THE EFFECTS OF ZINC INTAKE AND EXERCISE ON IRON STATUS OF PREGNANT SPRAGUE-DAWLEY RATS

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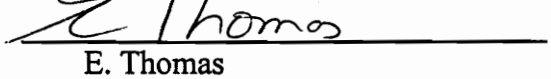

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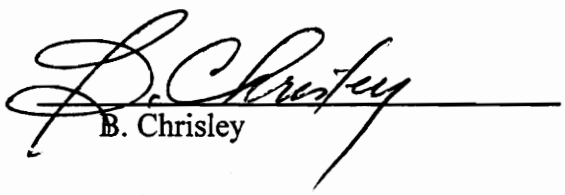

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(ABSTRACT)

The effects of marginal zinc intake and exercise on the iron status of Sprague-Dawley rats during pregnancy were observed. The dams were assigned to one of four groups: zinc marginal (6.18 ppm zinc sulfate), exercise or sedentary; zinc adequate (37.63 ppm), exercise or sedentary. The dams swam everyday for one hour until day 18. Data was collected on days 15, 18, and 21. Anorexia occurred by day 21 in the zinc marginal groups, significantly reducing dam body and organ weights and may be attributed to maternal tissue mobilization in response to increased fetal zinc demands late in gestation. Fetal and placental weights were unaffected. Hemoglobin and hematocrit decreased throughout gestation in the adequate group, and until day 18 in the marginal group, increasing significantly by day 21. Serum iron, TIBC, and maternal liver iron decreased throughout pregnancy although liver and spleen iron stores were higher in the zinc marginal dams. Fetal iron increased significantly independent of treatment. Increased iron status in the zinc marginal dams may be due to enhanced iron uptake when zinc nutriture is low. Overall decreases in serum iron, TIBC, and liver iron reflect the increasing demands of iron from the fetus. Heart weight was significantly higher in the swimmers. The sedentary group weighed more than the exercise group, their hematocrit increased by day 21, and spleen iron stores were significantly

higher. No change in hematocrit was found in the exercise dams and their TIBC was greater, revealing an increase demand for iron during exercise. It was concluded that a zinc marginal intake, its resulting anorexia, and the onset of strenuous exercise at conception, affected maternal iron status and weight without harming the fetus.

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I dedicate this work to the memory of my father, Thomas J. Waters, who would have been extremely happy to see the finished work and to the future of my children, Matthew and Meredith.

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I. INTRODUCTION

The benefits of exercise during pregnancy have received an increasing amount of attention over the past two decades as women continue to exercise after conception or view pregnancy as a time to start practicing healthy behaviors. Some of the benefits of exercise include maintenance or improvement of maternal fitness; control of excess weight gain; improved self-image; improved posture; increased energy; improved sleep; less problems with varicose veins; less water retention; decreased labor complications, and increased recovery (Paisley and Mellion, 1988). Pregnancy is in itself a hypermetabolic state where growth and development is rapid in both the mother and fetus.

Cardiorespiratory adaptations like increased oxygen consumption, cardiac output, and plasma volume, develop early in pregnancy preparing the mother for the work demands of the growing fetus (Bell et al, 1994). Maternal nutrient requirements increase to meet the demands of the developing fetus.

Iron and zinc are trace elements that play vital roles in assuring optimal pregnancy outcome. Iron demands increase to compensate for an increased red blood cell mass, growth in fetus and placenta, maternal basal losses, and blood loss during partuition (Schwartz and Thurnau, 1995; Roodenburg, 1995). Iron requirements increase throughout pregnancy, especially in the last trimester, where up to 5-6 mg of iron per day is required to meet fetal

needs (Schwartz and Thurnau, 1995). Adequate iron nutriture is a major concern during pregnancy due to the increased prevalence of iron deficiency anemia (IDA) in women of child-bearing years (Dallman et al, 1984; Expert Scientific Working Group, 1985). Deleterious effects of iron deficiency anemia include decreased birthweight, increased preterm labor, low maternal weight gain, and is evidenced by decreased maternal hemoglobin, hematocrit, and serum ferritin (Scholl and Hediger, 1994; Schwartz and Thurman, 1995).

It is important to distinguish iron deficiency anemia from the "physiologic anemia" which occurs in pregnancy. This hemodilution effect is the result of increasing plasma volume which exceeds red blood cell mass expansion (Scholl and Hediger, 1994; Roodenberg, 1995; Schwartz and Thurnau, 1995). To prevent the deleterious effects of IDA, diagnosis early in pregnancy is essential, since decreased birthweight and preterm delivery are associated with IDA during the first trimester (Scholl and Hediger, 1994; Schwartz and Thurnau, 1995). IDA is defined by a hemoglobin <11.0 g/dl and a hematocrit <33% in the first and third trimesters; hemoglobin <10.5 g/dl and hematocrit <32% in the second trimester; and a serum ferritn <12 μg/l (Institute of Medicine, 1990).

Zinc is necessary for organogenesis early in pregnancy (Breskin et al, 1983; Argemi et al, 1988; Oteiza et al, 1990); as a component in fetal growth and tissue synthesis (Simmer and Thompson, 1985); and for prostaglandin function as it relates to partuition and blood pressure (Hunt et al, 1984; Swanson and King, 1987). Like iron, zinc demands increase throughout pregnancy and are greatest in the last trimester (Swanson and King, 1987).

Although severe zinc deficiency affects both fetal and maternal outcome adversely, marginal zinc deficiency appears to be most common in human populations (Polberger et al, 1996). Marginal zinc deficiency throughout gestation mirrors that of severe deficiency in that there is an increased incidence of growth retardation, skeletal anomalies, delivery complications, and impaired immune function, but, unlike severe deficiency, rare congenital malformations are seen (Swanson and King, 1987).

Zinc and iron have an antagonistic effect on each other during absorption in the small intestines. This effect is related to the amount of each metal present in the diet, their form, and the body's demand for each. In studies of mineral supplementation, it was found that iron supplementation decreased zinc status (Bloxam et al, 1989) and zinc supplementation decreased iron status (Yadrick et al, 1989). This relationship applied primarily to the interaction of nonheme iron and inorganic zinc supplements and was not found when the two minerals were consumed in their organic form in food with a meal (Valberg et al, 1984; Storey and Greger, 1987; Davidsson et al, 1995). Concern rises during pregnancy when iron supplementation is common and zinc status is marginal.

Studies examining the effects of marginal zinc nutriture on iron status in pregnant subjects found that iron stores were preserved, and at times, enhanced throughout gestation in rats (Rogers et al, 1985; Reinstein et al, 1984) but were unaffected to slightly decreased in Rhesus monkeys (Golub et al, 1984). Fetal iron concentrations were higher in the zinc marginal rats but plasma iron was reduced in marginal zinc neonate monkeys. Clinical manifestations of marginal zinc deficiency appeared midpregnancy for all studies and were

associated with decreased weight and anorexia.

Iron is directly involved in exercise outcome by its function in hemoglobin and myoglobin to deliver oxygen to tissues (Hultman et al, 1988) and therefore iron demand during exercise is great. Like pregnancy, exercise is associated with an increase in oxygen consumption and cardiac output. A physiologic anemia, termed "sports anemia' is found in highly trained individuals and reflects a hemodilution effect involving plasma volume and red blood cell mass (Oscai et al, 1968; Convertino et al, 1980; Magnusson et al, 1984; Peterson, 1997) similiar to that of pregnancy. Again differentiation between sports anemia and IDA will ensure maximum exercise performance. Deleterious effects of exercise during pregnancy include decreased fetomaternal blood flow; ineffective thermoregulation; and decreased birthweight and appear to be related to weight-bearing versus nonweight-bearing exercise; the intensity of the exercise and its effect on maternal heart rate; prepregnancy conditioning of the mother; and whether intense exercise continues through the third trimester (Bell et al, 1994).

The purpose of this study was to examine the effects of initiation of zinc marginal intake and strenuous exercise at conception on iron status during pregnancy.

II. REVIEW OF LITERATURE

IRON STATUS AND METABOLISM

Iron Composition in the Body

Iron is a trace mineral that plays an important role in many biochemical processes. Total body iron in a normal adult ranges from approximately 3-5 grams (Finch and Huebers, 1982; Skikne, 1988) and can be classified as either essential (functional) or nonessential (storage) iron. Essential iron comprises approximately 70% of the total body iron and is found in cells and tissue enzymes as hemoglobin, myoglobin, heme enzymes, cofactors and transport proteins (Fairbanks and Beutler, 1988). The remaining 30% of total body iron is present as nonessential iron and is stored primarily in the liver, spleen and bone marrow as ferritin or hemosiderin. Body iron content is dependent on weight, hemoglobin concentration, sex, and storage compartment size. Approximate distributions of iron in a normal adult are depicted in Table 1.

Functional Forms of Iron

The functional or essential forms of iron are distributed in the body as follows: hemoglobin constitutes 85%, myoglobin 5%, heme enzymes or cofactors 10%, and <0.1% as iron bound to transferrin (Fairbanks and Beutler, 1988). Hemoglobin, the major component of red blood cells, is a protein that is responsible for the transport of O_2 and CO_2 to tissues and maintaining normal physiologic pH (Baldy, 1986). It is composed of four

heme groups which consist of a porphyrin ring system of four pyrrole units. These units are

Table 1. Distribution of iron throughout the body.

	Total (%)	Men (mg)	Women (mg)
Hemoglobin	60 - 70	2100	1750
Ferritin and Hemosiderin	15 - 30	1000	400
Tissue Enzymes	5 -15	350	300
Myoglobin	3 - 4	100	100
Transferrin	<1	4	4
Serum Ferritin	<1	0.3	0.1
Total		3554.3	2554.1

(Cook et al, 1990)

bound to a molecule of reduced ferrous iron which is also bound to four globin chains (Bohinski, 1987). Gas exchange occurs when the protein becomes oxygenated and O_2 binds to iron. Hemoglobin is incorporated into the red blood cell during erythropoiesis in the bone marrow (Cook et al 1990). In humans, the life span of a red blood cell is approximately 120 days. In the rat model, red blood cell turn-over is approximately 60 days (Dallman et al, 1982).

Myoglobin closely resembles the structure of hemoglobin except that it exists in a monomeric form (Fairbanks, 1982). It is present in striated skeletal and cardiac muscles and functions as a oxygen-binding protein which releases O_2 only at low oxygen tension.

Intracellular heme enzymes include the cytochromes, cytochrome oxidase, peroxidases, and catalase (Fairbanks and Beutler, 1988). The cytochromes are coenzymes present in the

mitochondrial membrane and endoplasmic reticulum of all cells and have iron-containing heme groups attached to a polypeptide (Bohinski, 1987). The cytochromes and cytochrome oxidase are involved in oxidation-reduction reactions that occur along the electron transport chain of cellular respiration. Catalases and peroxidases act by detoxifying cells of powerful oxidizing agents like superoxide (O_2^-) found in hydrogen peroxide (H_2O_2).

Iron is present in flavo-proteins like NADH dehydrogenase and succinic dehydrogenase and in iron-sulfur proteins that are involved in the transport of electrons throughout the respiratory chain (Woo et al, 1979). It can also be found as a cofactor in the conversion of beta carotene to vitamin A, collagen synthesis, production of purines, lipid removal from the blood, drug detoxification in the liver, antibody production, and carnitine synthesis (Cook et al, 1990).

Transferrin is the transport protein of plasma iron. It binds with iron ionically in the ferric state (Tietz, 1982) and transports it primarily to the bone marrow for erythropoiesis. Transferrin is synthesized in the liver and generally acquires iron from iron storage sites or from absorbed iron in the gastrointestinal tract (Skikne, 1988).

Storage Forms of Iron

Ferritin and hemosiderin are the major storage forms of iron and can be found primarily in the liver, spleen, and bone-marrow (Halberg, 1982; Finch and Huebers, 1982). Ferritin is a soluble metalloprotein that is capable of storing up to 4500 iron molecules (Finch and Huebers, 1982; Cook et al, 1990). Its relatively short life span of a few days provides a continuous pool of intracellular iron. Serum ferritin levels can be used as indicators of iron

stores. Hemosiderin consists of insoluble deposits of degraded protein and coalesced iron which are thought to be derived from ferritin (O'Connell et al, 1989) and are visible via histologic staining and examination of tissue. Hemosiderin's concentration of iron is approximately 50%, versus 20% found in ferritin, and the ratio of hemosiderin to ferritin varies depending upon the amount of iron stored in the body (Finch and Huebers, 1982). Studies have found however, that iron released from hemosiderin is less than that released from ferritin (O'Connell et al, 1989). In states where iron concentrations are low, ferritin stores predominate, while hemosiderin is present in greater quantities at higher iron concentrations (ie. iron overload).

Iron Balance : Losses and Absorption

Iron balance in the body is tightly controlled and maintained by the reutilization of iron from catabolized cells, the presence of iron stores, and the regulation of absorption depending on the body's daily iron requirements (Uchida, 1995). Iron atoms are recycled from plasma and extracellular fluid to the bone marrow where they are incorporated into hemoglobin during erythropoiesis (Fairbanks and Beutler, 1988). Mature red blood cells leave the bone marrow and circulate in the body for 120 days. As the red blood cells age, they loose their ability to maintain their potassium-sodium cation pump and the cations begin to leak out of the cells (Fairbanks, 1982). These senescent erythrocytes are taken up by the phagocytes of the reticuloendothelial system of the spleen where they are digested and iron is released into the plasma. Two thirds of this iron is transported via transferrin back to the bone marrow where it is again incorporated into erythropoiesis (Skikne, 1988).

The remaining iron goes into iron stores.

In addition to this internal recycling, iron balance is regulated via iron absorption and iron loss with iron loss playing a more fixed, limited role (Finch, 1994) . There are several sources of iron loss in the body including basal, menstrual, pregnancy demands and pathologic. Basal losses include desquamation of surface cells from the skin, gastrointestinal and urinary tracts, and gastrointestinal blood loss. Minimal losses are found in sweat (Jacob et al, 1981). Total basal losses in males are approximately 0.9 to 1.0 mg per day and 0.7 to 0.8 mg per day in females and are decreased during states of iron deficiency and increased during iron overload (Bothwell, 1995). Menstrual blood loss in females increases iron requirements to an average of 1.36 mg per day and these requirements are dependent on individual blood loss and the use of oral contraceptives versus intrauterine devices (Hallberg et al, 1995). Increased iron demands during pregnancy (approximately 840 mg) occur due to basal losses, increased red blood cell mass and fetal and placental growth (Roodenburg, 1995). Finally, pathologic losses, especially from parasitic infections like Necator americanus (Hookworm), can increase iron demands by 3 to 4 mg per day (Bothwell, 1995).

Since iron losses in the body are minimal in a normal individual, iron absorption is controlled to prevent the detrimental build-up of excess iron (Monsen, 1988). Absorption of iron appears to be the primary mechanism of iron balance and is regulated by the amount of iron stores present and the rate of erythropoiesis (Finch, 1994; Bothwell, 1995). Iron stores affect absorption inversely as evidenced in studies involving healthy, menustrating women (Hulten et al, 1995; Hallberg et al, 1995). There is increased absorption as iron

stores decrease, however, there is a critical point when iron stores become very low or depleted and the body is unable to absorb enough iron to compensate for losses. Iron deficiency anemia results. In the case of erythropoiesis, increased absorption occurs due to an increase demand for iron in the erythroid marrow and may be due to an increased rate of erythropoiesis, increased needs of an expanded red blood cell mass, or ineffective erythropoiesis. Of the two regulators, erythropoiesis has the greatest influence on the absorptive rate (Finch, 1994; Bothwell, 1995).

Maximum iron absorption occurs at the duodenum and the proximal part of the jejunum (Hartiti et al, 1994). The mucosal cells in the small intestines facilitate absorption by allowing uptake in the lumen based on nutrient needs. Any excess iron remaining is converted to ferritin and stored in the mucosal cell until the cell is exfoliated and excreted in the feces (Skikne, 1995). Generally more iron is taken up and stored in the mucosa as ferritin than is released to the circulation. Fecal ferritin concentration can be measured to determine the rate of absorption and is related to body iron stores. For example, in times of increased absorption (ie. low stores or increased erythropoiesis), fecal ferritin concentration is reduced. Interestingly, fecal ferritin is reduced in cases of genetic hemachromatosis which indicates that absorption control is shut off at the mucosa as reflected in abnormally high body stores.

Iron is absorbed as either heme or nonheme iron and each have their own pathways of uptake in the intestinal mucosa (Finch and Cook, 1984). Heme iron is absorbed as an intact iron porphyrin complex in the duodenum and jejunum of the small intestines (Monsen and

Balintfy, 1982; Skikne, 1988). Once in the intestinal lumen, the enzyme heme-oxygenase releases the iron from the heme complex (Fairbanks and Beutler, 1988). Heme iron is highly available and has a high bioavailability as compared to nonheme iron (Cook and Monsen, 1976; Hallberg et al, 1979; Hulten et al, 1995). Food sources are primarily meats (animal tissues). Heme content in meats vary somewhat; 30-40% of iron in pork, liver and fish compared to 50-60% in beef, lamb and chicken (Monsen et al, 1978).

Absorption of heme in meats has been found to be approximately 25% for 0.5 mg of heme iron (Halberg et al, 1979; Hallberg and Rossander, 1982). Heme absorption in humans does seem to be enhanced by the presence of meat in a meal (Hallberg et al, 1979) and by the the amino acid cysteine and the peptide glutathione (Layrisse et al 1984). Substituting soy protein for meat was found to cause a greater percentage of heme absorption even though total heme content was decreased (Lynch et al, 1985).

Although heme is an excellent source of absorbable iron, its content in meals in developing countries is low (Hallberg and Rossander, 1984) and its total contribution to Western meals, where meat intake is high, is negligible (Hallberg and Rossander, 1982a). Nonheme iron (inorganic iron) is the main source of iron in the diet (Hallberg, 1982) and is present in plant and animal foods in the ferric state (Skikne, 1988; Fairbanks and Beutler, 1988). Unlike heme iron, the bioavailability of nonheme iron is influenced by many factors.

When food enters the stomach, gastric acid reduces the insoluble ferric iron to a soluble ferrous state (Miller and Schricker, 1982). Concurrently, ligands are released from foods that form low molecular weight chelates with the ferrous iron keeping it in solution as it

passes into the duodenum, where conditions of increased pH can cause precipitation and/or polymerization of iron, making it unavailable for absorption (Miller and Schricker, 1982; Skikne, 1988). These low molecular weight chelates may then be absorbed intact or may transfer the iron to a receptor on the mucosal cell surface of the duodenum.

Ascorbic acid enhances nonheme iron absorption through its reducing properties and its ability to chelate with ferrous iron in acidic conditions (Hallberg and Rossander, 1982a; Leigh and Miller, 1983; Hallberg and Rossander, 1984; Gillooly et al, 1984; Hamdaoui, 1995). The actions of ascorbic acid on nonheme iron are greatly reduced however in oxidizing environments, during periods of prolonged warming or baking, and from extended storage times (Hallberg et al, 1982; Morck et al, 1982). Other organic acids that increase nonheme absorption include citric, L-malic, and tartaric acid (Gillooly et al, 1983). Ascorbic acid and citric acid appear to have an additive effect with citric acid acting at a pH near 6.0 (Kojima et al, 1981). Fructose, nitrolotriacetic acid, and EDTA form chelates with ferrous iron , increasing its availability (Carlson and Miller, 1983; Leigh and Miller, 1983). Meat present in a meal enhances the absorption of nonheme iron (Hallberg and Rossander, 1982a; Hallberg and Rossander, 1984; Hulten et al, 1995). It is thought that the amino acid cysteine, the peptide glutathione, and other intermediary products of meat digestion may chelate with nonheme soluble iron and present it to the gut mucosa (Layrisse et al, 1984; Taylor et al, 1986; Monsen, 1988).

Although cereals, grains, fruits and vegetables may be good sources of nonheme iron, the presence of certain inhibitors may decrease their bioavailability. For example, phytates

present in wheat, maize, bran and legumes decrease nonheme iron absorption (Reinhold et al, 1981; Simpson et al 1981; Lynch et al, 1984; Hallberg et al, 1987; Reddy et al, 1996). Lignins and psyllium have a high affinity for ferrous iron at pH values similar to those found in the duodenum (Fernandez and Phillips, 1982) and binding leads to the formation of an insoluble complex. Soy products reduce the absorption of nonheme iron although the exact mechanism of inhibition is unclear (Cook et al, 1981; Hallberg and Rossander, 1982; Gillooly et al, 1984; Shaw et al, 1995). The polyphenol (tannic acid) content of tea and certain vegetables (ie. spinach, brown and green lentils, beetroot greens) decrease the availability of nonheme iron by forming insoluble chelates which are excreted (De Alarcon et al, 1979; Gillooly et al, 1982; Kojima et al, 1981; Hamdaoui et al, 1995) as do the presence of calcium carbonate, calcium-phosphorous salts and phosphoproteins (ie. phosphovitin in egg yolks) (Peters et al, 1971;Monsen and Cook, 1976; Dawson-Hughes et al, 1986). Both tea and coffee oxidize nonheme iron to the ferric form which is not readily absorbed in man (Morck et al, 1983).

Iron Deficiency Anemia

Anemia is defined as a disorder in which the number of red blood cells, the quantity of hemoglobin, and the hematocrit (volume of packed red blood cells per 100 ml blood) are below normal limits (Baldy, 1986). It is classified according to the morphology and indices of the red blood cell and etiology of the disease. The morphology is based on the size of the red blood cell (ie. micro-, normo-, or macrocytic) and its color (hypo- or normochromic) which is a reflection of the concentration of hemoglobin in the red blood cell. The two

common etiologic sources of anemia include an increased red blood cell loss (via bleeding or internal hemolysis) and a decreased or defective production of red blood cells. Worldwide, anemia affects approximately 30% of the total population with an increased incidence in developing countries (36%) versus developed areas (8%) (Skikne, 1988).

Iron deficiency anemia is the most common form of anemia and affects 500 to 600 million people in the world (Bothwell, 1995). The prevalence of iron deficiency in the United States is fairly low and is found primarily in children aged 1 to 2 years, males aged 11 to 14 years, and females aged 15 to 44 years (Dallman et al, 1984; Expert Scientific Working Group, 1985). These three populations reflect four periods of life in which iron uptake is inadequate to meet nutrient needs of the body (Herbert, 1987; National Research Council, 1989). The first period is from approximately 6 months to 4 years and is due to the low iron content of milk, a rapid growth rate, and inadequate iron stores. The second period is in early adolescence when there is an increase in red blood cell mass and an increase need to deposit iron in myoglobin. The third period involves the female reproductive period when menstrual bleeding causes an increase in iron loss. Finally, pregnancy comprises the last period due to the increase in maternal blood volume, iron demands by the fetus and placenta, and blood losses during delivery.

There are three stages of impaired iron status in the body that lead to iron deficiency and subsequent anemia (Finch and Cook, 1984; Expert Scientific Working Group, 1985; National Research Council, 1989). The first stage involves a decrease in iron stores reflected by a reduction in serum ferritin in man and liver and spleen concentrations in rats (Dalman

et al, 1982). No functional impairment is noted. The second stage develops due to iron deficient erythropoiesis. There is a decrease in iron plasma supply to the tissues as evidenced by decreased transferrin saturation and a decrease in iron supply to the developing red blood cells as evidenced by an increase in red blood cell protoporphyrin. Hemoglobin is still within normal limits but a decreased work capacity becomes evident (Dallman et al, 1982). Iron deficiency anemia is a result of the third stage and is identified by a decrease in hemoglobin levels below normal ranges and the appearance of microcytic, hypochromic red blood cells on a peripheral blood smear. Manifestations of iron deficiency include gastrointestinal defects (atrophic gastritis and changes in small intestine epithelium), defects of immunity and decreased resistance to infection, impaired exercise and work performance, neurological disturbances, and inability to adapt to low temperatures caused by increased catecholamines and decreased conversion of thyroxine to triiodothryonine (Hagler et al, 1981; Hallberg, 1982; Dallman, 1987; Skikne, 1988). Table 2 depicts laboratory values present during the development of iron deficiency anemia.

IRON AND PREGNANCY

Iron Demands and Requirements during Pregnancy

Many physiologic changes occur in the body during pregnancy primarily to ensure proper development and growth of the fetus (Rosso, 1981; Ritchey and Taper, 1983). After implantation of the egg, placenta and fetal development begin and continue throughout the

first trimester with rapid growth occuring in the second and third trimesters. Placenta

Table 2. Sequence of Changes Induced by Depletion of Iron Content in the Body.

