ascorbic acid released from explants of migrating avian neural crest. Cell Differ. [Abstract]. 22, 107.

[n]Mover, H. and Ar, A. (1997). Antioxidant enzymatic activity in embryos and placenta of rats chronically exposed to hypoxia and hyperoxia. Comp. Biochem. Physiol. C. Pharmacol. Toxicol. Endocrinol. [Abstract]. 117, 151.

[o]Miki, A., Mizoguchi, A., and Mizoguti, H. 1988. Histochemical studies of enzymes of the energy metabolism in postimplantation rat embryos. Histochemistry. [Abstract]. 88, 489.

[p]Schaeren-Wiemers, N., Schaefer, C., Valenzuela, D.M., Yancopoulos, G.D., and Schwab, M.E. (1995). Identification of new oligodendrocytes- and myelin-specific genes by a differential screening approach. J. Neurochem. [Abstract]. 65, 10.

[q]Sherratt, A.J., Banet, D.E., and Prough, R.A. (1990). Glucocorticoid regulation of polycyclic aromatic hydrocarbon induction of cytochrome P450IA1, g;itatjopme S-transferases, and NAD(P)H: quinone oxidoreductase in cultured fetal rat hepatocytes. Mol. Pharmacol. [Abstract]. 37, 198.

[r]Santacana, M., Uttenthal, L.O., Bentura, M.L., Fernandez, A.P., Serrano, J., Martinez de Velasco, J., Alonso, D., Martinez-Murillo, R., and Rodrigo, J. (1998). Expression of neuronal nitric oxide synthase during embryonic development of the rat cerebral cortex. Brain Res. Dev. Brain Res. [Abstract]. 111, 205.

[s]Zimmerberg, J., Greengard, O., and Knox, W.E. (1975). Peptidyl proline hydroxylase in adult, developing, and neoplastic rat tissues. Cancer Res. [Abstract]. 35, 1009.

[t]Yeoh, G.C., Edkins, E., Mackenzie, K., Fuller, S., Mercer, J.F., Dahl, H.H. (1988). The development of phenylalanine hydroxylase in rat liver; in vivo, and in vitro studies utilizing fetal hepatocyte cultures. Differentiation. [Abstract]. 38, 42.

[u]Ventura, S.M. and Dachtler, S.L. (1980). Development and maturation of aldehyde oxidase levels in fetal hepatic tissue of C57BL/6J mice. Enzyme. [Abstract]. 25, 213.

[v]Wright, J.W., Schwinof, K.M., Snyder, M.A., and Copenhaver, P.F. (1998). A delayed role for nitric oxide-sensitive guanylate cyclases in a migratory population of embryonic neurons. Dev. Biol. [Abstract]. 204, 15.

VITA

Barbara D. Scaife was born in Rutland, Vermont on May 7, 1951, the daughter of George Dutton and Dorothy Dutton. After attending Otter Valley Union High School in Brandon, Vermont, she attended the University of Vermont in Burlington, Vermont where she graduated in 1973 with a Bachelor of Science degree in medical technology. She worked as a medical technologist at the Medical Center Hospital of Vermont in Burlington and as a research technician in the biochemistry department at the University of Vermont. Barbara moved to Beverly, Massachusetts, where she worked as a medical technologist at Salem Hospital in Salem, Massachusetts. She received her Master of Science degree in Foods and Nutrition in 1980 from Framingham State College, Framingham, Massachusetts. In 1980, she passed the registry exam for the Registered Dietitian credential. After about a decade as a "stay at home Mom", she worked in Austin as a research dietitian for the University of Texas Health Science Center – Houston from 1991 – 1997. In January 1998, she entered the Graduate School of Southwest Texas State University, San Marcos, TX.

Permanent address: 9514 San Diego Rd.
Austin, Texas 78737

This thesis was typed by Barbara D. Scaife