	Normal	Iron Depletion	Iron Deficient Erythropoiesis	Iron Deficiency Anemia
RE Marrow Iron	2-3+	0-1+	0	0
Transferrin Iron Binding Capacity (μg/100ml)	330 ± 30	360	390	410
Plasma Ferritin (μg/ml)	100 ± 60	20	10	<10
Iron Absorption (%)	5-10	10-15	10-20	10-20
Plasma Iron (μg/100ml)	115 ± 50	115	<60	<40
Transferrin Saturation (%)	35 ± 15	30	<15	<15
Sideroblasts (%)	40-60	40-60	<10	<10
RBC Protoporphyrin	30	30	100	200
Erythrocytes	Normal	Normal	Normal	Microcytic/ Hypochromic

(Herbert, 1987)

growth levels off early in the third trimester while the fetus continues to increase in size and weight. Maternal nutrient stores rise for the first and second trimesters. A large increase in plasma volume occurs between 6 to 24 weeks of gestation with a smaller increase in the third trimester (approximately 40% total) and is associated with a concomitant linear increase of red blood cell volume throughout pregnancy (approximately 30% total) (Lu et al, 1991; Schwartz and Thurnau, 1995). The plasma volume allows for adequate blood flow

to the uterus without compromising other major organ systems in the mother and is facilitated by an increase in cardiac output. The rate of uterine blood flow increases as the fetus grows (Rosso, 1981).

Iron demands during pregnancy are much greater than in the nonpregnant state and are due to an increase in red blood cell mass, growth in the fetus and placenta, basal iron losses by the mother, and blood loss during delivery (Schwartz and Thurnau, 1995; Roodenburg, 1995) (Table 3).

Table 3. Iron Requirement during Pregnancy and Immediate Postpartum

	Individual Requirement (mg)	Cumulative Requirement (mg)
During pregnancy		
Fetus	300	
Placenta	50	
Expansion of red cell mass	450	
Basal iron losses	240	1040
Maternal blood loss at delivery	250	1290
Contraction of maternal red cell mass immediate postpartum	450	840

(Roodenburg, 1995)

As mentioned previously, iron requirements for women in child-bearing years are approximately 2 mg per day. During pregnancy, daily requirements increase to 3 mg, with a maximum requirement of 5 to 6 mg per day in the third trimester when fetal iron demand is greatest. This is met in part by lack of menses throughout pregnancy and an increase rate of iron absorption from 10% in the nonpregnant state to 20% during pregnancy and as high

as 40% when iron deficiency anemia is present (Schwartz and Thurnau, 1995). The current recommended daily allowances for iron during pregnancy are 30 mg per day to ensure enough iron is absorbed to meet nutrient needs (National Research Council, 1989). Because generally only 50% of the needed iron is absorbed from the diet and there is increased incidence of iron deficiency in women of child-bearing years, supplementation (30 mg per day) is routinely encouraged at the beginning of the second trimester (Schwartz and Thurnau, 1995). The benefits of supplementation were found in the increase of maternal and fetal iron stores. No effects on pregnancy outcome, fetal growth, or maternal hemoglobin levels were noted (Thane-Toe and Thein-Than, 1982; Hemminki and Rimpela, 1991; Roodenburg, 1995). Current research involves looking at selectively supplementing only those women who exhibit iron deficiency throughout pregnancy.

Maternal-Fetal Iron Exchange

Exchange of iron from mother to fetus occurs at the placenta where iron goes from a low concentration gradient to a high concentration gradient (Reshetnikova, 1995). Early in pregnancy the placenta stores ferritin until the fetus develops a mature system for transfer, at which time, almost all of the stored placental iron is transferred to the fetus (Wong and Saha, 1990). Two thirds of the transferred iron is used to make fetal hemoglobin while the remaining one third is stored in the liver where it is used for iron requirements early in infancy (Schwartz and Thurnau, 1995). Fetal iron is derived primarily from maternal stores and if these stores are depleted then iron is obtained from the breakdown of maternal red blood cells and dietary sources. Much research has been done to see if maternal iron stores

affect fetal iron uptake and storage. It has been found that the fetus takes up iron from the mother until delivery regardless of maternal stores and fetal stores are generally unaffected (Bhargava, 1989; Wong and Saha, 1990; Lao et al, 1991; Roodenburg, 1995).

Iron Deficiency Anemia and Pregnancy

During pregnancy, a physiologic anemia occurs due to hemodilution where expansion of plasma volume exceeds red blood cell mass expansion and causes a decrease in hemoglobin and hematocrit (Scholl and Hediger, 1994; Roodenburg, 1995; Schwartz and Thurnau, 1995). This decrease in hemoglobin is seen until approximately 24 weeks gestation at which point the red cell mass starts to increase and hemoglobin concentrations begin to rise (Lu et al, 1991; Roodenburg, 1995).

Identification of iron deficiency anemia during pregnancy is difficult because of rapid turnover of iron to the fetus (as evidenced by decreased maternal serum iron and increased TIBC regardless of supplementation), hemodilution (increases mean corpuscular volume and decreases hemoglobin and hematocrit), and slow changes in red blood cell protoporphyrin (Beard, 1994;Schwartz and Thurnau, 1995). Assessment of iron deficiency is based on two sets of parameters- hemoglobin and hematocrit and serum ferritin. Anemia is diagnosed when hemoglobin is < 11.0 g/dl, hematocrit is <33% for the first and third trimesters and <10.5 g/dl and < 32% for the second trimester and ferritin is <12μg/l (Institute of Medicine, 1990). Because ferritin levels drop late in gestation regardless of iron status, plasma volume may dilute total serum levels, and due to its acute phase reactant characteristics, ferritin may not be a good indicator. It has been suggested that perhaps serum transferrin receptors

should be measured (Scholl and Hediger, 1994; Beard, 1994). Good clinical assessment of the mother may help determine the etiology of the anemia as well (ie. history of gastrointestinal bleeding, socioeconomic status, dietary intake, etc.).

Decreased birthweight and preterm delivery (<37 weeks) are two deleterious effects associated with iron deficiency anemia diagnosed in the first trimester but there is not an increased incidence when iron deficiency is diagnosed in the third trimester (Scholl and Hediger, 1994; Schwartz and Thurnau, 1995). This may be due in part to the protective mechanism of increased plasma volume later in pregnancy. Low maternal weight gain and iron deficiency anemia are significant throughout pregnancy. Identification and treatment of iron deficiency prior to conception or at its onset are important in preventing any complications later on in pregnancy.

Several studies have looked at pregnancy outcome associated with hemoglobin and hematocrit values without differentiatin between iron deficiency and other anemias (Lu et al, 1991; Mitchell and Lerner, 1992; Scholl and Hediger, 1994; Schwartz and Thurnau, 1995). Decreased hematocrits prior to 20 weeks and increased hemoglobins and hematocrits at the end of gestation were found to result in greater frequency of fetal growth retardation and preterm delivery. Mitchell and Lerner hypothesized that decreased parameters resulted in decreased O_2 carrying capacity and increased levels were most likely due to ineffective plasma volume expansion and subsequent decreased oxygen availability to the fetus.

ZINC STATUS AND ITS EFFECT ON IRON DURING PREGNANCY

Zinc Functions and Balance

Zinc is an important mineral that is necessary for cell growth, cell replication, sexual maturation, fertility and reproduction, night vision, immune defenses, taste and appetite. It functions in four ways- as a component of over 200 metalloenzymes in mammals; confirmation of polyribosomes for DNA synthesis and cell replication during protein synthesis; stabilizes membranes of circulating white blood cells, red blood cells and platelets; as a free ion in such processes as tubulin synthesis, activating thymic factors, and as a cofactor in enzymatic reactions (Solomons, 1988). Zinc is distributed primarily in the muscle, bone, skin and liver with the largest percent found in the bone and lean muscle (Nutrition Review, 1987). High concentrations are found in prostatic fluid and ocular tissues, although no storage form of zinc is present in the body (Solomons, 1988).

Like iron homeostasis, zinc balance is controlled by anabolic states (ie.growth and reproduction), basal losses, and bioavailability of zinc in the diet. Table 4 lists the Recommended Daily Allowances (NRC, 1989) for zinc based on maintenance, growth, surface losses, menses, pregnancy, and lactation and assuming a 20% rate of absorption to meet nutrient needs. Basal losses for zinc are due to excretion in the feces and, to a smaller extent, urine, desquamation of skin, hair growth, and sweat and average about 2.2 mg/day. In addition, semen ejaculum can contain 0.6-1.0 mg of zinc (Solomons, 1988; NRC, 1989).

Zinc is absorbed at all sites of the small intestines (Hartiti et al, 1994). Absorption is

regulated by the demand for zinc from the body and its bioavailability in the diet. Meals rich in red meat, organ meats, oysters and other meat products result in increased retention of zinc . This enhancement cannot be mimicked with a low meat diet that is fortified with zinc in amounts that would be comparable to the zinc content in meat (Hunt et al, 1995). The presence of phytate and other ligands (like casein and fiber), as well as high levels of calcium and iron, have been found to inhibit the absorption of zinc (Greger, 1987; Sandstead, 1995). Zinc rich pancreatic enzymes that are secreted into the intestines during absorption provide an endogenous source of zinc that is recycled back into the body (Solomons, 1988).

Zinc Status and Pregnancy

The demand for zinc during pregnancy (100 mg) is based on the needs of the fetus, placenta, amniotic fluid, uterus, mammary tissues and maternal blood. Approximately 60% of this zinc is found in the conceptus and 40% in maternal tissue. The average daily need for zinc increases throughout gestatation and can be catagorized as follows: 0.1 mg/day for the 1st 10 weeks; 0.2 mg/day for the 2nd 10 weeks; and 0.6 mg/day for the last 20 weeks (Swanson and King, 1987). This is evidenced by a decline in circulating zinc levels during pregnancy, of approximately 15 to 35%, that begins early in the first month of gestation, stabilizes during the second trimester, and begins a gradual decline after that (Breskin et al, 1983; Swanson and King, 1987; Bloxam et al, 1989). Zinc plays a role in organogenesis, a time of fast cellular growth changes in the embryo, when teratogenesis can occur (Breskin et al, 1983; Argemi et al, 1988; Oteiza et al, 1990). It is also an important component of fetal

growth and tissue synthesis (Simmer and Thompson, 1985) and maternal prostatglandin function, as it relates to maternal blood pressure and parturition outcome (Hunt et al, 1984; Swanson and King, 1987).

Zinc status during pregnancy is directly related to its outcome. Severe zinc deficiency has been associated with congenital malformations of the central nervous and skeletal systems, anencephaly, prolonged labor and atonic bleeding, increased pica and decreased taste perception (Hambridge et al, 1983; Sandstead, 1994). Severe zinc deficiency has been studied primarily in animal models, however, marginal zinc deficiency is more common in human populations (Polberger et al, 1996). Pregnant women are at an increased risk for zinc deficiency due to socioeconomic status or inappropriate food choices as evidenced by decreased consumption of red meat and increased incidence of vegetarianism (Hambridge et al, 1983; Hunt et al, 1984; Sandstead, 1995). Marginal zinc deficiency throughout gestation results in growth retardation, skeletal anomalies, delivery complications, and impaired immune function however no congenital malformations are seen (Swanson and King, 1987). Supplementation of zinc during pregnancy appears to have beneficial effects and decreases the adverse effects of severe and marginal zinc deficiency (Hunt et al, 1984; Simmer et al, 1990).

Table 4 . Recommended Daily Allowances (RDA) for Zinc

National Research Council, 1989

Category	Age (years) or Condition	RDA Zinc (mg)	Body Need (mg)
Infants	0.0-0.5	5	1
	0.5-1.0	5	1
Children	1-3	10	2
	4-6	10	2
	7-10	10	2
Males	11-14	15	3
	15-18	15	3
	19-24	15	3
	25-50	15	3
	51+	15	3
Females	11-14	12	2.4
	15-18	12	2.4
	19-24	12	2.4
	25-50	12	2.4
	51+	12	2.4
Pregnant		15	3
Lactating	1st 6 months	19	3.8
	2nd 6 months	16	3.2

Zinc-Iron Interactions During Absorption

It is believed that zinc and iron have an antagonistic relationship at the level of absorption and this relationship is directly affected by the intake of zinc and iron and their status in the body. When a group of pregnant women was supplemented with 47 mg of ferrous sulfate beginning at 16 weeks gestation, it was found that plasma zinc concentrations began to decrease rapidly within six weeks, long before increased iron status was noted in the supplemented group, as compared to the control group (nonsupplemented). Interestingly, this decline in plasma zinc became more gradual later in the study and was comparable to the nonsupplemented women. The initial inhibition of zinc at the absorptive level was perhaps reduced by a compensatory adaptation (ie.decreased urinary or pancreatico-biliary losses) to conserve zinc (Bloxam et al, 1989). The effect of zinc supplementation showed the same inhibitory effect on iron. Nonpregnant women, aged 25 to 40 years, were supplemented with either 25 mg zinc gluconate or 25 mg each of zinc gluconate and ferrous sulfate (1:1 Fe/Zn). Zinc supplementation caused a decrease in serum ferritin and hematocrit levels, and increased plasma zinc and salivary-sediment zinc (measured by atomic absorption spectrophotometry). Treatment with zinc and ferrous sulfate resulted in an increased serum ferritin, increased plasma zinc, but a decreased salivary-sediment zinc. This decrease in salivary-sediment zinc may reflect a state of zinc depletion and further supports an inhibitory affect of iron on zinc (Yadrick et al, 1989).

In a study of the effects of iron status on zinc absorption, 32 females, ages 19 to 50, with various degrees of iron nutriture, and one preschooler, receiving intramuscular iron therapy,

were given 2:1 Fe/Zn consisting of 50 mg ferrous iron to 25 mg of zinc. As the need for iron increased in these individuals, its enhanced passage into the mucosa reduced zinc uptake (Solomons, 1983). In the previously mentioned study by Yadrick et al, the effect of pretreatment iron and zinc status were measured and it was found that the greater the serum zinc concentration prior to zinc supplementation, the greater the decrease in serum ferritin after treatment. Increased hemoglobin and hematocrit levels prior to the study resulted in less of an increase of serum zinc with supplementation (Yadrick et al, 1989). Rats fed chronic excess iron had decreased zinc retention as compared to those rats fed excess iron in just one meal. A decrease in iron absorption and hematocrit (but not tissue iron levels) was seen when rats were fed chronic excess zinc when compared to excess zinc in one meal (Storey and Greger, 1987). Other studies have found however that subjects with a high capacity to absorb iron (ie. decreased serum ferritin) had the same % zinc absorption rate as those with a low capacity to absorb iron (ie. increased ferritin). It was suggested that in addition to a common binding site with zinc in the intestine, there must be sites present that are exclusively for iron, otherwise zinc absorption would have decreased when the demand for iron was great (ie. decreased ferritin) (Valberg et al, 1984). A study of the effects of iron supplementation on zinc absorption in Rhesus mokeys fed a zinc marginal diet (4μg/g) showed that there was an actual increase in zinc retention from the diet (Lonnerdal et al, 1990). It was noted that the iron supplemented groups had a decreased zinc turnover and that this decrease in turnover may be compensatory to an initial decreased zinc uptake in supplemented animals.

The inhibitory effects between iron and zinc vary depending on their composition and how much is present in the diet. The competitive interaction appears to be between inorganic zinc and nonheme iron. Ferrous sulfate in a 2:1 Fe/Zn solution decreased zinc absorption 1, 2, and 3 hours post dose while 2:1 Fe/Zn solution with ferric chloride did not affect zinc absorption until 3 hours post dose (Solomons et al, 1983). Ingestion of excess iron as a supplement or in fortified foods did not alter zinc absorption when zinc was fed in organic form or as a part of a meal (especially meat) (Valberg et al, 1984; Storey and Greger, 1987, Davidsson et al,1995). In most studies the doses of iron that are used to inhibit zinc absorption are much higher than those found normally in foods. In addition, zinc and iron are usually complexed to ligands in the diet which decrease the competitive action of the two metals for binding sites in the intestinal mucosa (Valberg et al, 1984; Davidson et al, 1995). Concern arises however in pregnancy, when maternal zinc status is usually low or depleted and iron supplementation is common.

Marginal Zinc Status During Pregnancy and Effects on Iron Nutriture

Studies in rats have looked at maternal and fetal iron status that result from deficient and marginal zinc intake during pregnancy (Reinstein et al, 1984; Rogers et al, 1985; Rogers et al 1987) . Reinstein et al examined the interactions of both zinc and copper intake while Rogers et al examined zinc intake only. Zinc deficient diets were composed of 0.5 to 1.0 μg/g (ppm) of zinc; zinc marginal diets were composed of 4.5 ppm; zinc adequate diets ranged from 10 to 1000 ppm. Hemoglobin and hematocrit levels increased in Sprague Dawley rats as zinc intake decreased (Reinstein et al, 1984) although no differences in

hematocrit were found in Long-Evans Hooded rats (Rogers et al, 1987). Plasma iron in Sprague Dawley rats was greater in the 1.0 ppm and 4.5 ppm zinc groups and was significantly increased in the 0.5 ppm zinc group in Long-Evans rats. Plasma iron in the zinc deficient rats did not increase until day 16 of gestation and no significant differences in plasma iron were found on day 12 between any level of zinc intake (Rogers et al, 1987). In contrast, plasma zinc concentrations were lower in the zinc deficient groups of both strains although no significant difference was found between this group and the marginal intake group. Dam liver iron was significantly higher in the zinc deficient mothers (Reinstein et al, 1984; Rogers et al, 1985) and in the deficient and marginal mothers (Rogers et al, 1987) as compared to the other groups. Maternal spleen iron concentrations were less in the deficient group when compared to the adequate zinc groups (Rogers et al , 1987). It is thought that zinc deficient dams may have ineffective erythropoiesis as evidenced by the spleen concentrations and the increased plasma iron levels.

Whole fetal iron concentrations were significantly higher in zinc deficient dams (Reinstein et al, 1984; Rogers et al, 1985) and zinc marginal dams (Rogers et al, 1987) as compared to adequate zinc intake. Conceptus iron concentration showed no differences among all dietary treatments on day 12 of gestation, and in conjunction with maternal plasma iron levels on that day, help to rule out a teratogenic effect of iron early in pregnancy. The conceptus zinc concentration on day 12, on the other hand, was significantly different between all groups (0.5 ppm< 4.5 ppm < 100 ppm zinc) and resulted in increased fetal malformations in the 0.5 ppm group only. By day 16 fetal zinc concentrations between

zinc marginal and zinc adequate dams were similar and significantly greater than concentrations in the deficient dams. For all three groups fetal zinc content increased significantly between days 16 and 19. (Rogers et al, 1987). Maternal zinc tissue levels were decreased for zinc deficient and zinc marginal groups, as compared to adequate groups, in all three studies.

Inadequate weight gain for both mothers and fetuses occurred in the zinc deficient groups, but it wasn't until day 18 of gestation that a decrease in maternal weight gain was seen in the 4.5 ppm zinc group (Rogers et al, 1985). This was reflected in a decreased diet intake on the same day. Insufficient intake in the zinc deficient dam occurs throughout gestation, although a substantial drop occurs on day 18 as well (Reinstein et al, 1984; Rogers et al, 1985). This occurs at a time when the permeability of the placenta to zinc increases. Approximately 80% of the fetal zinc accumulates at that time (Rogers et al, 1985). Without tissue catabolism, the zinc needs of the mother and fetus during this time could not be met. To support this terminal fetal growth phase, zinc is supplied to the fetus from the breakdown of maternal muscle (Masters et al, 1986).

A study in Rhesus monkeys examined the effects of zinc marginal status on pregnancy (Golub et al, 1984). Zinc marginal diets consisted of 4 μg/g of zinc and zinc adequate diets consisted of 100 μg/g of zinc. A group of monkeys on the zinc adequate diet were pair fed to the zinc marginal group to account for the inanition and calorie deprivation that may occur with zinc deficiency. A gradual decrease in the red blood cell count, hemoglobin and hematocrit occurred throughout gestation and was significant for all groups. Five of the 15

dams on the marginal diet, with the lowest plasma zinc concentrations, revealed decreases in hemoglobin (<12 g/dl) and hematocrit (<40%) by the third trimester that were indicative of iron deficiency. Plasma iron levels increased throughout the study but stayed within normal limits and no significant differences were found between groups.

The zinc marginal and adequate pair fed dams had an increased incidence of abortion, stillbirth, and neonatal death as opposed to the ad libitum 100 μg/g group (33%, 31%, and 10% respectively (Golub et al, 1984a). Seven of the eight male offspring in the zinc marginal group had significant and symmetrical growth retardation as opposed to the females in that group. Pair fed infants showed no signs of growth retardation and no differences in skinfold thickness, arm circumference, or head circumference were found among the offspring of all three groups. Plasma iron was reduced in the zinc marginal neonates although most of their hematology values were within normal range. Four of these infants had a decreased MCV (<85) and were offspring of dams reported anemic during the 3rd trimester. Three of the zinc marginal infants had decreased red blood cell count, hemoglogin and hematocrits and very low plasma irons as compared to the control.

By midpregnancy, clinical signs of zinc deficiency began to appear in the zinc marginal dams which continued to manifest throughout the third trimester (Golub et al, 1984). They included anorexia, dermatitis, and decreased plasma zinc levels. By the third trimester, many of the 4 μg/g dams failed to gain weight or to increase their diet intake. Four of the 15 zinc deficient dams showed signs of severe anorexia, with low food intake, weight loss and reduction in fat stores; these losses were reflected in the pair-fed 100μg/g dams.

EFFECTS OF EXERCISE ON IRON STATUS, ZINC STATUS, AND PREGNANCY

Iron Status During Exercise

Extensive research has examined the effects of exercise on iron status. Iron directly affects performance during exercise by its role in transporting and delivering oxygen to the mitochondria of cells via hemoglobin and myoglobin (Hultman et al, 1988). A study of male and female Sprague-Dawley rats looked at the effects of swimming on iron status over a 9 week period (Ruckman and Sherman, 1981). The training regimen included a 30 minute swim time for the first week; 60 minutes the second week; and 90 minutes weeks 3 through 9. Significant findings occurred only in the exercised males. Hemoglogin and hematocrit levels were increased reflecting the need for enhanced oxygen exchange and its utilization for energy in tissues. Depleted iron stores were found in the liver, spleen, and heart due perhaps to increased mobilization of iron to the bone marrow for red blood cell synthesis. Muscle iron levels were high in the exercised males because of increased myoglobin. Increased fecal iron loss was attributed to an increased excretion of iron in the bile and an adaptive decrease in absorption. Cardiomegaly and weight loss occurred in the exercised males. It was hypothesized that an exercise effect was not found in the females due to their propensity to float rather than swim which was attributed to their increased percent body fat.

These results were disputed somewhat in another study involving Sprague-Dawly female rats who were run to exhaustion on a treadmill everyday for 21 days and then allowed to rest for 14 days (Strause et al, 1983). Exercise had a direct effect on the absorption, distribution, and excretion of iron in the exercising females. No change in hemoglobin or hematocrit was

observed between the exercising and sedentary animals. Enhanced absorption of iron occurred with exercise and was distributed to the liver, spleen and heart, with significant uptake in the heart. This was supported by decreased urinary and fecal iron excretion. Liver and spleen iron decreased throughout the study in the exercise group with a 61% drop in liver iron and a 36% drop in spleen iron. Heart and soleus myoglobin were significantly greater in the exercise group. No weight differences were found between the exercise and sedentary groups. It was concluded that the body responds to strenuous activity by increasing absorption when iron intake is adequate and mobilizing iron from tissue stores (ie. liver and spleen) to compensate for the myoglobin needs of the heart and muscle.

The effect of moderate aerobic activity (dance aerobics) on the iron status of previously untrained women was examined throughout a 13 week period (Blum et al, 1986). The women participated in a class that met for 35 minutes a session, 4 days per week. Two, 3 day dietary records were collected on weeks 4 and 12 of the study, and were used to calculate total iron, total absorbable iron, ascorbic acid, protein, and energy intake. Hemoglobin and hematocrit levels increased between week 0 and week 6 and then dropped back to pretraining levels by week 13. All values were within normal limits. Ferritin decreased between weeks 0 and 6 and then stabilized throughout the remainder of the study. The stress upon initiation of exercise may have induced increased production of red blood cells thereby increasing hemoglobin and hematocrit from ferritin iron stores. Plasma iron, TIBC, and transferrin saturation were unaffected by the study. Incidence of iron deficient anemia between the exercise and sedentary groups was examined. Iron depletion was greatest in the

exercise groups but decreased and stabilized by week 6. Iron deficient erythropoiesis and subsequent anemia were increased in the sedentary subjects. Anemia was nonexistant during exercise. This study showed that the body adapts to the increased demand for iron-dependent compounds during exercise in order to maintain iron stores at a compromised level. This occurs when iron stores decrease and there is a decreased metabolism of iron-dependent compounds or feedback inhibition of iron mobilization; decreased iron losses via sweat, urine or feces; or increased absorption of iron.

Iron deficency anemia and nonanemic iron deficiency in athletes vary between men and women. In females it is due primarily to menstrual losses and inadequate intake in the diet (Parr et al, 1984; Strong et al, 1988; Strong et al, 1989). Hemorrhagic gastritis, especially in runners, accounts for the majority of losses in men (Moses, 1990; Peterson, 1997). 8% to 85% of runners have tested positive for fecal blood after runs of longer than 10 km and the occult blood may reflect a benign or malignant process (Peterson, 1997). Negligible iron losses have been found in the excretion of iron through sweat, and urine, and foot-strike hemolysis (Aruoma et al, 1988; Eichner, 1986).

Sports Anemia

"Sports anemia" is a phenomenon found in trained individuals where there is a decreased hemoglobin concentration which seems to enhance athletic performance (Peterson, 1997). Possible causes include hemodilution and increased destruction of red blood cells and hemoglobin degradation (Pate, 1983). The hemodilution effect observed with increased plasma volume is in part due to the body's conservation of salt and water (Peterson, 1997)

and to the fact that it is greater than the concomitant increase in red blood cell mass (Oscai et al, 1968; Convertino et al, 1980; Magnusson et al, 1984). The purpose of the plasma volume expansion is to increase total oxygen carrying capacity of the blood while decreasing its viscosity with the added benefits of increased cardiac stroke volume, increased resistance to dehydration, and increased sweating efficiency (Pate, 1983; Peterson, 1997).

An increase in intravascular hemolysis occurs during strenuous training in athletes as evidenced by a decrease in haptoglobin levels (Dufaux et al, 1981; Magnusson et al, 1984; Moore et al, 1993). This event is said to occur in the initial stage of training and may be related to an increased osmotic fragility of red blood cells (Yoshimura, 1970) and/or to mechanical damage to erythrocytes by the footstrike mechanism in running (Dufaux et al, 1981). The hemoglobin released from intravascular hemolysis is complexed with haptoglobin, thus preventing hemoglobinuria, and is taken up exclusively by the liver bypassing the reticuloendothelial system where ferritin and hemosiderin are produced (Magnusson et al, 1984a). Hemoglobin from hemolysis is incorporated into all tissues, especially skeletal and heart muscle, more readily than serum iron and may represent the body's increased utilization of this hemoglobin to produce muscle myoglobin and new red blood cells during exercise (Yoshimura, 1970).

Zinc Status During Exercise

Zinc plays an important role in exercise via its involvement in carbohydrate, lipid and protein metabolism (Campbell and Anderson, 1987). A study of 16 healthy women examined the effects of zinc on muscle strength and endurance (Krotkiewski et al, 1982).

Dynamic (isokinetic) and static (isometric) parameters were measured with and without zinc supplementation. There were significant increases in dynamic strength and static endurance with zinc supplementation. It was supposed that this may have been a result of zinc's effect on the enzyme lactate dehydrogenase (which decreases lactic acid build-up during anaerobic work) or by its influence on glucose metabolism via the pentose phosphate cycle).

Zinc response to strenuous exercise was measured in five healthy males by cycling to exhaustion on a bicycle ergometer in a zinc repleted state and a zinc depleted state (Lukaski et al, 1984). The subjects ate a diet adequate in zinc for 30 days (ie. 8.6 mg/day); a low zinc diet for 120 days (ie. 3.6 mg/day); and then a zinc supplemented diet for 30 days (ie. 33.6 mg/day). The results were adjusted by van Beaumont quotient (van Beaumont, 1973) due to the hemoconcentration effects that occur after brief, intensive muscular exercise in which plasma volume decreases and red blood cell volume remains unchanged. They found that in a replete state, zinc was retained in the plasma and mobilization of zinc occurred from tissues like muscle. In the depleted state, there was a net efflux of zinc from the plasma into the tissues. Reduced post-exercise plasma zinc reflected decreased body zinc pools, again most likely from the muscle. Hematocrit was significantly increased after exercise due to hemoconcentration but was unaffected by zinc intake.

The effects of training on plasma and red blood cell zinc were measured on 7 healthy, previously untrained male college students and consisted of running 5 km per day, six times per week for ten weeks (Ohno et al, 1990). The subjects consumed an ad libitum diet with no vitamin or mineral supplements. After the training period they found significant

increases in total red blood cell zinc, zinc derived from carbonic anhydrase isoenzyme I (CA-I), activity and concentration of carbonic anhydrase I immediately after exercise. Increased CA-I activity has been found in trained swimmers and runners and may be regarded as a significant part of available zinc during training. Albumin-bound zinc, serum iron and ferritin levels were all significantly reduced following exercise. The van Beaumont quotient for total zinc plasma had significantly dropped immediately after exercise at the end of the 10 week training period. This in conjunction with a decrease in albumin-bound zinc indicated there was an alteration in body zinc pools suggesting early zinc deficiency. Despite increased plasma levels immediately following exercise, researchers have found that zinc levels start to decrease significantly two hours post event and this may indicate a redistribution of zinc from serum into tissues or erythrocytes (Campbell and Anderson, 1987).

Exercise and Pregnacy

Early in pregnancy, maternal oxygen consumption, cardiac output, and plasma volume increase at rest. As the fetus grows, increased uterine pressure decreases venous return and cardiac output, especially in the supine position, and increased oxygen comsumption depletes oxygen reserves built early in gestation. (Bell et al, 1994). Exercise may affect these cardiorespiratory adaptations to pregnancy, and in addition, may cause detrimental effects due to increased maternal core temperatures, decreased uterine blood flow to the fetus, and decreased birthweight.

A study of the effects of moderate, nonweight bearing exercise via bicycle ergometer in

late gestation looked at these cardiorespiratory parameters (Morrow et al, 1989). The subjects exercised for 5 minutes and found an increased resistance of vessels that supply blood to the uterus, perhaps affecting fetal blood supply. A slight increase in maternal heart rate and blood pressure occurred. Fetal heart rate increased significantly until 20 minutes postexercise but no signs of fetal distress were found and vascular resistance on the fetal side of the placenta remained unchanged. Long-term treadmill training before and during pregnancy in Sprague-Dawley rats found no deleterious effects on blood flow to the placenta and uterus and this may have been due in part to the increase in blood flow to the placental and uterus that occurs naturally with increasing gestational age (Jones et al, 1990).

A study examining the birthweight of babies born to mothers who continued regular exercise at or above 50% of preconceptual limits found that birthweight was decreased in these infants and that this decrease in weight was due primarily to a decrease in neonatal fat mass (Clapp and Capeless, 1990). The pups of rats who were conditioned to swim 3 continuous hours six days per week (Savard et al, 1986) or to run on a treadmill 120 minutes per day 5 days per week (Mattola et al, 1989) did not weigh less than their sedentary counterparts. The exercisers were found to have decreased fat stores as compared to their sedentary counterparts and conditioning in the swimmers was confimed by significantly increased citrate synthase activity in the gastrocnemius muscle (Savard et al,1986). Citrate synthase activity measures improvements in mitochondrial oxidative capacity and can be used to demonstrate training effects of an exercise program (Kirwan et al, 1990). It was hypothesized that fetal weight was unaffected by exercise due to maternal conditioning prior

to conception.

At rest, there is an increased maternal heat production due to increased activity of the maternal tissues and heat production of the fetus and placenta. During exercise, extra heat is produced by the skeletal muscles as well (Belle et al,1994). A study of four aerobically conditioned females found that they were able to dissipate heat from both the fetus and mother efficiently during running (Jones et al, 1985). This may have been due in part to the increased plasma volume associated with pregnancy which helps to maintain fetomaternal heat transfer and dissipation and increased resting skin temperature that occurs throughout pregnancy. These results were supported in a study examining the effects of nonweight bearing exercise (ie. cycling) on land and in water on women who were physically active but were not participating in a regular exercise program (McMurray et al, 1993). Core temperature was slightly elevated on land as compared to water but thermoregulation was maintained regardless of environmental factors.

Swimming is recommended during pregnancy because it helps to dissipate heat, it is nonweight bearing, and supports the mother while exercising due to increased buoyancy (Berry et al, 1989). Fetal responses to cycle ergometry and swimming were examined in pregnant women at 25 and 35 weeks gestation (Watson et al, 1991). No change in maternal heart rate was found between the two groups. There was a smaller plasma volume decrease during swimming (18.9% versus 21.6% in cycling). This would allow for a decrease in uterine vascular resistance and may be due to volume expansion effect of immersion. Cooler water temperatures helped to dissipate heat. Fetal heart rate response was the same at weeks

25 and 35, with a decrease at one minute after exercise followed by an increase 10 to 20 minutes postexercise. Transient bradycardia occurred in one cycler and one swimmer at 25 weeks, and three cyclers and one swimmer at 35 weeks but was not associated with poor pregnancy outcome.

Zinc and iron play important roles during all phases of pregnancy. Concurrently, marginal to severe iron and marginal zinc deficiencies appear to be common among women of child-bearing years, due to an increased incidence of vegetarianism and/or decreased socioeconomic status. During pregnancy, deleterious effects on both the mother and fetus have been found when iron and/or zinc intake is decreased. Supplementing with iron and/or zinc appears to improve pregnancy outcome. Iron supplementation is common at the onset of pregnancy and women sometimes take 3 to 4 times more than the recommended 30 mg of iron per day. Concern with iron supplementation arises when zinc intake is marginal since iron and zinc have antagonistic effects on each other at the absorptive level and an abundance of iron in the diet (especially in the form of supplements) has been found to decrease zinc levels in the body. What would be the effects of iron status on the mother and fetus during pregnancy, if iron intake was adequate, but not supplemented, and marginal zinc intake developed after conception?

Studies concerning pregnancy and exercise are affected by many factors including conditioning of the mother prior to conception, type and duration of exercise during pregnancy, and whether exercise continues until delivery. Like pregnancy, exercise increases the demand for iron and zinc in the body. How would iron status be compromised in the

mother and fetus if strenuous exercise is initiated at conception and continues until late gestation?

This study will attempt to look at marginal zinc intake and strenuous exercise begun at the onset of pregnancy in rats and how maternal iron status is affected assuming an adequate (but not supplemented) iron intake throughout gestation.

III. MATERIALS AND METHODS

Animals

One hundred and fifty one weanling Sprague Dawley female rats and thirty males were obtained from Dominion Laboratories in Dublin, Virginia. The animals were housed in single-wide stainless steel cages and underwent 12 hour light/dark cycles at a constant temperature of 22° C until the experimental period began. During the adjustment phase, Agway PROLAB Rat/Mouse/Hamster 3000 chow (Agway, Inc., Syracuse, N.Y.) and tap water were given ad libitum. The animals were marked for identification with eartags.

Experimental Design

Animals were randomly assigned to particular diet/activity groups after mating had occurred. There were twelve groups in all and three different gestational ages at sacrifice. A total of 116 females had successful pregnancies. An attempt was made to have at least ten animals per experimental group. See Table 5 for presentation of the design.

Baseline data were collected from nonpregnant Sprague-Dawley females intermittently throughout the study. The animals used for this data were maintained on the PROLAB chow and tap water and remained sedentary.

Mating

When the female rats reached approximately 180 grams, they were mated with male Sprague-Dawley rats in triple-wide stainless steel cages. The cages were checked for copulation plugs at 8a.m. daily. Brown paper was placed under the mating cages for easy visibility of the plugs and was changed each afternoon. Males were rotated from the mating

cages every other day and allowed a 24 hour rest period.

Day 1 of gestation began with determination of conception; at this time the animals were assigned randomly to one of the twelve experimental groups and moved to single-wide wire bottom stainless-steel cages. Animals were weighed daily; total weight gain was determined as the difference between weight on the day of conception and weight on the day of sacrifice.

Diet

Sixty kilograms of purified diet were prepared for both the zinc marginal and zinc adequate groups (see Table 6 for diet composition). Zinc content of each diet was determined by atomic absorption spectrophotometry using a Perkin-Elmer 2100 AAS (Perkin-Elmer,Norwalk, Ct.) after wet-ashing via nitric-perchloric acid hydrolysis (Appendix I). The zinc marginal diet contained 6.18 ppm of zinc sulfate, while the zinc adequate diet contained 37.63 ppm. Protein content, calculated using the Kjeldahl procedure for nitrogen concentration (Appendix II), was 15.94% for the zinc marginal and 16.11% for the zinc adequate diet. Iron was present in the ICN Mineral Mixture as ferric citrate in the proportion of 6 grams (g) ferric citrate/1000g mineral mixture. Protein, vitamin and mineral contents were within guidelines recommended by the National Research Council (1978). Minimum nutrient requirements for iron and zinc in a pregnant rat are, respectively, 2.5 mg/100g and 0.4 mg/100g of diet (Baker, 1979). The purified diets were not analyzed for iron; iron content was calculated as 0.03g ferric citrate/100g purified diet. Total iron intake (g) was estimated by multiplying average intake (g) per gestational period by 0.0003g ferric

citrate/g purified diet.

Porcelain feed cups were used to feed the zinc marginal groups. Prior to the study, the cups were acid-washed in a 10% solution of HCl and rinsed with deionized water. Stainless steel cups were used for the zinc adequate groups. The cups were weighed and labeled with the number

Table 5 Experimental Design.

Diet	Activity	Gestational Age (Day)	Number of Animals per Group
Zinc Marginal[1]	Exercise	15	10
Zinc Marginal	Sedentary	15	10
Zinc Adequate[2]	Exercise	15	10
Zinc Adequate	Sedentary	15	9
Zinc Marginal	Exercise	18	10
Zinc Marginal	Sedentary	18	10
Zinc Adequate	Exercise	18	8
Zinc Adequate	Sedentary	18	10
Zinc Marginal	Exercise	21	10
Zinc Marginal	Sedentary	21	10
Zinc Adequate	Exercise	21	9
Zinc Adequate	Sedentary	21	10

[1] Zinc Marginal = 6.18 ppm.
[2] Zinc Adequate= 37.63 ppm.

corresponding to the animal's eartag. Once a week, the cups were washed and rinsed with deionized water.

The animals began eating the purified diets at Day 1 of gestation. The feed cups were weighed and fresh food was offered daily (approximately 20 grams per day). If the animals consumed all of the diet and no spills were present, more was given at the next feeding. Any significant spills found under the cages were measured and adjustments were made to accurately determine the amount of diet consumed.

The animals were given deionized water ad libitum at the onset of pregnancy. The water was changed every 2 to 3 days. Iron and zinc analysis of the water was determined via atomic absorption spectrophotometry using the Perkin-Elmer 2100 AAS (Perkin-Elmer, Norwalk, Ct.).

Activity

After the environmental adjustment period, all females were acclimatized to swimming for three days in 38°C to 40°C water, that was approximately 1/2 foot deep, for 10 to 15 minutes a session (Appendix III). The water was at a depth such that the animals were forced to wade; but, it was not over their heads. There were 10 to 15 animals per tub.

The animals were removed from the tubs after the designated time period and were dried with cotton bath towels and placed in 50 gallon garbage cans with at least one dry towel lining the bottom of the can. Heat lamps were attached to the top of each can and the animals were taken back to their cages after they had completely dried (approximately 30-35 minutes).

Table 6 Composition of Experimental Diet.

Dietary Ingredient	Zinc Marginal (g/100 g)	Zinc Adequate (g/100 g)
Egg White Solids[1]	20.000	20.000
Cornstarch[1]	33.100	33.100
Cerelose[2]	33.100	33.100
Fat (Corn Oil)[1]	5.000	5.000
Fiber[1]	3.000	3.000
Choline Chloride[1]	0.300	0.300
AIN Mineral Mixture 76[1] (Zinc-deficient)	5.000	5.000
Ferric Citrate[1,3]	0.030	0.030
AIN Vitamin Mixture 76[1]	0.500	0.500
Zinc sulfate[1]	0.0003	0.003

[1]ICN Biochemical, Costa Mesa, California
[2]Corn Products Co., Argo, Illinois
[3]Calculated (6g Ferric Citrate/1000g AIN Mineral Mixture 76)

The animals began their exercise protocol on Day 1 of gestation. The initial intent of this experiment was to swim the pregnant animals for 1 1/2 hours per day in 38° to 40°C water at a depth of approximately 3 feet as previously performed by Asente and Cameron (1985). The schedule began with a 30 minute session and was increased by 15 minutes per day until 90 minutes was reached.

Early on in the study one of the 90 minute swimmers drowned and seven other 90 minute swimmers became stressed while exercising. After evaluating the situation, the design was changed so that the animals swam for a maximum of 60 minutes per session beginning with

30 minutes and increasing by 15 minute increments per day. Although 38° to 40°C has been indicated as the optimal water temperature for peak swimming time in rats (Dawson and Horvath, 1970), the water temperature in this experiment was lowered to 35°C to prevent heat exhaustion and further stress on the animals. Due to an increased incidence of vaginal bleeding in either exercise groups, animals assigned to the Day 21 gestation groups swam until Day 18 and then remained sedentary until sacrificed, to prevent miscarriage.

There were no more than 10 rats per swim tub during each session. The animals were monitored carefully at this time. During the exercise periods, some females would attempt to rest by clinging to the corners of the swim tub or by piggybacking other swimmers. These animals would be gently pried away from the corners or fellow swimmers with a stick, and exercise would continue. The animals became more buoyant with gestational age and would float rather than swim. It was again necessary to prod them. After the allotted time period, the animals were dried as described previously. While the exercised females swam, the sedentary females remained in their cages and had their feed cups and water bottles removed until the exercise sessions were completed.

Animal Sacrifice

Pregnant rats were sacrificed on Days 15, 18 or 21 of gestation. On the morning of sacrifice, the animals were weighed and their feed cups and water bottles were removed from the cages. Animals in the exercise groups did not exercise on that day.

The rats were anaesthetized with CO_2 until they became unconscious and pentobarbitol was injected into the peritoneal cavity. The dose of pentobarbitol administered was 80 mg

of pentobarbitol per kg of body weight (Appendix IV).

A thoracotomy was performed, once the animal's reflexes had diminished, and blood was obtained through the left ventricle of the heart via cardiac puncture (Appendix V). Blood was collected in a 10 ml polyethylene syringe using a 23 gauge hypodermic needle and immediately transferred to one mineral-free, no additive test tube and one mineral-free, sodium heparin test tube. Approximately 5 to 10 mls of blood were obtained from each animal. To insure that the syringes were mineral-free, a sample of deionized water was collected in a syringe and analyzed for iron and zinc content via atomic absorption spectrophotometry (Appendix I).

After exsanguination was completed, the right atrium of the heart was nicked and the body organs were slowly perfused with a solution of sodium heparin and 0.9% normal saline (Appendix V). Several organs and tissues were collected following perfusion including the left gastrocnemius muscle, the liver, spleen, heart, uterus, fetus, and placenta (Appendix VI). The gastrocnemius muscle was removed from the body as quickly as possible, rinsed with normal saline, blotted dry, weighed, wrapped in aluminum foil, and submerged in liquid nitrogen for 8 seconds for rapid freezing. The heart was removed, rinsed with normal saline, blotted dry, weighed and then discarded. The liver, spleen, uterus, fetus and placenta were collected, rinsed with saline, dried, weighed, transferred to a freezer bag, and placed on ice until they could be stored in a -18℃ freezer. It is important to note that the uterus, fetus, and placenta were first weighed together and then separated and weighed individually. Fetal resorptions were noted while examining the uterus.

The stomach was removed and cut open. If blood was present in the stomach, it was rinsed off and placed in 10% formalin. The sample was sent to the Virginia-Maryland Regional College of Veterinary Medicine for histologic examination.

Blood Analysis

Hematocrit and hemoglobin determinations were performed on the heparinized whole blood on the day of sacrifice. Hematocrit analysis was obtained via the Microhematocrit method (Appendix VII) and all samples were run in triplicate. The cyanmethemoglobin technique was used for hemoglobin evaluation (Sigma Diagnostics, St. Louis, Missouri) (Appendix VIII) and absorbances were read on the Milton Roy Company Spectrophotometer 501.

Clotted blood from the mineral-free, no additive test tubes was spun down in a table top centrifuge for approximately 20 minutes at 2000 x g speed on the day of sacrifice. The serum was transferred into a polypropylene test tube and stored in a -18°C freezer.

Serum iron and total iron binding capacity (TIBC) were determined via a colorimetric test according to the ferrozine methodology developed by Persijn et al (1971) and Stookey (1970) (Stanbio Laboratory, Inc., San Antonio, Texas) (Appendix IX). All reagent and sample amounts were cut back by 1/2 of what the methodology required due to a limited amount of serum available for analysis. Serachem I normal controls by Fisher Scientific products (Norcross, Georgia) were run with each iron and TIBC procedure to insure good quality control. All test samples were run in duplicate.

Tissue Analysis

The liver, spleen, and fetal tissues were freeze-dried. Each organ was cut up prior to freeze-drying to increase the surface area of the sample. It was found to be beneficial to further cut the samples daily to speed up the drying process. The liver required approximately 4 days of drying, the spleen 2 to 3 days, and the fetus 2 to 4 days depending on sample size. The tissues were ground to a fine powder using an acid-washed porcelain mortar and pestle, placed in polycarbonate sample cups, and stored in a dessicator until iron analysis was performed. The dried sample weight of each tissue was determined by subtracting the cup weight (grams) from the cup with sample weight (grams).

The freeze-dried samples were wet ashed using a nitric acid, perchloric acid digestion procedure (Appendix X) and iron content was determined via atomic absorption spectrophotometry using the Perkin Elmer 2100 AAS (Perkin-Elmer, Norwalk, Ct.) (Appendix I).

Citrate Synthase Analysis

The left gastrocnemius muscle was homogenized according to methods described by Costill et al (1979) (Appendix XI). A Kinematica homogenizer was used and samples were spun down in an Eppendorf 5415C centrifuge at 11750 x g for five minutes. Citrate synthase analysis was performed via the 5,5'-dithiobis-(2-nitrobenzoate) (DTNB) described by Srere (1969) (Appendix XII). Samples were read over a three minute span of time on a Hitachi 100-10 spectrophotometer with attached plotter. Citrate Synthase activity was used to measure muscle adaptation to swimming.

Statistical Analysis

Analysis of variance (ANOVA) to determine significance (a = 0.05) of main effects and interactions was performed on the following: hemoglobin, hematocrit, serum iron and TIBC, liver, spleen and total fetal iron concentration, weight of maternal liver and spleen, average dietary intake during gestational period including estimated iron intake, maternal weight at conception and sacrifice, total maternal weight gain, weight of fetus, placenta, and uterus at sacrifice, number of pups and resorptions present, average fetal weight defined as total fetal weight divided by total number of pups, gastrocnemius muscle weight, heart weight, and citrate syntase activity. The mean and standard deviation of all variables for each experimental group were calculated. Normality and constant variance assumptions for the ANOVA were verified and the data transformed when either condition was violated. As there were some missing observations type III partial sums of squares were examined. The means were compared via least squares means with the probability of differences (pdiff) option to determine significant interactions. This allowed adjustment of the true means for unbalanced sample sizes present within each variable. To insure an overall experimentwise error rate of a= 0.05, the Bonferroni method was used to determine p-values for significance testing with multiple comparisons (SAS/STAT User's Guide, Volume 2).

IV. RESULTS AND DISCUSSION

Observations for three dams were discarded prior to statistical analysis due to abnormal circumstances and/or lab values. Two dams, each belonging to a zinc adequate exercising group, exhibited severe vaginal bleeding on the day of sacrifice (days 15 and 18) with resulting hemoglobins less than 4.0 g/dl. The third dam, who was zinc marginal and sedentary, had only 2 fetuses which was much lower than the average number of pups per litter (see proceeding section pertaining to number of pups).

Upon perfoming statistical analysis of the data, several parameters did not satisfy the assumptions for normality and constant variance and adjustments had to be made to avoid reporting invalid conclusions. Hemoglobin, hematocrit, and fetal weight were not normal at $p>0.01$. Variance problems were seen with residual vs. predicted means plots for hemoglobin, hematocrit, fetal weight and fetal, placental, and uterine weight.

The problem with normality and constant variance for hemoglobin and hematocrit was due to extreme outliers for the two groups. Hemoglobin had three outliers while hematocrit revealed two. The outliers for hematocrit were the same as those for hemoglobin. It was decided to rerun the statistics after removing the outliers since the outlier data was obtained on animals who exhibited vaginal and/or gastrointestinal bleeding at sacrifice which caused a drastic decrease in hemoglobin and hematocrit. All outliers belonged to a zinc marginal exercise group (days 15, 18, and 21). The assumptions for normality and constant variance were then met for each parameter.

Several transformations were performed on the fetal weight data. The square root of fetal

weight met the assumptions of normality ($p > 0.01$) but constant variance was still a problem. Since the null hypothesis for fetal weight was unacceptable, it was decided to examine the average fetal weight defined as fetal weight divided by the number of pups per litter. A log transformation of average fetal weight met the assumptions for normality and constant variance. A square root transformation for fetal, placental and uterine weight met the requirements for constant variance and the null hypothesis was accepted.

When examining the data, there were several significant main effect interactions and two factor interactions among the three treatment groups (ie. zinc, exercise, and gestation) but no significant three factor interactions were found. Throughout these results and discussion, significant main effect interactions will be described only when there is no corresponding two factor interaction, since main effect interactions are not independent of each other in a two factor interaction. Graphic representation of main effect and two factor interactions can be found in Appendix XIV. (All data depicted in the figures correspond to significant interactions presented in the tables throughout this section and letters for each data point on the figures correspond to the superscripts describing the differences between these measurements.)

Dietary Intake of Dams

Mean values and significant interactions for dam average daily intake of total diet and iron for the three treatment groups can be seen in Table 7. Total daily intake revealed main effect and two factor interactions for gestation, zinc x exercise, and zinc x gestation. Group means for zinc x exercise can be seen in Table 8. Total daily intake did not differ between

Table 7. Main Effect and Two Factor Interactions for Daily Dietary Intake of Purified Diet.

Treatment	Total Daily Intake[1] (g)	N	p-value[2]	Daily Intake of Iron[1] (mg)	N	p-value
Zinc marginal[3]	19.79 ± 2.08	59	N.S.[4]	5.94 ± 0.62	59	N.S.
Zinc adequate[5]	20.35 ± 1.64	53		6.10 ± 0.49	53	
Sedentary	20.02 ± 1.81	58	N.S.	6.01 ± 0.54	58	N.S.
Exercise	20.09 ± 1.99	54		6.03 ± 0.60	54	
Day 15	20.49 ± 1.67[A]	37	**	6.15 ± 0.50[A]	37	**
Day 18	20.40 ± 1.90[A]	37		6.12 ± 0.57[A]	37	
Day 21	19.29 ± 1.90[B]	38		5.78 ± 0.57[B]	38	
Zinc x Exercise			**			**
Zinc x Gestation			**			**
Exercise x Gestation			N.S.			N.S.

[1]Average for the group ± the standard deviation.
[2]Significant differences via ANOVA for main effect and two factor interactions; *p0.05-0.01; **p0.01-0.001; ***p<0.001.
[3]Zinc marginal diet = 6.18 ppm.
[4]N.S. = Nonsignificant (p>0.05).
[5]Zinc adequate diet = 37.63 ppm.

[A-B]Measures are different (Least Squares Means via pdiff; p≤ 0.0167) within each group when superscripts are different.

Table 8. Zinc-Exercise Interactions for Total Daily Intake of Purified Diet.
Group Means.

Zinc	Activity	N	Average Total Daily Intake[1]
Marginal[2]	Sedentary	29	20.20 ± 2.13^{A}
Marginal	Exercise	30	19.40 ± 1.98^{AB}
Adequate[3]	Sedentary	29	19.84 ± 1.44^{A}
Adequate	Exercise	24	20.97 ± 1.68^{AC}

[1]Average for the group ± the standard deviation.
[2]Zinc marginal diet = 6.18 ppm.
[3]Zinc adequate diet = 37.63 ppm.

[A-C]Measures are different (Least Squares Means via pdiff; $p \leq 0.00625$) within each group when superscripts are different.

the sedentary and exercise groups for either the zinc marginal or zinc adequate diets. The total intake of the sedentary dams was approximately the same regardless of zinc treatment. However, the zinc marginal exercisers ate significantly less than the zinc adequate exercisers.

Mean data for the zinc x gestation interactions are found in Table 9. Total daily intake for the zinc adequate dams did not change regardless of gestational age but there was a significant decrease in intake for the zinc marginal dams on day 21 as compared to days 15 and 18. No differences in intake between the zinc marginal and zinc adequate groups were found on days 15 and 18, but on day 21, the zinc marginal group was much lower than that of the zinc adequate group ($p=0.0002$). The data for the average daily iron intake for the dams mirrors that of the total daily dietary intake (Tables 10 and 11).

Inanition and decreased dietary intake were to found to occur in rats (Reinstein et al, 1984; Rogers et al, 1985) and monkeys (Golub et al, 1984) by midpregnancy. Our findings agree with the previous studies. The increased intake seen with the zinc adequate exercise group may have been in response to the increased energy needs imposed on the animal by both exercise and pregnancy.

Hematology Parameters

Mean values and significant interactions of dam hemoglobin and hematocrit for the three treatment groups (zinc, exercise, and gestation) can be seen in Table 12. Main effect and two factor interactions for hemoglobin were significant for zinc, gestation, and zinc x gestation ($p<0.001$) . No significant effect of exercise was seen for hemoglobin.

Table 9. Zinc-Gestation Interactions for Total Daily Intake of Purified Diet.
Group Means.

Zinc	Gestational Age (Day)	N	Average Total Daily Intake[1] (g)
Marginal[2]	15	19	20.72 ± 1.82^{A}
Marginal	18	20	20.37 ± 1.99^{A}
Marginal	21	20	18.34 ± 1.62^{B}
Adequate[3]	15	18	20.26 ± 1.51^{A}
Adequate	18	17	20.44 ± 1.85^{A}
Adequate	21	18	20.35 ± 1.63^{A}

[1]Average for the group ± the standard deviation.
[2]Zinc marginal diet = 6.18 ppm.
[3]Zinc adequate diet = 37.63 ppm.

[A-B]Measures are different (Least Squares Means via pdiff; $p \leq 0.004$) within each group when superscripts are different.

Table 10. Zinc-Exercise Interactions for Daily Intake of Iron. Group Means.

Zinc	Activity	N	Daily Iron Intake[1] (mg)
Marginal[2]	Sedentary	29	6.06 ± 0.64^{A}
Marginal	Exercise	30	5.82 ± 0.59^{AB}
Adequate[3]	Sedentary	29	5.95 ± 0.43^{A}
Adequate	Exercise	24	6.29 ± 0.50^{AC}

[1]Average for the group ± the standard deviation.
[2]Zinc marginal diet = 6.18 ppm.
[3]Zinc adequate diet = 37.63 ppm.

[A-C]Measures are different (Least Squares Means via pdiff; $p \leq 0.00625$) within each group when superscripts are different.

Table 11. Zinc-Gestation Interactions for Daily Intake of Iron. Group Means.

Zinc	Gestational Age (Day)	N	Daily Iron Intake[1] (mg)
Marginal[2]	15	19	6.22 ± 0.55^{A}
Marginal	18	20	6.11 ± 0.60^{A}
Marginal	21	20	5.50 ± 0.49^{B}
Adequate[3]	15	18	6.08 ± 0.45^{A}
Adequate	18	17	6.13 ± 0.56^{A}
Adequate	21	18	6.11 ± 0.49^{A}

[1]Average for the group ± the standard deviation.
[2]Zinc marginal diet = 6.18 ppm.
[3]Zinc adequate diet = 37.63 ppm.

[A-B]Measures are different (Least Squares Means via pdiff; $p \leq 0.004$) within each group when superscripts are different.

Table 12. Main Effect and Two Factor Interactions for Hemoglobin and Hematocrit.

Treatment	Hemoglobin[1] (g/dl)	N	p-value[2]	Hematocrit[1] (%)	N	p-value[2]
Zinc marginal[3]	13.6 ± 1.1	56	***	40.5 ± 3.1	47	***
Zinc adequate[4]	12.8 ± 1.0	53		38.2 ± 2.9	48	
Sedentary	13.2 ± 1.2	58	N.S.[5]	39.4 ± 3.5	50	N.S.
Exercise	13.2 ± 1.0	51		39.2 ± 2.8	45	
Day 15	13.7 ± 0.9[A]	36	***	40.9 ± 2.2[A]	27	***
Day 18	12.8 ± 0.8[B]	36		38.1 ± 2.0[B]	32	
Day 21	13.3 ± 1.4[B]	37		39.2 ± 4.1[B]	36	
Zinc x Exercise			N.S.			N.S.
Zinc x Gestation			***			***
Exercise x Gestation			N.S.			*

[1]Average for the group ± the standard deviation.
[2]Significant differences via ANOVA for main effect and two factor interactions; *p0.05-0.01; **p0.01-0.001; ***p<0.001.
[3]Zinc marginal diet = 6.18 ppm.
[4]Zinc adequate diet = 37.63 ppm.
[5]N.S. = Nonsignificant (p>0.05).
[A-B]Measures are different (Least Squares Means via pdiff; p≤0.0167) within each group when superscripts are different.

Table 13. Zinc-Gestation Interactions for Hemoglobin. Group Means.

Zinc Treatment	Gestational Age (Day)	N	Hemoglobin[1] (g/dl)
Marginal [2]	15	18	13.7 ± 0.9 [A]
Marginal	18	19	12.8 ± 0.8 [B]
Marginal	21	19	14.4 ± 1.0 [A]
Adequate [3]	15	18	13.7 ± 1.0 [A]
Adequate	18	17	12.7 ± 0.8 [B]
Adequate	21	18	12.0 ± 0.5 [B]

[1]Average for the group ± the standard deviation.
[2]Zinc marginal = 6.18 ppm.
[3]Zinc adequate = 37.63 ppm.

[A-B] Measures are different (Least Squares Means via pdiff; $p \leq 0.004$) within each group when superscripts are different.

Group means for the zinc x gestation interactions for hemoglobin can be seen in Table 13. For the zinc marginal group, it was found that the hemoglobin values decreased between days 15 and 18 and increased again by day 21. Day 18 of gestation was significantly lower compared to days 15 and 21. Hemoglobin for the zinc adequate group decreased throughout pregnancy and was significantly higher on day 15 than on days 18 and 21. No differences in hemoglobin were found between the zinc marginal and zinc adequate groups on days 15 or 18. However, on day 21, the zinc marginal group had a significantly higher hemoglobin when compared to the zinc adequate group.

Main effect and two factor interactions for hematocrit were significant for zinc, gestation, zinc x gestation, and exercise x gestation. Group means for the zinc x gestation interaction can be seen in Table 14 . This interaction is similiar to the one seen with hemoglobin. The hematocrit for the zinc marginal dams began to decrease between days 15 and 18 but increased significantly by day 21. The hematocrit for the zinc adequate group decreased throughout pregnancy and was significantly higher at day 15 than at days 18 and 21. There were no significant differences found between days 18 and 21 for the zinc adequate group. No differences in hematocrit were found between the zinc marginal and zinc adequate groups on days 15 or 18 of gestation. On day 21, the zinc marginal mean was significantly greater than the zinc adequate mean.

Reistein et al (1984) found that as zinc status decreased in pregnant Sprague-Dawley rats, hemoglobin and hematocrit levels increased. Golub et al (1984) found that hemoglobin and hematocrit values decreased throughout pregnancy regardless of zinc status. In this study,

Table 14. Zinc-Gestation Interactions for Hematocrit. Group Means.

Zinc Treatment	Gestational Age (Day)	N	Hematocrit[1] (%)
Marginal[2]	15	13	40.6± 2.3^{A}
Marginal	18	16	38.3± 2.1^{AC}
Marginal	21	18	42.4± 3.1^{ADE}
Adequate[3]	15	14	41.2± 2.1^{ADE}
Adequate	18	16	37.9± 1.9^{BCG}
Adequate	21	18	35.9± 1.9^{BDFG}

[1]Average for the group ± the standard deviation.
[2]Zinc marginal diet = 6.18 ppm.
[3]Zinc adequate diet = 37.63 ppm.

[A-G]Measures are different (Least Squares Means via pdiff; $p \leq 0.004$) within each group when superscripts are different.

the increase in hemoglobin and hematocrit from day 18 to day 21 indicates that zinc marginal deficiency did not affect the dam until midpregnancy which is the time when the placental permeability to zinc greatly increases (Reinstein et al, 1984; Rogers et al, 1985) The gradual decline in hemoglobin and hematocrit in the zinc adequate group is in agreement with Golub et al.

Hematocrit group means for the effect of exercise x gestation can be seen in Table 15. The hematocrit for the sedentary dams on day 15 was significantly greater than day 18 and seemed to rise slightly by day 21 although no differences were found between the hematocrits on day 21 and days 15 or 18. Hematocrits for the exercise group remained basically the same at all three gestational ages. No gestation difference on hematocrit was found between the sedentary and exercise groups.

Hemoglobin, and concomittantly, hematocrit values decrease until midgestation when the red cell mass increases more proportionately to plasma volume and levels begin to rise (Lu et al, 1991; Roodenburg, 1995). Ruckman and Sherman (1981) found that hematocrits in swimming males increased over time to enhance oxygen delivery to tissues. Perhaps it was the increased demand for oxygen in the exercise dams that counteracted the hemodilution effect of early pregnancy and prevented the drop seen between days 15 and 18 of the sedentary group.

Mean values and significant interactions for serum iron and total iron binding capacity (TIBC) can be seen in Table 16. There were no significant two factor interactions for serum iron concentration or TIBC. A gestational effect ($p<0.001$) for serum iron was seen with

Table 15. Exercise-Gestation Interactions for Hematocrit. Group Means.

Treatment		N	Hematocrit[1] (%)
Activity	Gestational Age (Day)		
Sedentary	15	14	41.7 ± 1.9^{A}
Sedentary	18	17	37.5 ± 1.8^{B}
Sedentary	21	19	39.4 ± 4.6^{AB}
Exercise	15	13	40.1 ± 2.2^{AB}
Exercise	18	15	38.8 ± 2.0^{B}
Exercise	21	17	38.9 ± 3.7^{B}

[1] Average for the group ± the standard deviation.

[A-B] Measures are different (Least Squares Means via pdiff; $p \leq 0.004$) within each group when superscripts are different.

Table 16. Main Effect and Two Factor Interactions for Serum Iron and Total Iron Binding Capacity.

Treatment	Serum Iron[1] (μg/dl)	N	p-value[2]	TIBC[1,3] (μg/dl)	N	p-value
Zinc marginal[4]	119.2± 63.9	46	N.S.[5]	397.7 ± 68.5	46	N.S.
Zinc adequate[6]	106.4 ± 76.4	40		409.0 ± 69.4	39	
Sedentary	103.9 ± 63.2	45	N.S.	384.0 ± 68.4	45	**
Exercise	123.5 ± 76.0	41		424.1 ± 63.3	40	
Day 15	180.4 ± 62.4[A]	31	***	442.1 ± 55.3[A]	31	***
Day 18	89.5 ± 34.3[B]	28		389.0 ± 55.6[B]	28	
Day 21	60.6 ± 37.3[B]	27		371.1 ± 75.3[B]	26	
Zinc x Exercise			N.S.			N.S.
Zinc x Gestation			N.S.			N.S.
Exercise x Gestation			N.S.			N.S.

[1]Average for the group ± the standard deviation.
[2]Significant differences via ANOVA for main effect and two factor interactions; *p0.05-0.01; **p0.01-0.001; ***p<0.001.
[3]TIBC = Total Iron Binding Capacity.
[4]Zinc marginal diet = 6.18 ppm.
[5]N.S. = Nonsignificant (p>0.05).
[6]Zinc adequate diet = 37.63 ppm.

[A-B]Measures are different (Least Squares Means via pdiff; p≤0.0167) within each group when superscripts are different.

iron concentrations decreasing throughout pregnancy. The serum iron concentration on day 15 was significantly greater than on day 18 and day 21. In turn, day 18 was significantly greater than day 21.

Exercise and gestation effects for TIBC were found. The TIBC for the sedentary group was significantly lower than for the exercise group. Like serum iron concentration, TIBC results revealed significantly higher values on day 15 of gestation as compared to days 18 or 21. No differences were found between days 18 and 21.

Decreasing serum iron reflects the increased demands of iron throughout gestation and the rapid turnover of iron to the fetus by the mother (Schwartz and Thurnau, 1995). In humans, TIBC greatly increases throughout pregnancy, but in rats, TIBC decreases with advancing gestational age (Morgan, 1961). This phenomenon may be due to a decrease in the rate of synthesis and/or an increase in the rate of destruction of transferrin, or possibly due to the placental transfer of transferrin to the fetus and was found throughout this study. The increased TIBC demonstrated in the exercise dams as compared to the sedentary group may be a reflection of the increased demand for iron during exercise (Hultman et al, 1988). Serum iron values were slightly higher in the exercise group but they were not statistically significant.

Tissue Analysis of Dam Liver and Spleen

Mean values and significant interactions for the weight and iron content of dam liver over the three treatment groups can be seen in Table 17 . Main effect and two factor interactions for dam liver weight (g wet tissue) were significant for zinc, gestation, and zinc x gestation.

Table 17. Main Effect and Two Factor Interactions for Dam Liver Weight and Iron Content of Dam Liver.

Treatment	Liver weight[1] (g wet tissue)	N	p-value[2]	Liver Iron Concentration[1] (μg/g dry tissue)	N	p-value
Zinc marginal[3]	10.670± 1.276	59	***	606.06± 199.23	59	***
Zinc adequate[4]	11.833± 2.025	53		527.24± 173.57	53	
Sedentary	11.307± 1.902	58	N.S.[5]	565.99± 181.46	58	N.S.
Exercise	11.128± 1.616	54		571.73± 202.09	54	
Day 15	10.473 ± 1.119[A]	37	***	720.25± 107.51[A]	37	***
Day 18	11.487± 1.301[B]	37		619.13± 115.53[B]	37	
Day 21	11.689± 2.370[B]	38		372.22± 140.92[C]	38	
Zinc x Exercise			N.S.			N.S.
Zinc x Gestation			***			N.S.
Exercise x Gestation			N.S.			N.S.

[1]Average for the group ± the standard deviation.
[2]Significant differences via ANOVA for main effect and two factor interactions; *p0.05-0.01; **p0.01-0.001; ***p<0.001.
[3]Zinc marginal diet = 6.18 ppm.
[4]Zinc adequate diet = 37.63 ppm.
[5]N.S. = Nonsignificant (p>0.05).

[A-C]Measures are different (Least Squares Means via pdiff; p≤0.0167) within each group when superscripts are different.

Table 18. Zinc-Gestation Interactions for Dam Liver Weight. Group Means.

Zinc	Gestational Age (Day)	N	Liver Weight[1] (g wet tissue)
Marginal[2]	15	19	10.477 ± 1.187^{A}
Marginal	18	20	11.218 ± 1.507^{A}
Marginal	21	20	10.307 ± 0.935^{AC}
Adequate[3]	15	18	10.468 ± 1.078^{A}
Adequate	18	17	11.805 ± 0.956^{ABD}
Adequate	21	18	13.225 ± 2.550^{B}

[1]Average for the group ± the standard deviation.
[2]Zinc marginal diet = 6.18 ppm.
[3]Zinc adequate diet = 37.63 ppm.

[A-D]Measures are different (Least Squares Means via pdiff; $p \leq 0.004$) within each group when superscripts are different.

Mean values for zinc x gestation can be seen in Table 18 . Statistically no differences in liver weight for the zinc marginal group were found between the three periods of gestation although the weight increased somewhat from day 15 to 18 and began to decrease again by day 21. The liver weight of the zinc adequate group rose over time and was significantly lower on day 15 compared to day 21. The liver weight of the zinc marginal dams versus the zinc adequate dams revealed no differences for days 15 and 18 of gestation. On day 21, the zinc marginal liver weight was significantly lower than the zinc adequate liver weight. Increasing liver weight occurs normally throughout pregnancy in rats but dietary restriction can cause significant decreases in liver weight and if the restriction is severe enough, a net loss in weight can be seen (Reynolds et al, 1984). Liver weight did not increase with the zinc marginal group because tissue catabolism occurs in the marginally zinc deficient dam, late in gestation, to satisfy the zinc demands of the fetus (Masters et al, 1986). In addition, it was found that the dietary intake of the zinc marginal group decreased significantly by day 21.

Main effect interactions for iron content of the dam liver (μg/g dry tissue) were significant for zinc and gestation. The zinc marginal group had a significantly higher liver iron content than the zinc adequate group. Increased liver iron stores have been noted previously in marginally deficient dams (Rogers et al, 1987) and may be due in part to enhanced absorption of iron (Solomons, 1983; Bloxom et al, 1989). As a whole, liver iron content decreased throughout pregnancy and statistical significance was found among all three days of gestation. This decrease in stores may reflect the increased demands of of iron throughout gestation.

Table 19. Main Effect and Two Factor Interactions for Dam Spleen Weight and Iron Content of Dam Spleen.

Treatment	Spleen weight[1] (g wet tissue)	N	p-value[2]	Spleen Iron Content[1] (mg/g dry tissue)	N	p-value
Zinc marginal[3]	0.760 ± 0.221	59	***	2.58 ± 0.99	59	N.S.[4]
Zinc adequate[5]	0.856 ± 0.126	53		2.55 ± 0.73	53	
Sedentary	0.819 ± 0.199	58	N.S.	2.73 ± 0.93	58	*
Exercise	0.791 ± 0.175	54		2.39 ± 0.78	54	
Day 15	0.902 ± 0.098[A]	37	***	2.39 ± 0.69[A]	37	*
Day 18	0.882 ± 0.118[A]	37		2.42 ± 0.79[A]	37	
Day 21	0.635 ± 0.194[B]	38		2.87 ± 1.04[A]	38	
Zinc x Exercise			N.S.			N.S.
Zinc x Gestation			***			*
Exercise x Gestation			N.S.			N.S.

[1]Average for the group ± the standard deviation.
[2]Significant differences via ANOVA for main effect and two factor interactions; *p0.05-0.01; **p0.01-0.001; ***p<0.001.
[3]Zinc marginal diet = 6.18 ppm.
[4]N.S. = Nonsignificant (p>0.05).
[5]Zinc adequate diet = 37.63 ppm.

[A-B]Measures are different (Least Squares Means via pdiff; p≤ 0.0167) within each group when superscripts are different.

Table 20. Zinc-Gestation Interactions for Dam Spleen Weight. Group Means.

Zinc	Gestational Age (Day)	N	Spleen Weight[1] (g wet tissue)
Marginal[2]	15	19	0.924 ± 0.089^{A}
Marginal	18	20	0.860 ± 0.115^{AC}
Marginal	21	20	0.503 ± 0.144^{B}
Adequate[3]	15	18	0.879 ± 0.103^{AC}
Adequate	18	17	0.910 ± 0.119^{A}
Adequate	21	18	0.783 ± 0.123^{C}

[1]Average for the group ± the standard deviation.
[2]Zinc marginal diet = 6.18 ppm.
[3]Zinc marginal diet = 37.63 ppm.

[A-C] Measures are different (Least Squares Means via pdiff; $p \leq 0.004$) within each group when superscripts are different.

Mean values and significant interactions for dam spleen weight and iron content over the three treatment groups can be seen in Table 19. Analysis of dam spleen weight (g wet tissue) revealed main effect and two factor interactions for zinc, gestation,and zinc x gestation. The group means for zinc x gestation are seen in Table 20. The spleen weight for the zinc marginal group decreased throughout gestation and was significantly lower on day 21 as opposed to days 15 and 18. The weight for the zinc adequate group was lower on day 21 when compared to day 18 ($p=0.0027$) but no differences were seen between day 15 and days 18 or 21. No gestation effect between the zinc marginal and zinc adequate dams for days 15 and 18 were seen but on day 21 the zinc marginal spleen weight was significantly less than the zinc adequate weight. Like liver weight, the decreased spleen weight observed by day 21 was in response to the increased zinc demands of the fetus and mobilization of zinc from the tissues to the fetus. The lower spleen weight on day 21 of the zinc adequate dam may be after increased red blood cell mass expansion and release of the red blood cells from the spleen to the circulation (Lu et al, 1991; Roodenburg, 1995) since red blood cells are stored in great quantities in the spleen (Romer and Parsons, 1978).

The dam spleen iron content (mg/g dry tissue) revealed main effect and two factor interactions for exercise, gestation, and zinc x gestation. When examining the exercise effect, the spleen iron content for the sedentary group was significantly higher than for the exercise group and is in agreement with Ruckman and Sherman (1981) who hypothesized that this iron was mobilized to the bone marrow for increased red blood cell synthesis during exercise.

Table 21. Zinc-Gestation Interactions for Dam Spleen Iron Content. Group Means.

Zinc	Gestational Age (Day)	N	Spleen Iron Content[1] (mg/g dry tissue)
Marginal[2]	15	19	2.25 ± 0.77^{A}
Marginal	18	20	2.31 ± 0.83^{A}
Marginal	21	20	3.16 ± 1.11^{B}
Adequate[3]	15	18	2.54 ± 0.57^{AB}
Adequate	18	17	2.56 ± 0.75^{AB}
Adequate	21	18	2.55 ± 0.88^{AB}

[1]Average for the group ± the standard deviation.
[2]Zinc marginal diet = 6.18 ppm.
[3]Zinc adequate diet = 37.63 ppm.

[A-B]Measures are different (Least Squares Means via pdiff; $p \leq 0.004$) within each group when superscripts are different.

Although the ANOVA for the gestation effect revealed a p-value < 0.05, the least squares means analysis via pdiff (with a Bonferroni's correction factor = 0.0167) revealed no significant differences between the three gestational ages for spleen iron content . This is inconsequential since there was a significant two factor interaction between zinc and gestation. The group means for zinc x gestation interactions can be seen in Table 21. Dam spleen iron content for the zinc marginal dams increased throughout pregnancy with the content on day 21 significantly higher than on days 15 and 18. The spleen stores greatly increased at the point where the effects of marginal deficiency became evident and again may be a reflection of enhanced iron absorption. These findings disagree with those of Rogers et al (1987) who found decreased spleen iron stores in the marginally deficient dam. Spleen iron content for the zinc adequate group remained fairly constant throughout the study. When comparing the content of the zinc marginal group to the zinc adequate group for each gestation day, no statistical significance was found.

Dam Total Body Weight Values

Mean values and significant interactions for dam body weight throughout the study can be seen in Table 22. No differences (main effect or two factor) were found for weight at conception.

Dam weight at sacrifice and total weight gain revealed main effect interactions for zinc, exercise, and gestation and a two factor interaction for zinc x gestation. The exercise effect for weight at sacrifice and total weight gain revealed that the weight of the exercise group was lower than the sedentary group ($p<0.05$) . The same findings were reported by Cameron

Table 22. Main Effect and Two Factor Interactions for Weight Gain of Dams.

Treatment	Weight at Conception[1] (g)	N	p-value[2]	Weight at Sacrifice[1] (g)	N	p-value	Total Weight Gain[1] (g)	N	p-value
Zinc marginal[3]	245 ± 20	59	N.S.[4]	303 ± 26	59	***	58 ± 17	59	***
Zinc adequate[5]	244 ± 20	53		324 ± 38	53		80 ± 31	53	
Sedentary	243 ± 19	58	N.S.	320 ± 33	58	*	77 ± 25	58	***
Exercise	246 ± 21	54		305 ± 33	54		59 ± 25	54	
Day 15	245 ± 20[A]	37	N.S.	294 ± 22[A]	37	***	49 ± 10[A]	37	***
Day 18	243 ± 19[A]	37		316 ± 25[B]	37		73 ± 16[B]	37	
Day 21	246 ± 21[A]	38		328 ± 42[C]	38		82 ± 35[C]	38	
Zinc x Exercise			N.S.			N.S.			N.S.
Zinc x Gestation			N.S.			***			***
Exercise x Gestation			N.S.			N.S.			N.S.

[1]Average for the group ± the standard deviation.
[2]Significant differences via ANOVA for main effect and two factor interactions; *p0.05-0.01; **p0.01-0.001; ***p<0.001.
[3]Zinc marginal diet = 6.18 ppm.
[4]N.S. = Nonsignificant (p>0.05).
[5]Zinc adequate diet = 37.63 ppm.

[A-C]Measures are different (Least Squares Means via pdiff; p≤0.0167) within each group when superscripts are different.

Table 23. Zinc-Gestation Interactions for Dam Weight at Sacrifice. Group Means.

Zinc	Gestational Age (Day)	N	Dam Weight at Sacrifice[1] (g)
Marginal[2]	15	19	298 ± 24^{A}
Marginal	18	20	309 ± 28^{A}
Marginal	21	20	301 ± 26^{A}
Adequate[3]	15	18	289 ± 19^{AC}
Adequate	18	17	324 ± 18^{AD}
Adequate	21	18	359 ± 35^{B}

[1]Average for the group ± the standard deviation.
[2]Zinc marginal diet = 6.18 ppm.
[3]Zinc adequate diet = 37.63 ppm.

[A-D]Measures are different (Least Squares Means via pdiff; $p \leq 0.004$) within each group when superscripts are different.

(1985), who swam pregnant rats, and Clapp and Dickstein (1984), who studied the effects of endurance exercise on pregnant women. Savard et al (1986) found that swimming during pregnancy, decreased fat stores in dam rats.

Zinc x gestation group means for dam weight at sacrifice can be seen in Table 23. Weight did not change for the zinc marginal groups at all three gestational ages. However, weight for the zinc adequate dams increased with age and all three weights were significantly different from each other. Comparisons of weight at sacrifice showed no effect between the zinc marginal and zinc adequate groups on days 15 and 18 of gestation. On day 21, the weight of the zinc marginal group was significantly lower than the zinc adequate group.

Group means for the zinc x gestation interaction for total weight gain can be seen in Table 24. The zinc marginal dams gained weight between days 15 and 18 ($p=0.0001$) but by day 21 their weight had decreased and was no different from the weight of the dams on day 15. Weight gain for the zinc adequate dams continued throughout pregnancy and was significant at all three ages. Like dam weight at sacrifice, that total weight gain of the zinc marginal dams and the zinc adequate dams was approximately the same on days 15 and 18. On day 21, the weight gain of the zinc marginal group was less than half the gain of the zinc adequate group ($p=0.0001$).

Weight gain during pregnancy is vital in ensuring a good outcome (Institute of Medicine, 1990). Marginal zinc intake during pregnancy is associated with lack of weight gain and at times even weight loss (Reinstein et al, 1984; Rogers et al, 1985; Golub et al, 1984) and held

Table 24. Zinc-Gestation Interactions for Total Weight Gain of Dams. Group Means.

Zinc	Gestational Age (Day)	N	Total Weight Gain of Dam[1] (g)
Marginal[2]	15	19	50 ± 11^{A}
Marginal	18	20	70 ± 17^{B}
Marginal	21	20	54 ± 17^{A}
Adequate[3]	15	18	48 ± 9^{A}
Adequate	18	17	77 ± 14^{B}
Adequate	21	18	114 ± 19^{C}

[1]Average for the group ± the standard deviation.
[2]Zinc marginal diet = 6.18 ppm.
[3]Zinc adequate diet = 37.63 ppm.

[A-C]Measures are different (Least Squares Means via pdiff; $p \leq 0.004$) within each group when superscripts are different.

Table 25. Main Effect and Two Factor Interactions for Measurements of Exercise Training of Dams.

Treatment	Citrate Synthase Activity of Gastrocnemius Muscle[1] (μM/min/g wet tissue)	N	p-value[2]	Weight of Left Gastrocnemius Muscle[1] (g wet tissue)	N	p-value	Heart Weight[1] (g wet tissue)	N	p-value
Zinc marginal[3]	5645.6 ± 1826.6	31	N.S.[4]	1.116 ± 0.128	59	*	1.090 ± 0.120	49	N.S.
Zinc adequate[5]	5695.8 ± 2094.1	27		1.166 ± 0.111	52		1.096 ± 0.129	48	
Sedentary	5936.0 ± 2029.7	31	N.S.	1.138 ± 0.123	57	N.S.	1.051 ± 0.119	50	***
Exercise	5362.3 ± 1816.6	27		1.140 ± 0.123	54		1.137 ± 0.114	47	
Day 15	5812.5 ± 1841.5^{A}	17	N.S.	1.156 ± 0.112^{A}	37	**	1.139 ± 0.131^{A}	28	**
Day 18	5632.4 ± 1876.9^{A}	21		1.178 ± 0.101^{A}	36		1.102 ± 0.098^{AB}	33	
Day 21	5585.3 ± 2163.2^{A}	20		1.086 ± 0.135^{B}	38		1.048 ± 0.128^{B}	36	
Zinc x Exercise			N.S.			N.S.			N.S.
Zinc x Gestation			N.S.			*			N.S.
Exercise x Gestation			N.S.			N.S.			N.S.

[1]Average for the group ± the standard deviation.
[2]Significant differences via ANOVA for main effect and two factor interactions; *p0.05-0.01; **p0.01-0.001; ***p<0.001.
[3]Zinc marginal diet = 6.18 ppm.
[4]N.S. = Nonsignificant (p>0.05).
[5]Zinc adequate diet = 37.63 ppm.

[A-B]Measures are different (Least Squares Means via pdiff; p≤0.0167) within each group when superscripts are different.

true with a zinc marginal intake of 6.17 ppm in this study.

Measurements of Exercise Training

Mean values and significant interactions for the parameters used to follow exercise training (citrate synthase activity, gastrocnemius muscle and heart weight) can be seen in Table 25. No effects were seen on citrate synthase acitivity for any of the three treatment groups. This is in disagreement with Savard et al (1986) who found significant increases in citrate synthase activity. The dams, in that study however, were trained before pregnancy, their exercise regime was more strenuous (6 hours/day; 6 days/week), and they exercised until day 21. Gagne (1985) found citrate synthase response in older, female rats who swam 1 hour/day for six weeks. Lack of response in this present study may have been due to the cessation of exercise after day 18 or in sample collection. The gastrocnemius is composed of a mixture of white and red muscle fibers, with the highest oxidative capacity in the red fibers (Baldwin et al, 1972). Optimal results may have been obtained if the muscle had been separated to the white and red fiber components.

Muscle weight measurements for the left gastrocnemius muscle did reveal main effect and two factor interactions for zinc, gestation, and zinc x gestation. Group means for zinc x gestation are listed in Table 26. Muscle weight for the zinc marginal group decreased with age and was significantly lower on day 21 as compared to day 15. No differences in muscle weight were found in the zinc adequate group over the three gestational ages. When comparing the muscle weights of the zinc marginal to zinc adequate groups, no differences were found on days 15 and 18, however on day 21, the zinc marginal muscle weighed

Table 26. Zinc-Gestation Interactions for Weight of Dam Gastrocnemius Muscle.
Group Means.

Zinc	Gestational Age (Day)	N	Weight of Gastrocnemius Muscle[1] (g wet tissue)
Marginal[2]	15	19	1.176 ± 0.107^{A}
Marginal	18	20	1.139 ± 0.100^{AB}
Marginal	21	20	1.035 ± 0.135^{B}
Adequate[3]	15	18	1.135 ± 0.117^{AB}
Adequate	18	16	1.225 ± 0.081^{A}
Adequate	21	18	1.144 ± 0.112^{A}

[1]Average for the group ± the standard deviation.
[2]Zinc marginal diet = 6.18 ppm.
[3]Zinc adequate diet = 37.63 ppm.

[A-B]Measures are different (Least Squares Means via pdiff; $p \leq 0.004$) within each group when superscripts are different.

significantly less than the zinc adequate muscle. Again, this is most likely a reflection of catabolism of tissues during zinc deficiency. It is thought that zinc mobilized to the fetus late in pregnancy during a zinc deficient state is derived primarily from the muscle (Masters et al, 1986).

Analysis for dam heart weight showed two main effect interactions with exercise and gestation. The heart weight for the exercise group was greater than that of the sedentary group ($p<0.05$) and correlates to studies by Oscai et al (1971) and Gagne (1985) in rats. Cardiomegaly in exercise occurs due to increased capacity of the heart to deliver blood and oxygen to the muscles (Ruckman and Sherman, 1981). Heart weight decreased with gestational age independent of zinc or exercise treatment with a significant difference in weight between days 15 and 21. When examining heart weights of the baseline rats, it was discovered that an average heart weight of a nonpregnant rat was 0.96 g wet tissue. Heart weight in dams increased to 1.139 ± 0.131 g wet tissue by day 15 of gestation revealing a pregnancy induced cardiomegaly resulting from increased cardiac output, (due to increased stroke volume and heart rate), and an increased plasma volume (Nageotte and Porto, 1988). The pregnant body eventually compensates by decreasing peripheral vascular resistance and thus decreasing work by the heart. Since the heart is a muscle, perhaps this decreased work allowed for the heart weight loss seen with advancing gestational age.

Weight of Products of Conception and Dam Uterus

Table 27 shows mean values and significant interactions for placental weight, uterine weight, and weight of fetus, placenta, and uterus combined. When measuring placental

Table 27. Main Effect and Two Factor Interactions for the Weights of the Placenta, Uterus and Fetus.

Treatment	Placenta weight[1] (g wet tissue)	N	p-value[2]	Uterus weight[1] (g wet tissue)	N	p-value	Placenta, Uterus, and Fetus Weight[1] (g wet tissue)	N	p-value
Zinc marginal[3]	4.249 ± 1.502	59	N.S.[4]	3.469 ± 0.753	59	**	35.238 ± 19.944	58	***
Zinc adequate[5]	4.319 ± 1.840	53		3.745 ± 1.075	53		40.569 ± 27.625	52	
Sedentary	4.323 ± 1.674	58	N.S.	3.672 ± 0.943	58	N.S.	38.242 ± 23.741	57	N.S.
Exercise	4.238 ± 1.666	54		3.521 ± 0.909	54		37.238 ± 24.331	53	
Day 15	2.373 ± 0.722[A]	37	***	2.540 ± 0.553[A]	37	***	11.858 ± 2.501[A]	35	***
Day 18	4.569 ± 0.794[B]	37		4.061 ± 0.492[B]	37		32.674 ± 4.638[B]	37	
Day 21	5.862 ± 0.942[C]	38		4.182 ± 0.596[B]	38		66.564 ± 12.103[C]	38	
Zinc x Exercise			N.S.			N.S.			N.S.
Zinc x Gestation			N.S.			*			***
Exercise x Gestation			N.S.			N.S.			N.S.

[1]Average for the group ± the standard deviation.
[2]Significant differences via ANOVA for main effect and two factor interactions; *p0.05-0.01; **p0.01-0.001; ***p<0.001.
[3]Zinc marginal diet = 6.18 ppm.
[4]N.S. = Nonsignificant (p>0.05)
[5]Zinc adequate diet = 37.63 ppm.

[A-C]Measures are different (Least Squares Means via pdiff; p≤0.0167) within each group when superscripts are different.

Table 28. Zinc-Gestation Interactions for Dam Uterus Weight. Group Means.

Zinc	Gestational Age (Day)	N	Dam Uterus Weight[1] (g wet tissue)
Marginal[2]	15	19	2.573 ± 0.381^{A}
Marginal	18	20	3.905 ± 0.436^{B}
Marginal	21	20	3.890 ± 0.467^{B}
Adequate[3]	15	18	2.506 ± 0.700^{A}
Adequate	18	17	4.244 ± 0.503^{BC}
Adequate	21	18	4.511 ± 0.558^{C}

[1]Average for the group ± the standard deviation.
[2]Zinc marginal diet = 6.18 ppm.
[3]Zinc adequate diet = 37.63 ppm.

[A-C]Measures are different (Least Squares Means via pdiff; $p \leq 0.004$) within each group when superscripts are different.

weight of the dams, an effect was seen with gestation only. The placenta weight increased with gestational age and each day was significantly different from the other. This corresponds to the natural progression of tissue growth and weight gain during pregnancy (Institute of Medicine, 1990).

Uterus weight was affected by both zinc and gestation and showed a significant zn x gestation interaction. Group means can be seen in Table 28. The uterus weight of both zinc marginal and zinc adequate dams weighed significantly less at day 15 when compared to days 18 and 21 and reflects the tissue growth demands of pregnancy. Comparison of the zinc marginal to zinc adequate uterine weight revealed no differences on days 15 and 18. The weight of the zinc marginal uterus on day 21 was much lower than the zinc adequate uterus ($p=0.0004$) and, as seen with all maternal organ weights, is probably due to mobilization of zinc from dam tissues to the fetus.

The data for total weight of the fetus, placenta and uterus showed interactions for zinc, gestation, and zinc x gestation ($p<0.0001$). Group means for zinc x gestation are listed in Table 29. The total weight increased at each gestational age regardless of zinc treatment. No weight differences were seen between zinc marginal and zinc adequate groups on days 15 and 18 however the total weight on day 21 was significantly lower for the zinc marginal group when compared to the zinc adequate group.

Mean values and significant interactions for total number of pups and resorptions are shown in Table 30. An effect of zinc x gestation was seen with total number of pups per litter. Group means are listed in Table 31. No differences were found among the zinc

Table 29. Zinc-Gestation Interactions for the Total Weight of Placenta, Uterus, and Fetus.
Group Means.

Zinc	Gestational Age (Day)	N	Weight of Placenta, Uterus, and Fetus[1] (g wet tissue)
Marginal[2]	15	18	12.508 ± 2.318^{A}
Marginal	18	20	31.728 ± 4.049^{B}
Marginal	21	20	59.206 ± 8.061^{C}
Adequate[3]	15	17	11.170 ± 2.570^{A}
Adequate	18	17	33.787 ± 5.147^{B}
Adequate	21	18	74.740 ± 10.569^{D}

[1]Average for the group ± the standard deviation.
[2]Zinc marginal diet = 6.18 ppm.
[3]Zinc adequate diet = 37.63 ppm.

[A-D]Measures are different (Least Squares Means via pdiff; $p \leq 0.004$) within each group when superscripts are different.

Table 30. Main Effect and Two Factor Interactions for Total Number of Pups and Resorptions.

Treatment	Total Number of Pups per Litter[1]	N	p-value[2]	Total Number of Resorptions[1]	N	p-value
Zinc marginal[3]	13 ± 3	59	N.S.[4]	0.8 ± 1.1	59	N.S.
Zinc adequate[5]	13 ± 3	53		0.7 ± 0.8	53	
Sedentary	13 ± 3	58	N.S.	0.7 ± 1.0	58	N.S.
Exercise	13 ± 3	54		0.8 ± 1.0	54	
Day 15	13 ± 3	37	N.S.	0.9 ± 1.0	37	N.S.
Day 18	14 ± 2	37		0.7 ± 0.7	37	
Day 21	13 ± 2	38		0.7 ± 1.2	38	
Zinc x Exercise			N.S.			N.S.
Zinc x Gestation			**			N.S.
Exercise x Gestation			N.S.			N.S.

[1]Average for the group ± the standard deviation.
[2]Significant differences via ANOVA for main effect and two factor interactions; *p0.05-0.01; **p0.01-0.001; ***p<0.001.
[3]Zinc marginal diet = 6.18 ppm.
[4]N.S. = Nonsignificant (p>0.05).
[5]Zinc adequate diet = 37.63 ppm.

Table 31. Zinc-Gestation Interactions for Total Number of Pups per Litter.
Group Means.

Zinc	Gestational Age (Day)	N	Total Number of Pups per Litter[1]
Marginal[2]	15	19	14 ± 3^{A}
Marginal	18	20	13 ± 2^{A}
Marginal	21	20	12 ± 2^{A}
Adequate[3]	15	18	12 ± 3^{AB}
Adequate	18	17	15 ± 2^{AC}
Adequate	21	18	14 ± 2^{A}

[1]Average for the group ± the standard deviation.
[2]Zinc marginal diet = 6.18 ppm.
[3]Zinc adequate diet = 37.63 ppm.

[A-C]Measures are different (Least Squares Means via pdiff; $p \leq 0.004$) within each group when superscripts are different.

marginal group at any gestational age. Total number of pups for the zinc adequate group was significantly higher at day 18 when compared to day 15 of gestation. Comparison of the number of pups per zinc marginal litter versus zinc adequate litter was nonsignificant at all gestational ages.

No significant main effect or two factor interactions wer found for total number of resorptions.

Average Fetal Weight and Fetal Iron Concentration

Table 32 displays mean average fetal weight and fetal iron concentrations and significant interactions for all three treatments. Significant interactions for average fetal weight occurred with gestation and zinc x gestation. Group means for zinc x gestation are listed in Table 33. As with placental weight, average fetal weight significantly increased at each gestational age for both zinc marginal and zinc adequate fetuses. No weight differences were seen between zinc marginal and adequate groups at any of the three gestational days.

Experimental effects were seen with zinc, gestation, and zinc x gestation for fetal iron. The group means for zinc x gestation are seen in Table 34. The fetal iron concentration for both the zinc marginal and zinc adequate fetuses increased significantly between days 15 and 18 in both the zinc marginal and adequate groups. Iron demands increase greatly at the beginning of the third trimester in humans (Schwartz and Thurnau, 1995) and day 18 coincides with this time. Throughout the present data, day 18 has been the day in which diminished zinc status has become apparent. The uptake of iron by the zinc marginal fetus was greatly enhanced at this point as compared to day 21. Perhaps the fetus adjusted the

transfer of iron across the placenta to better satisfy its needs, since fetal iron concentration was not significantly higher in the marginal group compared to the adequate fetuses on day 21. Rogers et al (1987) found fetal iron concentrations were much higher in the marginal fetus and there were no significant differences in fetal iron concentration early in gestation when teratogenesis occurs. The current data agrees with this point at midpregnancy only.

Histologic Exam of the Stomach

Of the 121 gastrointestinal tracts examined, 8 stomachs were sent to the Virginia-Maryland Regional College of Veterinary Medicine for histologic exam of the gastric mucosa. All eight tissue specimens belonged to dams that exercised and belonged to either the zinc marginal or zinc adequate diet group. In six of the stomachs, some degree of superficial to deep mucosal errosion was found. No statistical analysis was performed on this data because of the small cell size examined. (Appendix XIII). Hemorrhagic gastritis has been found in long distance runners (Moses, 1990) however most likely the gastrointestinal effects found in this study were due to the stress of forced swimming (Nishimura, 1988).

Table 32. Main Effect and Two Factor Interactions for Average Fetal Weight and Iron Content of Fetus.

Treatment	Average Fetal Weight[1] Fetal weight (g wet tissue) / number of pups per litter	N	p-value[2]	Fetal Iron Content[1] (μg/g dry tissue)	N	p-value
Zinc marginal[3]	1.600 ± 1.373	59	N.S.[4]	414.59 ± 123.89	59	**
Zinc adequate[5]	1.634 ± 1.521	52		372.05 ± 101.66	53	
Sedentary	1.680 ± 1.464	58	N.S.	394.79 ± 112.80	58	N.S.
Exercise	1.546 ± 1.419	53		394.10 ± 119.18	54	
Day 15	0.231 ± 0.031^{A}	37	***	262.49 ± 43.61^{A}	37	***
Day 18	1.067 ± 0.125^{B}	37		485.59 ± 84.38^{B}	37	
Day 21	3.550 ± 0.415^{C}	37		434.21 ± 61.86^{C}	38	
Zinc x Exercise			N.S.			N.S.
Zinc x Gestation			*			**
Exercise x Gestation			N.S.			N.S.

[1]Average for the group ± the standard deviation.
[2]Significant differences via ANOVA for main effect and two factor interactions; *p0.05-0.01; **p0.01-0.001; ***p<0.001.
[3]Zinc marginal diet = 6.18 ppm.
[4]N.S. = Nonsignificant (p>0.05).
[5]Zinc adequate diet = 37.63 ppm.

[A-C]Measures are different (Least Squares Means via pdiff; p≤0.0167) within each group when superscripts are different.

Table 33. Zinc-Gestation Interactions for Average Fetal Weight. Group Means.

Zinc	Gestational Age (Day)	N	Average Fetal Weight[1] Fetal weight (g wet tissue) / Number of pups per litter
Marginal[2]	15	19	0.229 ± 0.026^{A}
Marginal	18	20	1.100 ± 0.133^{B}
Marginal	21	20	3.406 ± 0.394^{C}
Adequate[3]	15	18	0.233 ± 0.037^{A}
Adequate	18	17	1.031 ± 0.109^{B}
Adequate	21	17	3.719 ± 0.384^{C}

[1]Average for the group ± the standard deviation.
[2]Zinc marginal diet = 6.18 ppm.
[3]Zinc adequate diet = 37.63 ppm.

[A-C]Measures are different (Least Squares Means via pdiff; $p \leq 0.004$) within each group when superscripts are different.

Table 34. Zinc-Gestation Interactions for Fetal Iron Concentration. Group Means.

Zinc	Gestational Age (Day)	N	Fetal Iron Concentration[1] (μg/g of dry tissue)
Marginal[2]	15	19	256.11 ± 28.90^{A}
Marginal	18	20	522.21 ± 70.68^{B}
Marginal	21	20	457.52 ± 42.63^{C}
Adequate[3]	15	18	269.23 ± 55.22^{A}
Adequate	18	17	442.51 ± 80.26^{C}
Adequate	21	18	408.31 ± 70.35^{C}

[1]Average for the group ± the standard deviation.
[2]Zinc marginal diet = 6.18 ppm.
[3]Zinc adequate diet = 37.63 ppm.

[A-C]Measures are different (Least Squares Means via pdiff; $p \leq 0.004$) within each group when superscripts are different.

SUMMARY

The effects of a zinc marginal diet and exercise on the iron status of pregnant Sprague-Dawley rats were studied. The dams were fed a diet consisting of either a marginal (6.17 ppm) or adequate (37.63 ppm) amount of zinc sulfate. Exercise consisted of swimming one hour per day, 7 days per week until day 18 of gestation. One half of the animals swam, while the other remained sedentary throughout the study. Data was collected, on days 15, 18, or 21 of gestation, to examine the iron status of the animals; maternal weight gain; diet intake; effects of training; fetal weight gain and total fetal iron content.

The effects of a marginal zinc intake during pregnancy became apparent between days 18 and 21 of gestation and affected many maternal parameters. Significant differences were found with exercise but zinc intake seemed to predominate the results. Perhaps more of an exercise effect would have been found if the dams had continued to exercise until day 21.

The iron status of the dams was determined by hemoglobin, hematocrit, serum iron and TIBC, and liver and spleen iron content. Hemoglobin and hematocrits of the zinc adequate dams declined throughout pregnancy. This was seen with the zinc marginal dams until day 18, when levels began to rise. The hemoglobin and hematocrits of the zinc marginal dams were greater than those of the adequate dams on day 21. Liver and spleen iron concentrations were greatest in the zinc marginal dams. The decreased zinc status favored uptake and storage of iron. It would have been helpful to measure fecal iron excretion to see if enhanced absorption did occur or if the increase in liver and spleen iron content may have

been due to their decreased tissue weights and a resulting concentration effect in the smaller tissues.

The exercise dams' hematocrit values remained fairly stable throughout the study, while those of the sedentary dams' declined until day 18 and began to rise by day 21. The hematocrit response of the sedentary group is a normal response at midpregnancy when the red blood cell mass begins to "catch up" with the increasing plasma volume. The increased demands for oxygen, and thus red blood cells, during exercise may have generated a larger red cell mass earlier in pregnancy preventing the expected decline in hematocrit. The TIBC was greater, and spleen iron less, in the exercise group, again reflecting the increase demand for iron with exercise.

Serum iron and TIBC declined throughout pregnancy regardless of zinc intake or exercise.

Intake of purified diet decreased by day 21 in the zinc marginal dams and was significantly less than the intake in the zinc adequate group for the same day. Total weight gain increased throughout gestation in the zinc adequate rats. However, no changes were seen from day 15 to 21 in the zinc marginal group. The total weight gain was significantly less on day 21 as compared to the same day in the adequate group. This trend was seen in the zinc marginal group with all the maternal organ weights measured and is a reflection of diminished zinc nutriture. A group of dams pair-fed to the marginal zinc group would have helped determine whether marginal zinc intake affected maternal iron status and weights or if differences were due to the anorexia found later in pregnancy in the zinc marginal group.

The heart weight decreased regardless of zinc or exercise status and reflected a cardiac hypertrophy that began to resolve after day 15.

The only parameter that reflected a training effect was maternal heart weight which was increased in the exercise group. Difficulties in obtaining a complete gastrocnemius sample early in the study, may have skewed the results. In future studies, it may be beneficial to assay either red or white muscle fibers in the gastrocnemius since the oxidative capacity of the red is much higher than the white. Another suggestion would be to measure heart and muscle myoglobin levels to better determine training effects and their demands on iron. It would have been interesting to see how the dams would have been affected if they continued to swim until day 21 of gestation.

The fetus and placenta were for the most part unhampered by the experimental protocol. Fetal iron concentrations on day 18 were significantly higher in the zinc marginal than the zinc adequate and reflects the point when signs of maternal zinc deficiency occurred. This is also when fetal demands for iron are great. In retrospect, greater care in examining the fetuses for malformations would have helped determine any further zinc effects. Placental iron concentration would have been a good indicator of iron transfer to the fetus throughout gestation.

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APPENDIX I

Nitric-Perchloric Wet Acid Oxidation

Procedure:

1. Weigh 50 mg of sample into 50ml clean (acid washed) beakers and cover with watchglass.

2. Prepare two beakers for blanks.

3. Add 3.0ml of redistilled nitric acid and allow to sit for approximately 12 hours.

4. Turn on perchloric hood and hotplate to 150°F.

5. Add 1.5ml of 70% perchloric acid and heat at 150°F until sample dissolves and foaming ceases.

6. Increase temperature slowly to a maximum of 325°F and allow samples to reflux in acid until clear.

7. Remove watchglasses and dry down to a white ash. If charring occurs, add nitric acid dropwise, after the beaker is cooled, and allow to reflux with watchglass on.

8. When samples have dried to a white ash, dissolve in 10ml of 10% HCl (from 6M Ultrapure HCl).

9. Cover beakers containing ash and 10% HCl with parafilm and allow to sit at least 2 hours before emptying into labelled polypropylene tubes. Stopper tubes tightly and refrigerate.

10. Make appropriate mineral standards.

11. Shake samples well and allow them to reach room temperature.

12. Samples are now ready to be read on an Atomic Absorption Spectrophotometer.

Atomic Absorption Spectrophotometry

1. Start up the instrument according to the procedure specified for the Perkin-Elmer M2100 AAS (Perkin-Elmer,Norwalk,Ct.) in the procedure manual.

2. Construct standard curve using Zinc and Iron standards.
   Zinc standards: 0.5ppm, 1ppm, 2ppm
   Iron standards: 4ppm, 6ppm, 10ppm

3. Run samples. Percent error between duplicates should be no greater than 10%.

4. Once sample run is completed, follow shutdown procedure outlined in the procedure manual.

APPENDIX II

Kjeldahl Nitrogen Determination

A. Block Digestion Procedure

1. Turn block digestor ON - Takes 1 to 1 1/2 hours to warm up. Begin with temperature set on 390°F.

2. Place sample in block digestion tube. (Approximately 750mg of diet run in duplicate from each batch prepared).

3. Add 10 gms Na_2SO_4 - $CuSO_4$ mixture (one measure) and 4 glass beads to each tube. (Add this and filter paper to 2 clean, dry tubes to be used as blanks).

4. Pour 25 mls H_2SO_4 down the side of the tube (slowly turning tube as you pour) to wash down any sample adhering to the neck of the tube.

5. Set rack containing tubes into block digestor. Turn water on and turn heat up to 440°F.

6. Let samples digest until a clear light, green color is obtained. Heat for additional 30 minutes. Remove rack from block digestor and allow samples to cool until they can be handled by hand.

7. Slowly add distilled water (250 mls) down the side of tube and up to white line on neck of tube.

8. Pour into labeled Kjeldahl flask and stopper tightly. Allow samples to cool before continuing distillation.

B. Distillation Procedue

1. Pour about 25 ml of 4% boric acid and 25 ml H_2O into 500 ml Erlenmeyer flask (use graduated cylinder).

2. Add 4 drops of mixed Indicator (methyl red-methylene blue).

3. Place flask under the distillation rack. Turn on water to the condenser. Check to be sure cold water is flowing.

4. Turn on burners to 2 to let them warm up. Swirl each flask to mix contents.

5. To each Kjeldahl flask add approximately 1/16 teaspoon of granular zinc, then immediately add 85 to 90 ml of 50% NaOH. Add the NaOH slowly down the side of the flask to layer the NaOH below the diluted sulfuric acid digest.

6. Connect the flask with the distillation rack. Swirl slowly and then vigorously to mix the contents of the flask. Turn the heat up to 5 immediately and place the label on the receiving flask. If the mixture does not turn blue the acid was not neutralized and more NaOH should be added to the samples.

7. Distill until about 150 mls are in the collection flask. Lower the collection flask so the tube is out of the liquid. Distill to 200 mls total.

8. Turn off the heat. Turn off water about 15 minutes after heat has been turned off.

C. Titration Procedure

1. Titrate samples in the order of coming off the distillation rack, titrating the blanks first.

2. Place a magnetic stir bar into the flask to be titrated. Titrate samples with 0.1N HCl until a slight purple color appears matching the end-point of the blank.

3. Record the volume of 0.1N HCl used to reach the endpoint.

D. Calculations

1. Determine weight of nitrogen present. Calculate how much nitrogen would be in one ml solution having same normality of the HCl solution used in titration.

One ml of one N ammonia solution would contain 0.014 gm nitrogen or 0.014 x normality of the titration solution.

0.014 x normality x ml of titration solution = gm nitrogen present

2. Converting nitrogen to protein

gm nitrogen x 6.25 = gm protein present

3. Calculating percentage protein

$$\frac{\text{gm protein}}{\text{weight of sample}} \times 100 = \text{percentage protein}$$

APPENDIX III

Aclimatization, Exercise Equipment and Procedure

A. Equipment

2- Celsius Thermometers

2- 62.2 X 92.7 X 71.1 cm Plastic Tubs

4- 50 gallon Garbage Cans

2- Heat Lamps

Absorbent Cotton Bath Towels

2- Hot Plates

2- Dutch Ovens or Large Saucepans

B. Procedure

1. Warm tap water to approximately 35°C. If tap water is cool, supplement with water heated on hot plates so that desired temperature is reached.

2. Fill plastic tubs with the 35°C water to a depth of approximately 3 feet for swimming or 1/2 foot for aclimatization.

3. During aclimatization, 10 to 15 rats per tub is adequate. While swimming, no more than 10 rats per tub should be allowed.

4. Time the animals for their designated swim and aclimatization periods. The aclimatization schedule, where the animals are allowed to wade in in the water, lasts from 10 to 15 minutes for three consecutive days. The swimming schedule begins at 30 minutes and is advanced by 15 minute increments daily until 60 minutes has been reached. Observe the animals carefully during the swimming phase to assure that they exercise continuously.

5. After the aclimatization and swimming processes, hand dry the animals with a clean dry towel and place them into a trash can lined with clean, dry towels.

6. Turn on the heat lamps above the trash cans.

7. Once the animals are completely dry (approximately 30 to 35 minutes) return them to their cages.

APPENDIX IV

Sacrifice Procedure

A. Equipment

-CO_2 gas tank

-Gas cage/barrel

-Pentobarbitol (65mg/ ml)

-5 ml polyethylene syringe

-23g hypodermic needle

-Latex gloves

-Dissecting tools: scissors, scalpel, wax-lined tray

B. Procedure

1. Weigh animal to be sacrificed in a.m. and record weight. Remove food and water from cage after weighing. All animals will be sedentary on day of sacrifice.

2. Anaesthetize animal with CO_2 until it becomes unconscious and the breathing slows down.

3. Calculate amount of pentobarbitol needed (ie. 80 mg/kg body weight) and inject dose into the animal's peritoneal cavity after CO_2 has been administered.

4. Observe the animal until its' reflexes have diminished and perform a thoracotomy by making a small incision below the xiphoid process and cutting up through the sternum.

APPENDIX V

Cardiac Puncture and Tissue Perfusion Procedures

A. Cardiac Puncture (Exsanguination)

1. Equipment

1- Mineral-free, No Additive Vacutainer Test Tube

1- Mineral-free, Sodium Heparin Vacutainer Test Tube

1- 10 ml polyethylene syringe

1- 23g hypodermic needle

2. Procedure

a. Obtain blood from the left ventricle of the heart by inserting hypodermic needle and syringe. Pull back on the plunger of the syringe slowly to avoid hemolysis.

b. Once blood has been collected, detach the syringe from the needle and immediately transfer approximately 3 mls of blood into the sodium heparin tube and the remaining volume into the no additive tube.

B. Tissue Perfusion

1. Equipment

1- 60 ml polyethylene syringe

0.9% normal saline (500 ml deionized H_2O + 4.5gm NaCl)

Anhydrous sodium heparin

Scissors

2. Procedure

a. Prepare a solution of 1000U/ml of sodium heparin by mixing the anhydrous sodium heparin with 0.9% normal saline.
ie. 166U/mg anhydrous sodium heparin
$\frac{1000U}{166U/mg}$ = 6mg anhydrous sodium heparin/500 ml saline

b. Make a small incision in the right atrium of the heart.

c. Attach the 60 ml syringe containing the heparinized saline to the needle in the left ventricle and slowly inject the saline into the heart.

d. Continue administering the heparinized saline until all the blood has been perfused from the organs and body cavity.

APPENDIX VI

Tissue Collection

A. Equipment

-Metler balance

-0.9% normal saline

-Lint-free tissues (ie. Kimwipes)

-Dissecting tools

-Freezer bags

-Aluminum foil

-Ice chest with ice

-Liquid nitrogen

B. Procedure

1. After perfusion, dissect organs from the body. Clean as much of the fat and connective tissue away from the organs as possible.

2. Rinse each organ with normal saline and blot dry with lint-free tissues.

3. Weigh each organ and record the weight.

4. Collect sample in a labeled specimen bag and keep on ice until the sample can be stored in a -18°C freezer.

*** **Left Gastrocnemius Muscle:** Remove from the animal as soon as possible. Rinse, blot and weigh specimen and wrap in several layers of aluminum foil. Submerge muscle in liquid nitrogen for approximately 8 seconds. Keep on ice until stored in freezer.

*** **Fetus, Placenta, and Uterus:** Remove the uterus from the female intact and weigh. Carefully separate the fetus, placenta and uterus. Look for resorbed sites on the uterus. Rinse, blot, and weigh individually.

APPENDIX VII

Microhematocrit Procedure[1]

A. Equipment

-Capillary hematocrit tubes approximately 7 cm long, bore width of 1mm with no anticoagulant present

-Clay

-Centrifuge capable of 11,500 to 15,000 rpms with a capillary hematocrit head

-Microhematocrit tube reader

B. Specimen

Whole blood using EDTA, heparin, or ammonium-potassium oxalate as the anticoagulant.

C. Procedure

1. Mix whole blood sample thoroughly. Allow blood sample to enter three capillary hematocrit tube until they are approximately 2/3 filled with blood. (Air bubbles denote poor technique but do not affect the results of the test).

2. Seal an end of the hematocrit tube with clay.

3. Place the hematocrit tubes in the radial grooves of the centrifuge head exactly opposite each other, with the sealed end away from the center of the centrifuge.

4. Centrifuge for 5 minutes at 10,000 to 12,000g's or 10 minutes at $5000g^2$.

5. Remove the hematocrit tubes as soon as the centrifuge has stopped spinning. Obtain the results for all the hematocrits using the microhematocrit reading device. Results should agree within a 2 percent error rate. If they do not, repeat

the procedure.

[1]Brown, Barbara. Hematology: Principles and Procedures. Ch. 3. Routine Hematology Procedures. p.73-75. Lee and Febiger ed. 1980.

[2]Todd, Sanford, Davidsohn. 1979. Ch. 27. Basic Methodology. p.872-874.

APPENDIX VIII

Total Hemoglobin Analysis- Cyanmethemoglobin Procedure
Sigma Diagnostics

A. Equipment and Reagents

-Drabkin's Reagent: Sodium bicarbonate, 100 parts, potassium ferricyanide, 20 parts, and potassium cyanide, 5 parts.

-Brij-35 Solution: Brij-35, 30 g/dl.

-Hemoglobin Standard: Lyophilized human methemoglobin. Equivalent to hemoglobin concentration of 18 g/dl whole blood when reconstituted.

-Milton Roy Company Spectrophotometer 501

-Test Tubes: Polypropylene, 10 ml.

-20 microliter (ul) capillary tubes or micropipet

-Autopipeter calibrated to 5 ml.

B. Specimen

Whole blood using EDTA, oxalate, citrate or heparin as the anticoagulant.

C. Procedure

1. Prepare working standards by pipeting and mixing thoroughly the solutions indicated below.

Tube No.	Cyanmethemoglobin Standard Solution (ml)	Drabkin's Solution (ml)	Blood Hemoglobin (g/dl)
1	0.0	6.0	0.0
2	2.0	4.0	6.0
3	4.0	2.0	12.0
4	6.0	0.0	18.0

2. Read absorbance ot tubes 2-4 vs. tube 1 as a reference at a wavelength of 540 nm.

3. Record absorbance values.

4. Plot a calibration curve of absorbance values (y) vs. blood hemoglobin (g/dl) (x) in column 4. The curve is linear, passing through the origin.

5. Label test tubes- Blank, Test Specimens (in duplicate).

6. To all tubes, add 5.0 ml Drabkin's Solution.

7. To Test Specimens, add 20 ul whole blood, rinsing capillary tube or pipet 3-4 times with reagent. Mix well. Allow to stand at least 15 minutes at room temperature (18-26°C).

8. Read and record absorbance (A) of Test Specimen vs. Blank as reference at the same wavelength and in the same instrument as used in the preparation of the calibration curve.

9. Determine total hemoglobin concentration (g/dl) of Test Specimen directly from the calibration curve. Color of the reaction is stable for several hours. Percent error between duplicates should be less than or equal to 5%.

APPENDIX IX

Iron and Total Iron Binding Capacity
Ferrozine Methodology
Colorimetric Test
Stanbio Laboratory, Inc.

A. Equipment and Reagents

-Iron-Ferrozine Color Reagent

-Iron HA buffer

-Iron-Binding TRIS Buffer

-Iron Standard

-Fisher Diagnostics Serachem I control

-Cuvets and lids (polypropylene)

-Timer

-Water Bath at 37^0C

B. Specimen

Serum collected in a mineral free test tube. Samples were frozen for up to one year before analysis.

C. Procedure

Note: Due to limited sample size, the procedure performed was adjusted by one half to insure that all test samples could be run in duplicate.

Serum Iron

1. To appropriately marked cuvets, add 0.3 (0.15) ml iron-free water (Blank or "B"), 0.3 (0.15) ml Iron Standard ("S"), and 0.3 (0.15) ml serum (Unknown or "U").

2. Add 2.3 (1.15) ml Iron HA Buffer to each cuvet, mix well and allow to stand for at least 1 minute (permits air bubbles to rise).

3. Read absorbance (A^1) of S and U vs B at 560 nm.

4. Add 0.1 (0.05) ml Iron-Ferrozine Color Reagent to each cuvet, mix well and incubate 10 minutes at 37°C.

5. Cool 2 minutes in a water bath at room temperature and again read absorbance (A^2) as in Step 3.

Iron Binding Capacity

1. Add 0.6 (0.3) ml iron-free water to a cuvet marked "B". To a second cuvet ("S") add 0.3 (0.15) ml iron-free water plus 0.3 (.15) ml Iron Standard and to a third cuvet ("U") 0.3 (0.15) ml serum plus 0.3 (0.15) ml Iron Standard.

2. To each cuvet, add 2.2 (1.1) ml Iron-Binding TRIS Buffer, mix well and allow to stand for at least 1 minute.

3. Read absorbance (A^1) of S and U vs B at 560 nm.

4. Add 0.1 (0.05) ml Iron-Ferrozine Color Reagent to each tube, mix well and incubate 10 minutes at 37°C.

5. Cool 2 minutes in a water bath at room temperature and again read absorbance (A^2) as in Step 3.

Results

Values were derived from the following calculations:

1. Serum Iron (ug/dl) = $\frac{(A^2 - A^1)\ \text{Unknown}}{(A^2 - A^1)\ \text{Standard}} \times 500$

2. Iron-Binding Capacity:

   a. Excess Iron (ug/dl) = $\frac{(A^2 - A^1)\ \text{Unknown}}{(A^2 - A^1)\ \text{Standard}} \times 500$

b. UIBC (ug/dl) = 500(total iron added in ug/dl) - Excess Iron (ug/dl)

c. TIBC (ug/dl) = Serum Iron (ug/dl) + UIBC (ug/dl)

3. Percent error between duplicates should be no greater than 10%.

APPENDIX X

Acid-Wash and Wet Ashing Procedure for Iron Determination of Tissue Samples

Acid-Wash Procedure

A. Equipment and Reagents

-50 ml test tubes used in wet ashing

-Large plastic dish pans or tubs

-Test tube brush

-100 ml acid-washed beaker

-Rubber gloves

-Safety goggles

-Deionized water

-50% solution of H_2SO_4

-Kimwipes or other lint-free tissue

-Dish drain or drying rack

B. Procedure

1. Immediately after transferring wet ashed sample to a polypropylene test tube, add deionized water to the 50 ml test tube and scrub thoroughly with a clean test tube brush. Discard water.

2. Put test tubes in appropriate test tube racks and place in a large dish pan or tub (plastic).

3. Under a hood, fill each test tube to the rim with a 50% solution of H_2SO_4 using an acid-washed beaker. Be sure that safety goggles and rubber gloves are worn during this step.

Allow test tubes to soak for at least one hour.

4. After the tubes have soaked, pour the sulfuric acid back into its container and place the tubes in a plastic tube half filled with deionized water. When done, cover the tubes with deionized water and let them rinse for approximately 1/2 hour.

5. Rinse each tube (exterior and interior) 5 to 6 times with deionized water.

6. Place each tube on a drying rack lined with lint-free tissues and allow to air dry. **Note:** Tubes can be dried in an oven if necessary. Be sure to dry the exterior of each tube before placing in the oven for 30 to 45 minutes.

Wet Ashing Procedure

A. Equipment and Reagents

-50 ml wet ashing test tubes

-80 ml acid washed beaker

-Parafilm

-Scissors

-Kim wipes

-Rubber gloves

-Safety goggles

-Glass pipette with rubber top

-10% HCL (made with ultrapure HCL)

-Redistilled Nitric Acid

-Perchloric Acid

-Heating block with hood

-Polypropylene test tubes and caps (20 ml)

B. Specimen

-Freeze-dried tissue samples, ground to a fine powder

C. Procedure

1. Weigh 100 mg samples into each tube. Run each test sample in duplicate. **Note:** It may be necessary to run smaller tissue samples depending on the original size of each organ. For example, sample size for fetal and spleen tissues were at times less than 50 mg each. Allow for at least one blank tube per run.

2. Add 3.0 ml of redistilled nitric acid and allow to digest over night.

3. In a.m., turn on heating block and allow to heat.

4. Under a hood, pipet 1.0 ml of redistilled perchloric acid to each tube beginning with the blanks. Place each test tube on the heating block. **Note:** Safety goggles and rubber gloves should be worn at all times during this procedure and when handling the test tubes during the actual digestion process.

5. Allow the samples to digest. A brown smoke will appear. Begin swirling each tube after the formation of the brown smoke has stabilized (approximately 30 minutes to 1 hour into the procedure). Lift the tubes slightly out of the heating block so that the contents in the bottom of the tube can be seen and gently agitate.

6. Continue agitating the samples every 15 minutes until the smoke disappears. Sample color changes from a dark brown to a yellow during this phase. Once the samples are yellow, increase the heat on the block.

7. As the temperature increases, the samples will develop a

white smoke. Continue to swirl the samples every 15 minutes.

8. When the sample size has reduced down to slightly below the curve of the bottom of the test tube and the contents turn clear upon swirling without formation of bubbles, the digestion procedure has been completed.

9. Remove the samples from the heating block and allow to cool under the hood.

10. Once samples have cooled, carefully add 10% HCL until volume reaches 12.5 ml graduation on the test tube. (Use the acid-washed beaker and glass pipet to dispense the HCl into each sample).

11. Cover each tube with parafilm until ready to transfer samples into polypropylene tubes. Do not transfer samples for at least two hours after the HCl has been added to the sample. Store, refridgerated, at 2-6^{0}C.

12. Once all samples have completed the digestion procedure, unplug the heating block and wash down the hood.

APPENDIX XI

Homogenate Preparation for Animal Muscle Tissue

Costil, D.L. et al
Human Performance Laboratory
Ball State University
Muncie, Indiana

A. Equipment and Reagents

-Kinematica Gimbalt homogenizer

-Eppendorf Centrifuge 5415C

-Scissors, forceps

-Polypropylene test tubes

-Eppendorf centrifuge tubes

-175 mM KCl + 2 mM EDTA (ml)

-Crushed ice

B. Specimen

Gastrocnemius muscle sample cleaned of connective and adipose tissue. **Please note:** Samples are stored frozen until assay and thawed out immediately prior to homogenization.

C. Procedure

1. 0.1 - 0.2 g of finely minced muscle is weighed. For optimum results, red and white muscle tissue should be separated before homogenization.

2. Muscle weight (g) x 19 = ml 175 mM KCl + 2 mM EDTA to prepare a 1:20 dilution of homogenate

3. Add 3/4 of above volume to the test tube containing the tissue and homogenate the sample using the Kinematica unit. After the tissue has been homogenized, the remaining volume of KCl-EDTA is used to rinse the shaft of the homogenizer.

4. The homogenate is transferred to Eppendorf centrifuge tubes were it is spun down at 11750 x g for five minutes. The supernatant is removed and stored on ice until it is analyzed for enzymatic activity.

5. The shaft of the homogenizer should be cleaned of any remaining connective tissue and rinsed with deionized water.

D. Reagent Preparation for 175 mM KCl + 2 mM EDTA stock solution, pH = 7.5

1. Weigh out 13.048 g KCl (Sigma grade III, MW 74.6, Anhydrous) and 0.7444 g EDTA (Sigma ED 2 SS, MW 336.2)

2. Dissolve KCl and EDTA in distilled water and bring to a total volume of 90 ml.

APPENDIX XII

DTNB Method for Citrate Synthase Activity

P.A. Srere
Methods in Enzymology Vol. XIII, p.3, 1969.

A. Equipment and Reagents

-Hitachi Spectrophotometer 100-10

-100 mM Tris Buffer (Store in refridgerator) pH = 8.35
7.88 g/ 500 ml deionized water

-1 mM DTNB (Prepare daily) pH = 8.1
3.96 mg DTNB / 10 ml 100mM Tris Buffer

-10 mM Oxaloacetic acid (OAA) (Prepare daily) pH = 7.5
1.321 mg OAA / 1 ml 100mM Tris Buffer

-3mM Acetyl CoA (Prepare daily) pH = 8.0
2.88 mg Acetyl CoA / 1 ml deionized water

-Eppendorf pipettes

-1 ml cuvettes

B. Specimen

Homogenized muscle tissue sample.

C. Procedure

1. Add the following to cuvette:
   0.1 ml DTNB
   0.1 ml Acetyl CoA
   0.74 ml Tris Buffer
   0.01 ml sample (enzyme)

2. Start citrate synthase reaction by adding 0.05 ml of OAA.
   Follow absorption at 412 nm for 3 minutes (time in which

rates should remain linear).

3. Determine the change in absorbance (OD) over the three minutes.

D. Calculations

1. Citrate synthase activity = $\frac{\text{OD/min x DF}}{13.6\ \text{mM}^{-1}}$ = mM/min

   Where DF = dilution factor of homogenate
   Extinction coefficient = 13.6 mM

2. $\frac{\text{Citrate synthase activity (mM/ min)}}{\text{weight of muscle sample analyzed (g)}}$ = mM/min/g

APPENDIX XIII

Histology Reports
Virginia-Maryland Regional College of Veterinary Medicine
Blacksburg, Virginia

Stomach of Dam 151: Some superficial, erosive gastritis and slight congestion.

Stomach of Dam 60: Mostly normal mucosa but has localized area of erosion.

Stomach of Dam 70: Superficial to deep mucosal erosion and cystic dililation of deeper gastric glands.

Stomach of Dam 77: Some superficial erosion of mucosa.

Stomach of Dam 2 : Fairly normal

Stomach of Dam 71: Some erosion of antrum. Corpus is mostly normal, with slight evidence of erosion.

Stomach of Dam 12: Mostly normal. Very slight evidence of corporal very superficial damage.

Stomach of Dam 56: Generalized superficial erosive gastritis (diffuse).

APPENDIX XIV

Graphic Representation of Main Effect and Two Factor Interactions.

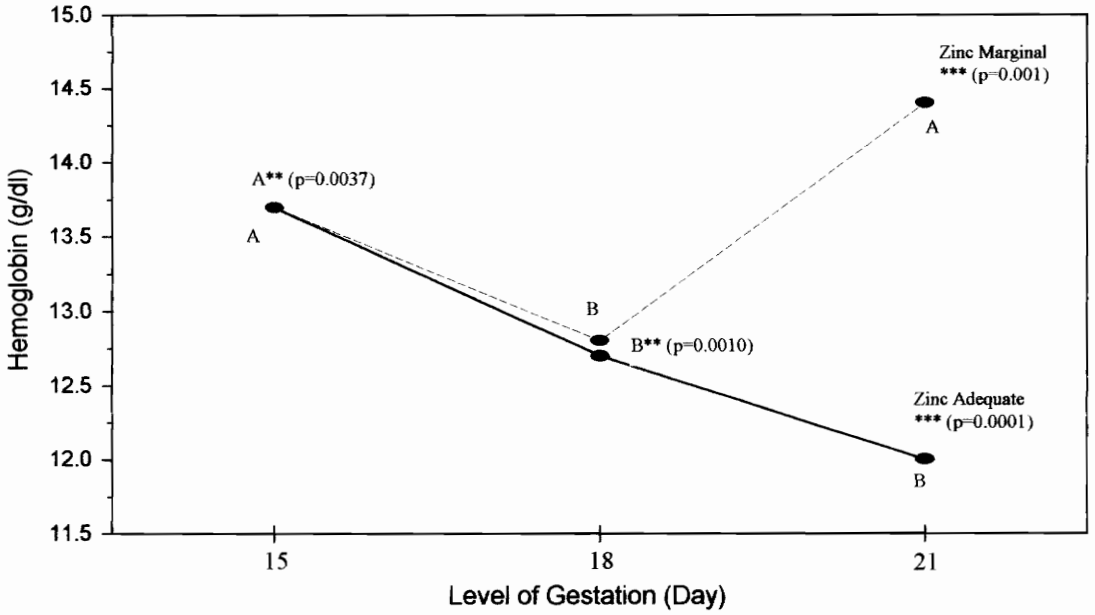

Figure 1 Zinc-Gestation Interactions for Hemoglobin- Zinc Effect

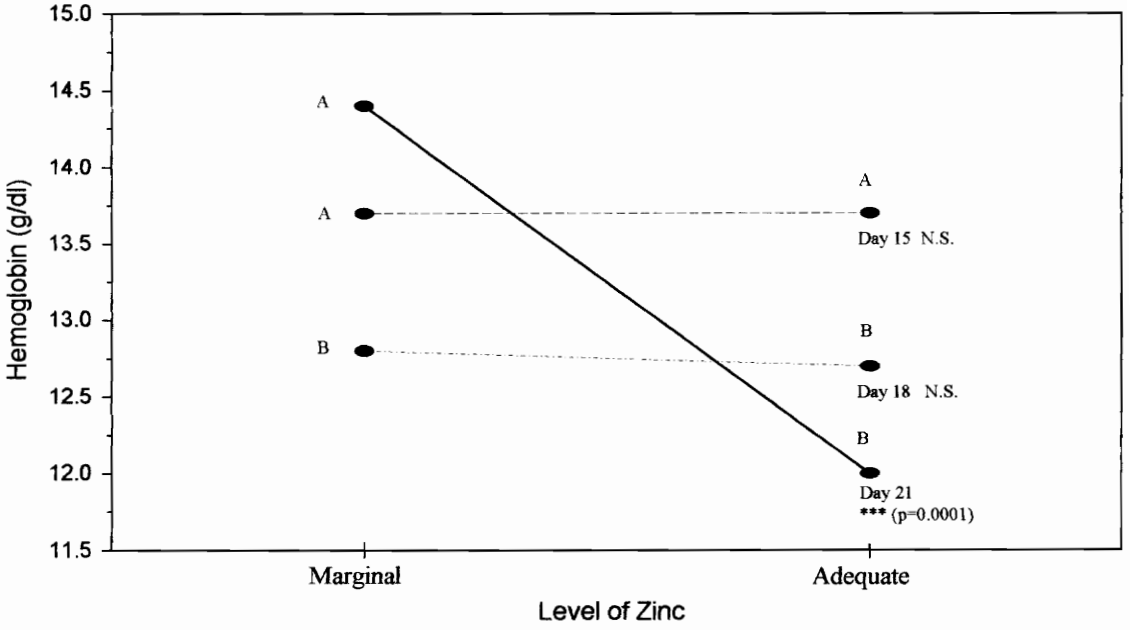

Figure 2 Zinc-Gestation Interactions for Hemoglobin- Gestation Effect.

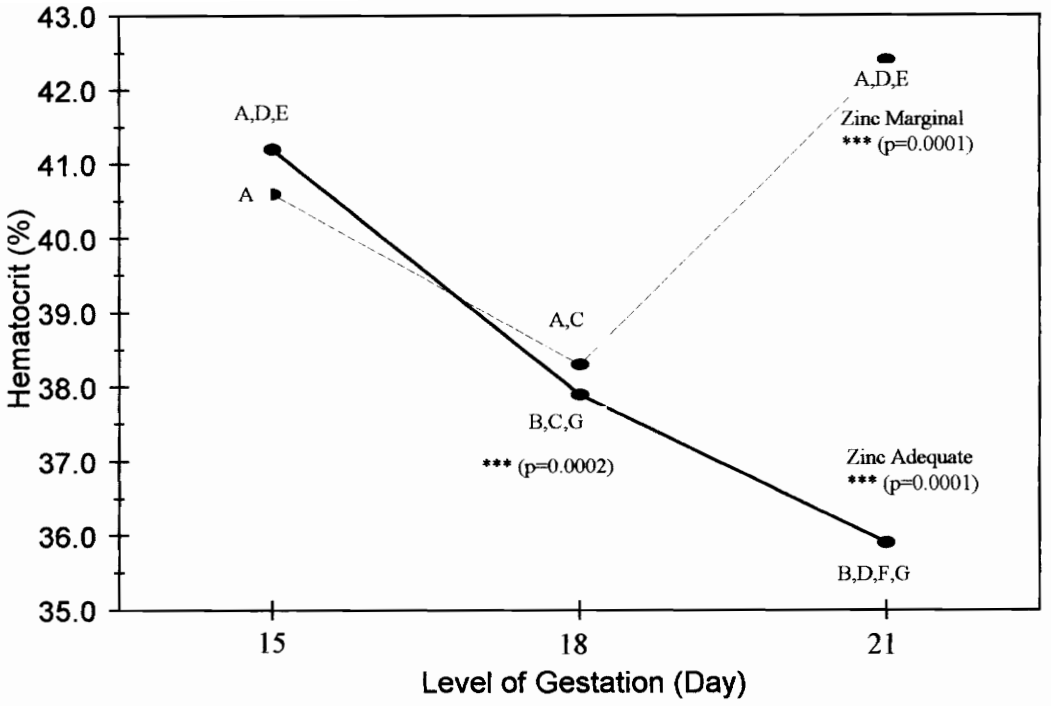

Figure 3 Zinc-Gestation Interactions for Hematocrit- Zinc Effect.

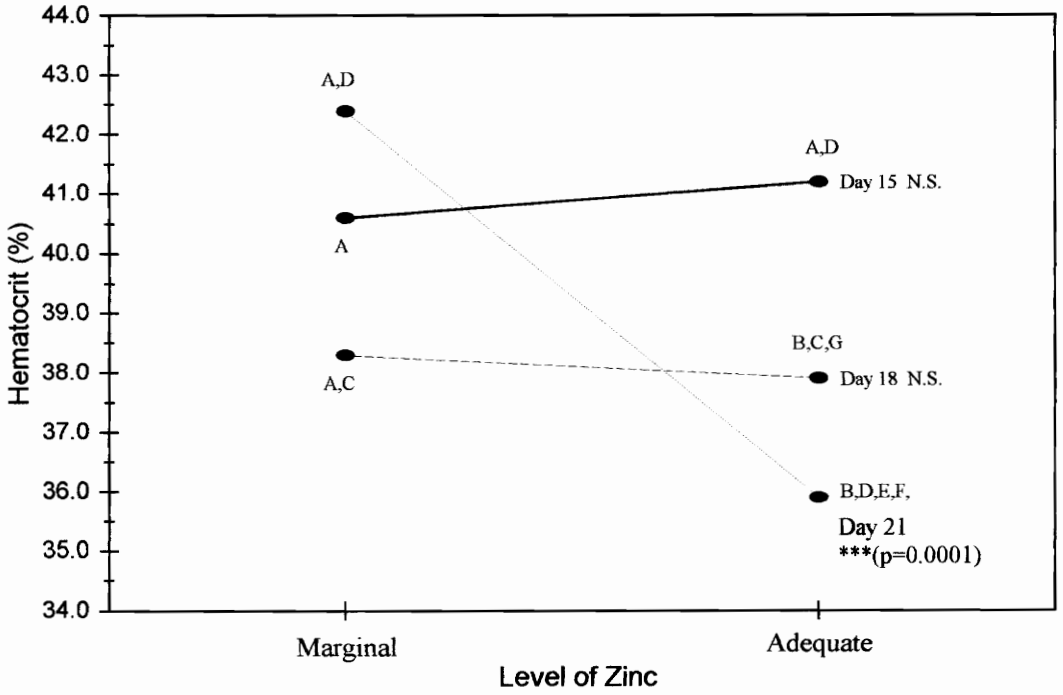

Figure 4 Zinc-Gestation Interactions for Hematocrit- Gestation Effect.

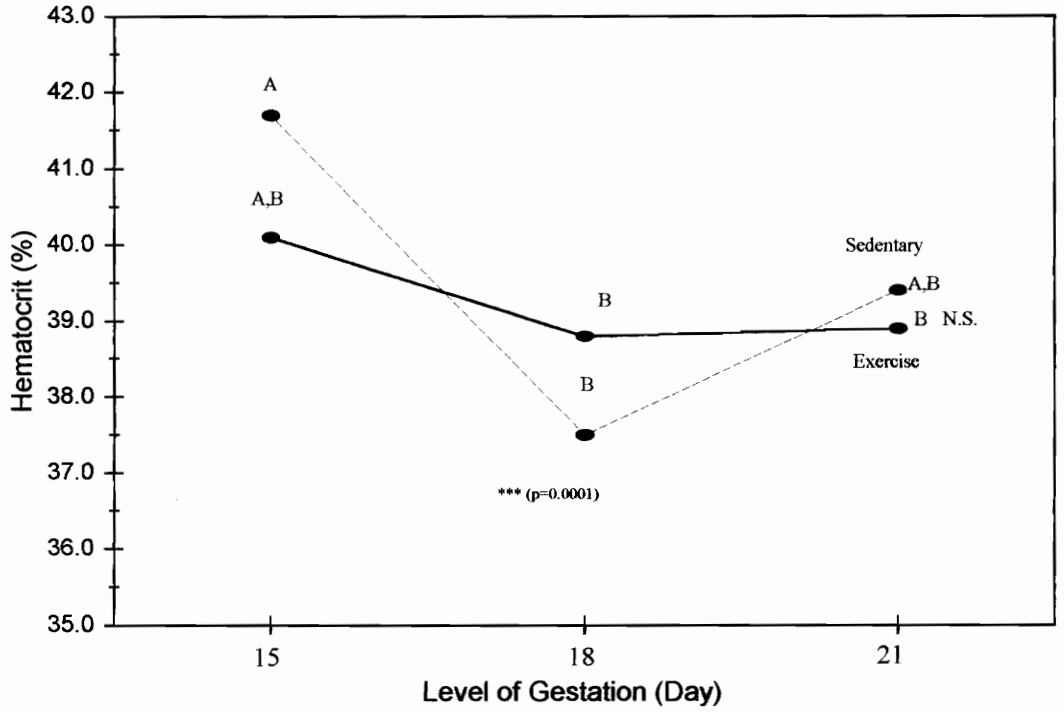

Figure 5 Exercise- Gestation Interactions for Hematocrit- Exercise Effect.

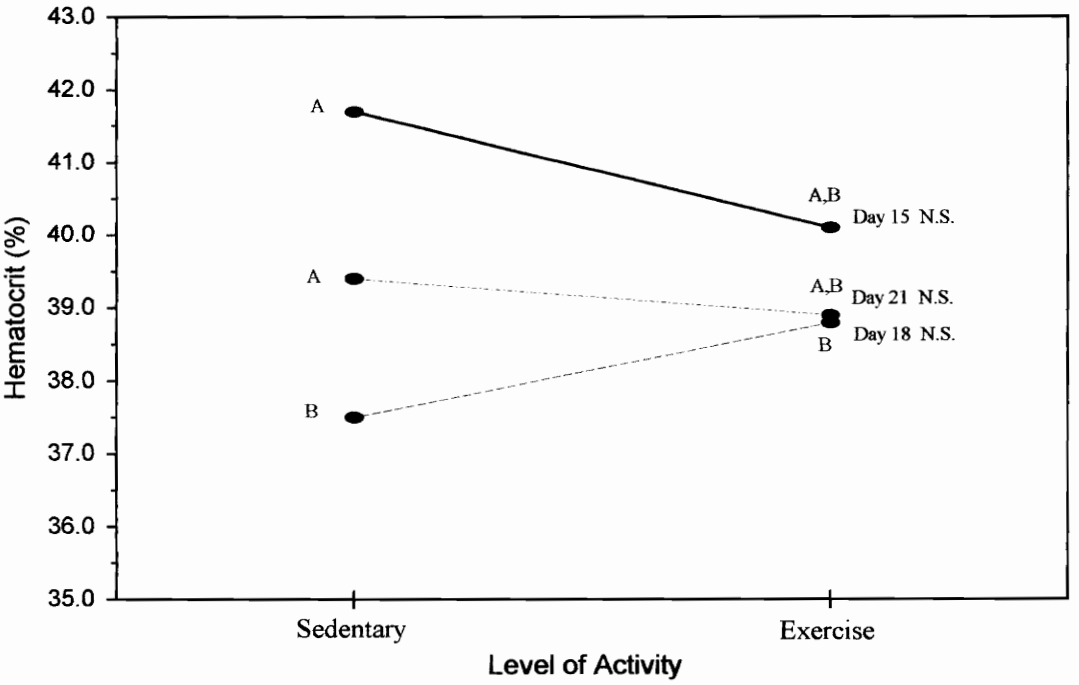

Figure 6 Exercise-Gestation Interactions for Hematocrit- Gestation Effect.

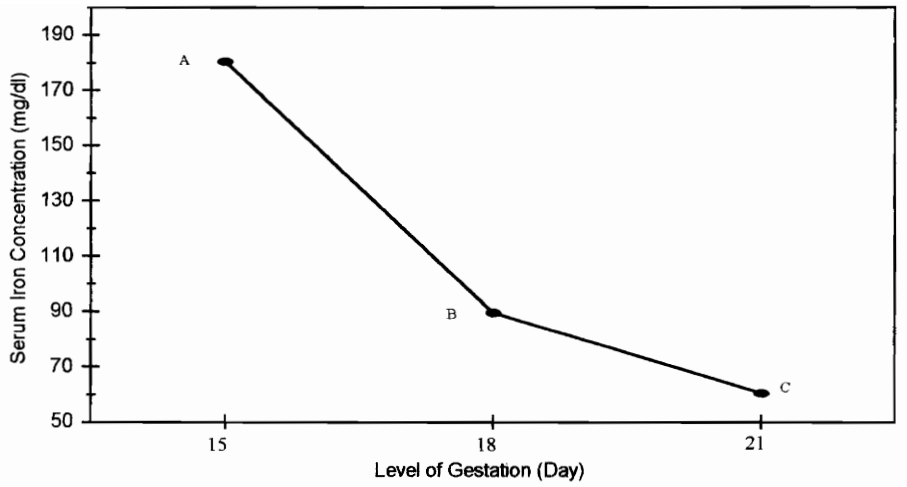

Figure 7 Gestation Analysis for Dam Serum Iron Concentration.

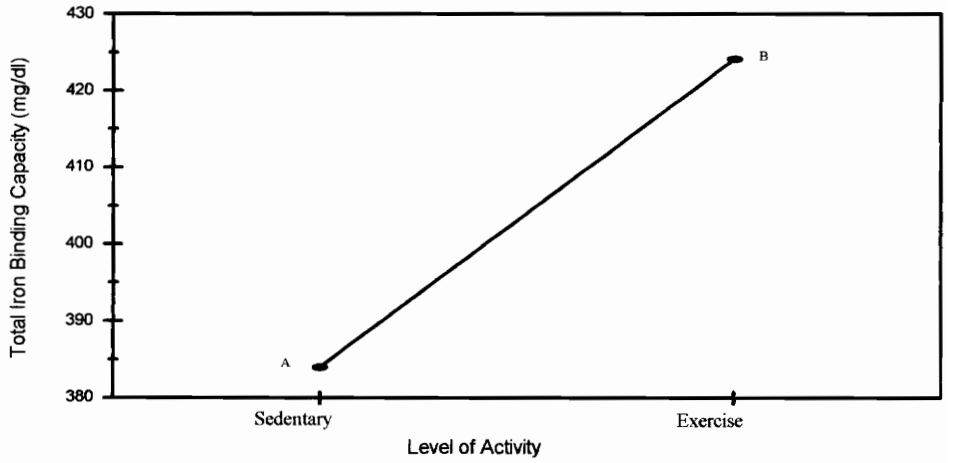

Figure 8 Exercise Analysis For Dam Serum Total Iron Binding Capacity.

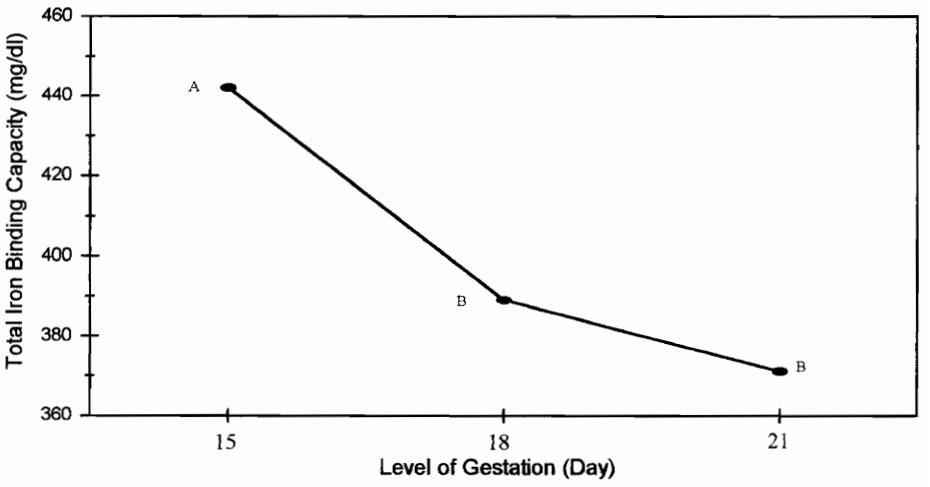

Figure 9 Gestation Analysis for Dam Serum Total Iron Binding Capacity.

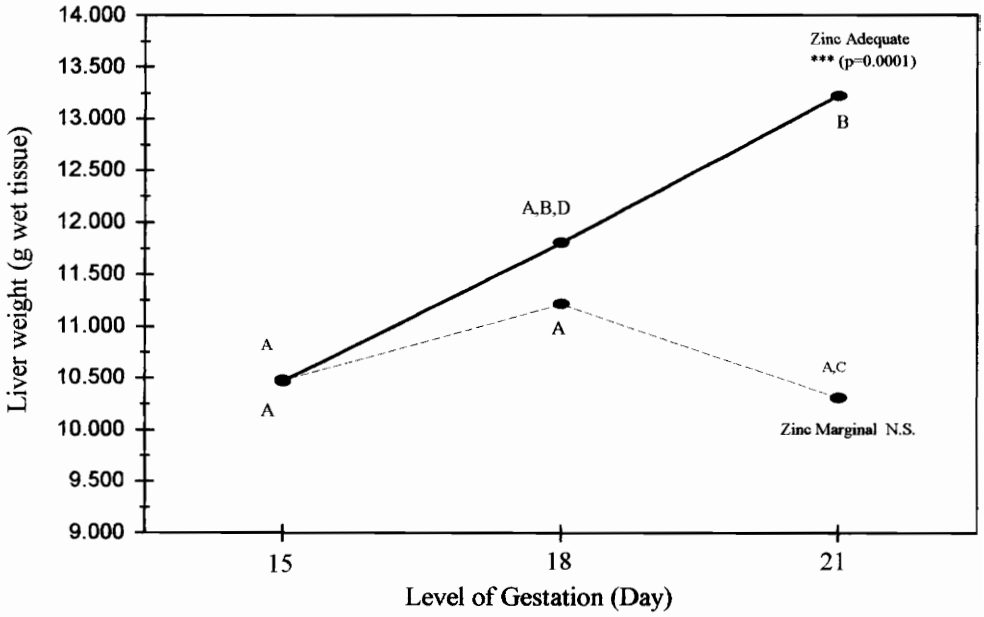

Figure 10 Zinc-Gestation Interactions for Dam Liver Weight- Zinc Effect.

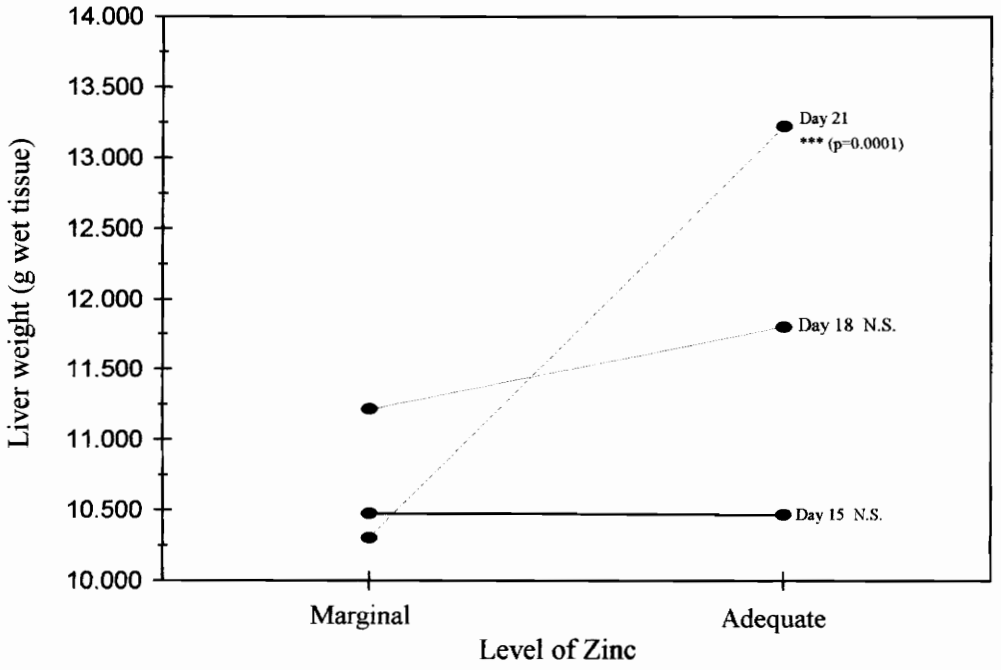

Figure 11 Zinc-Gestation Interactions for Dam Liver Weight- Gestation Effect.

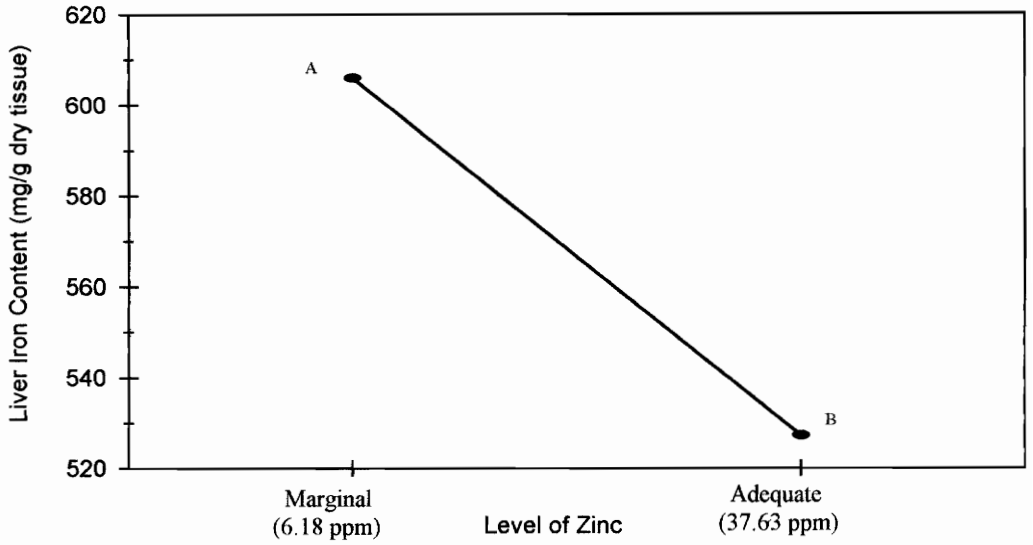

Figure 12 Zinc Analysis for Dam Liver Iron Content.

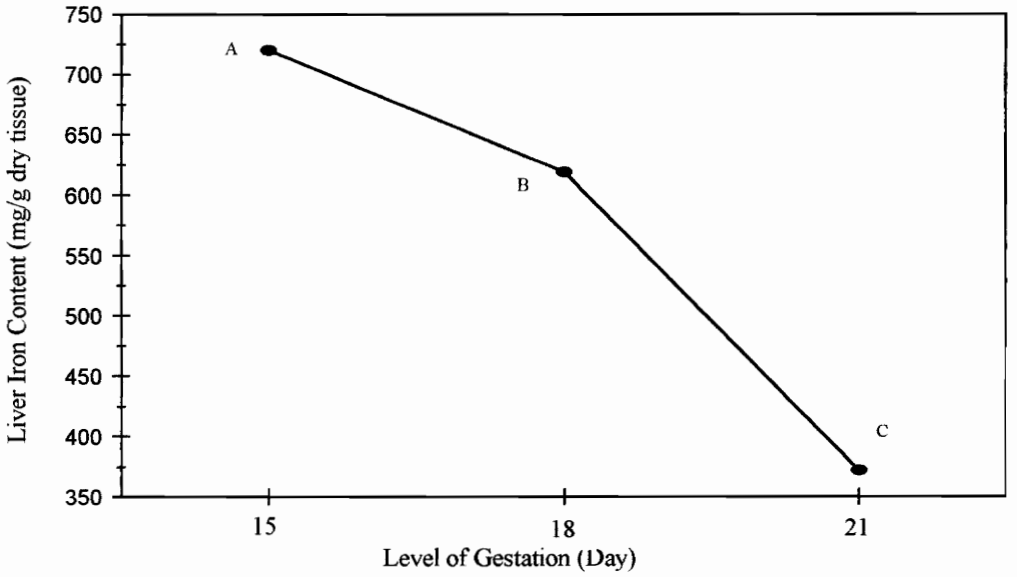

Figure 13 Gestation Analysis for Dam Liver Iron Content.

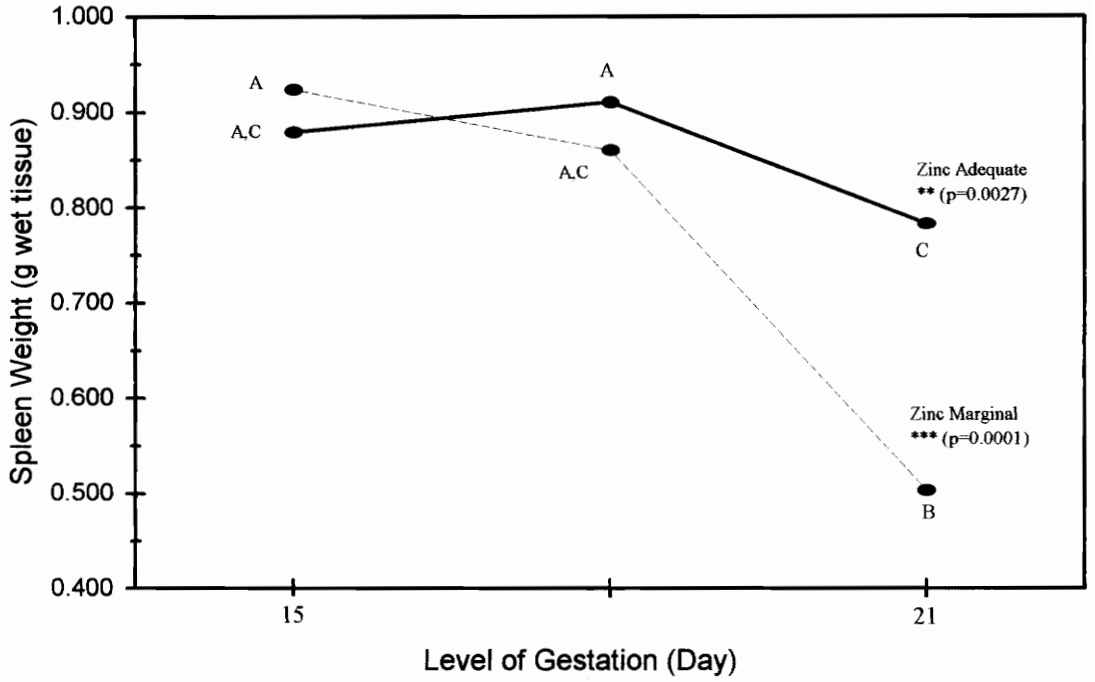

Figure 14 Zinc-Gestation Interactions for Dam Spleen Weight- Zinc Effect

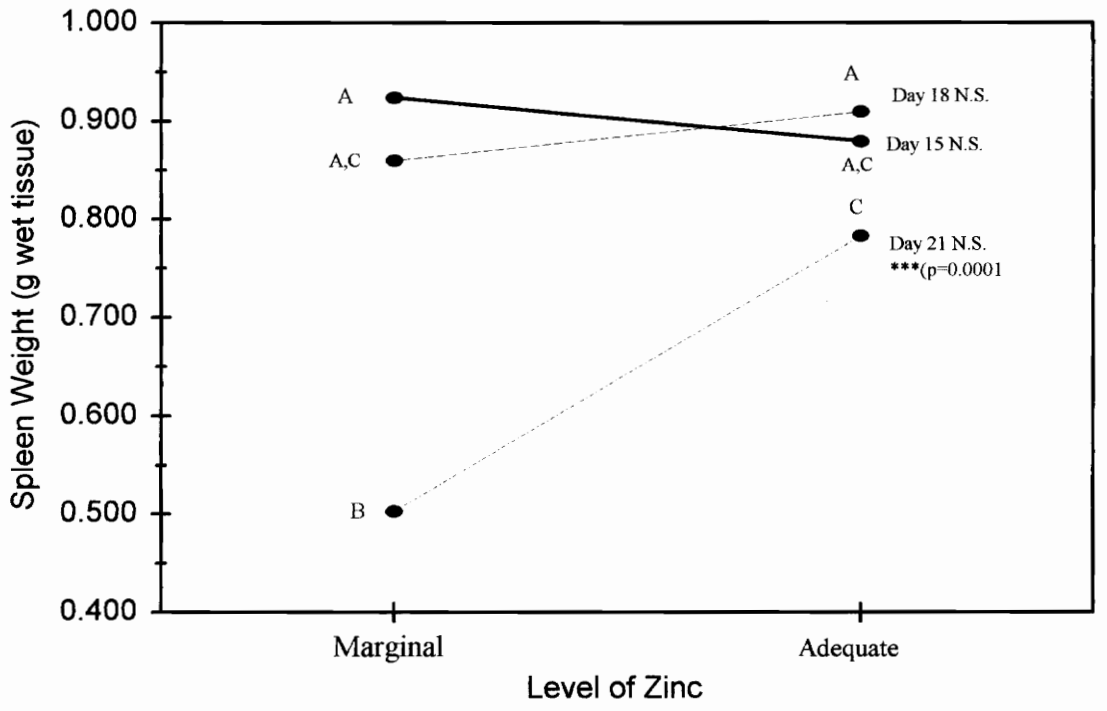

Figure 15 Zinc-Gestation Interactions for Dam Spleen Weight- Gestation Effect.

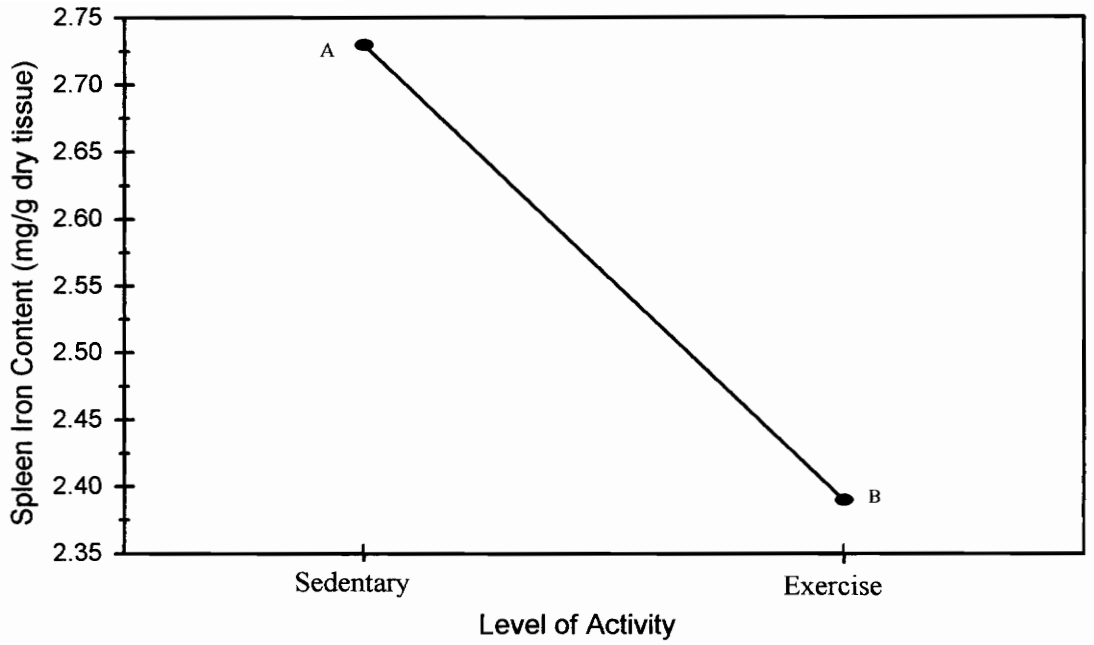

Figure 16 Exercise Analysis for Dam Spleen Iron Content.

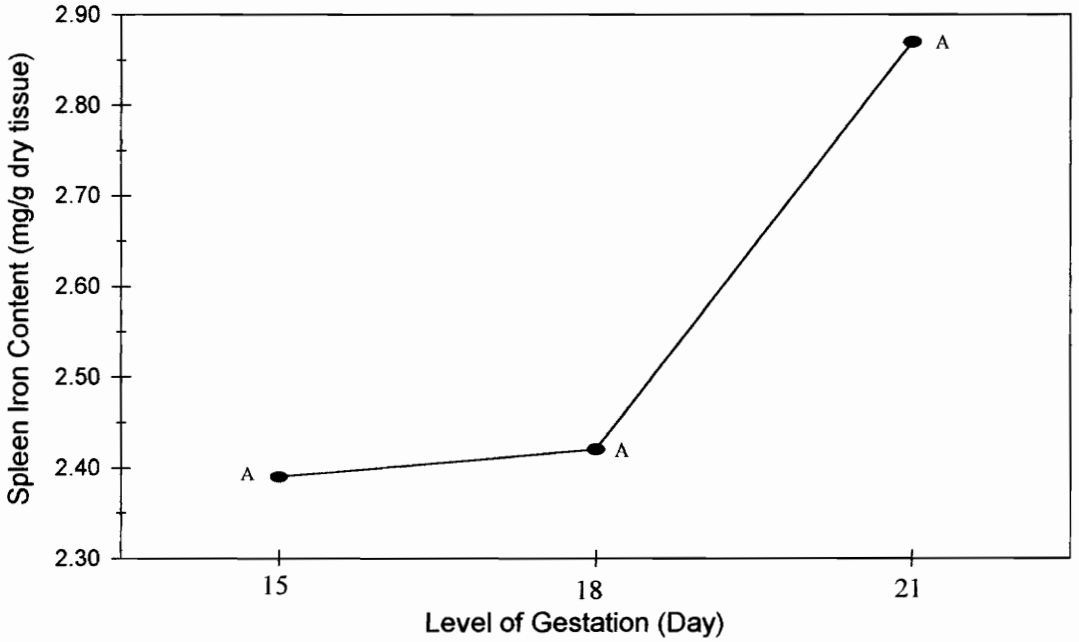

Figure 17 Gestation Analysis for Dam Spleen Iron Content.

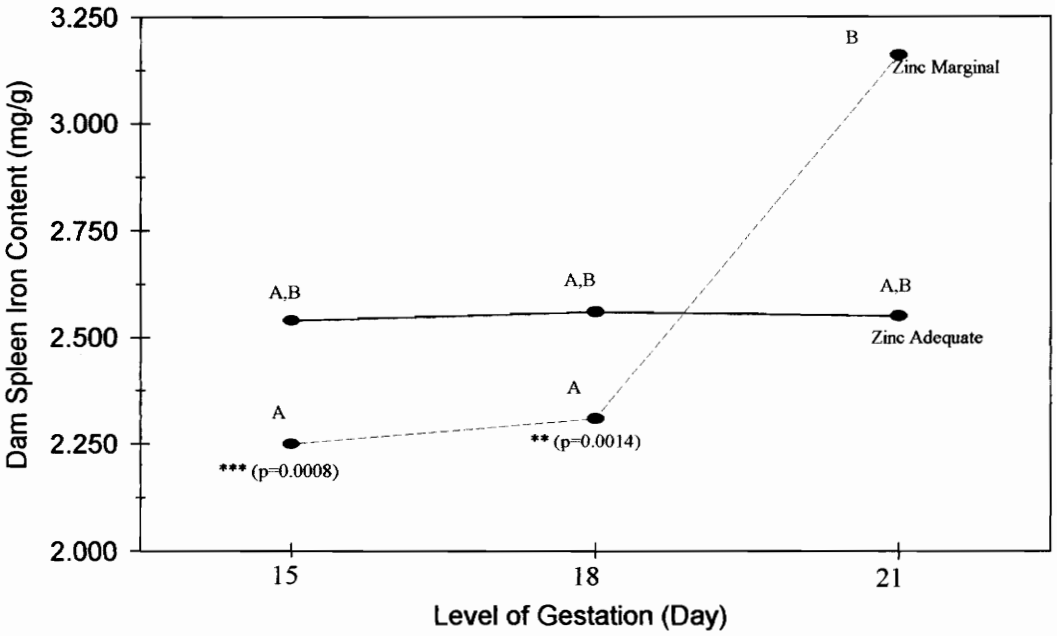

Figure 18 Zinc-Gestation Interactions for Dam Spleen Iron Content- Zinc Effect.

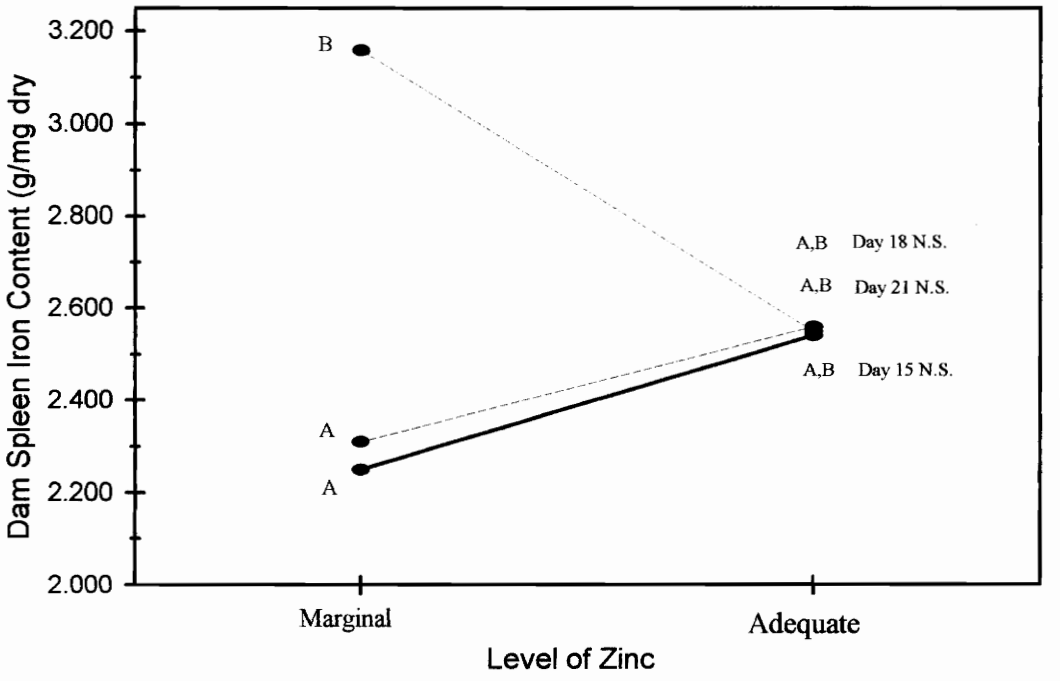

Figure 19 Zinc-Gestation Interactions for Dam Spleen Iron Content- Gestation Effect.

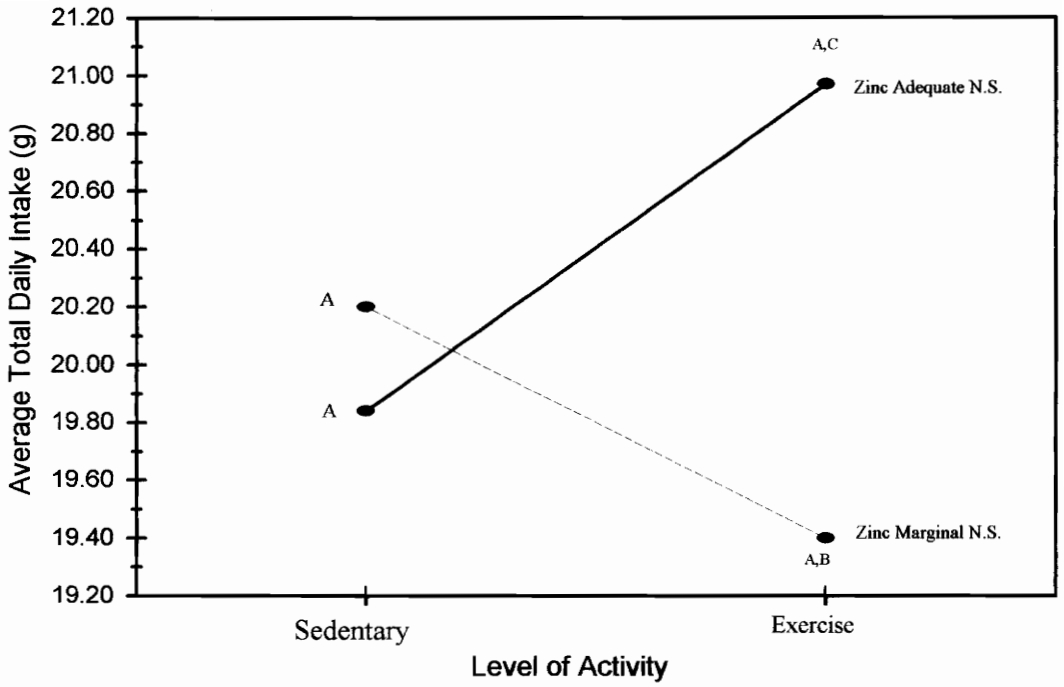

Figure 20 Zinc-Exercise Interactions for Total Daily Intake- Zinc Effect.

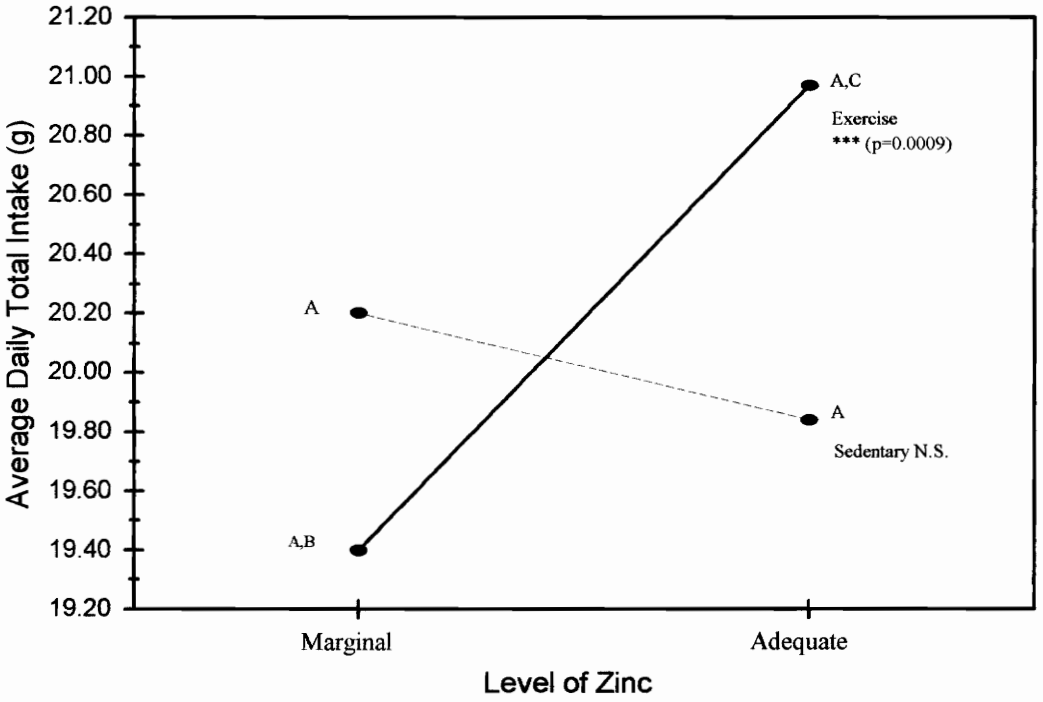

Figure 21 Zinc-Exercise Interactions for Total Daily Intake- Exercise Effect.

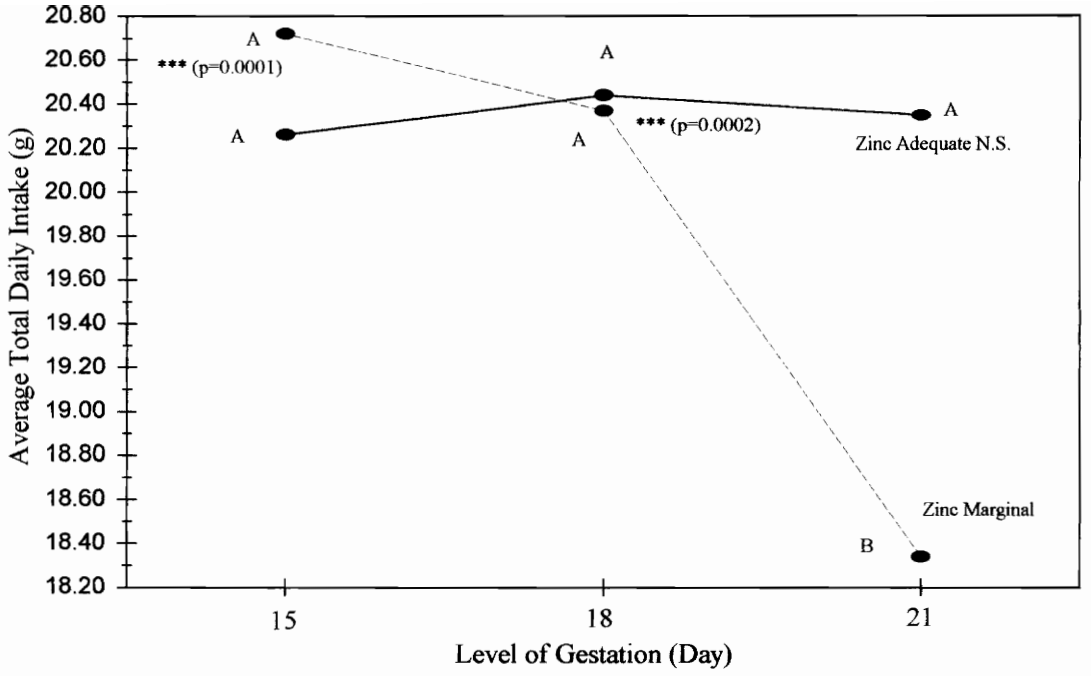

Figure 22 Zinc-Gestation Interactions for Total Daily Intake- Zinc Effect.

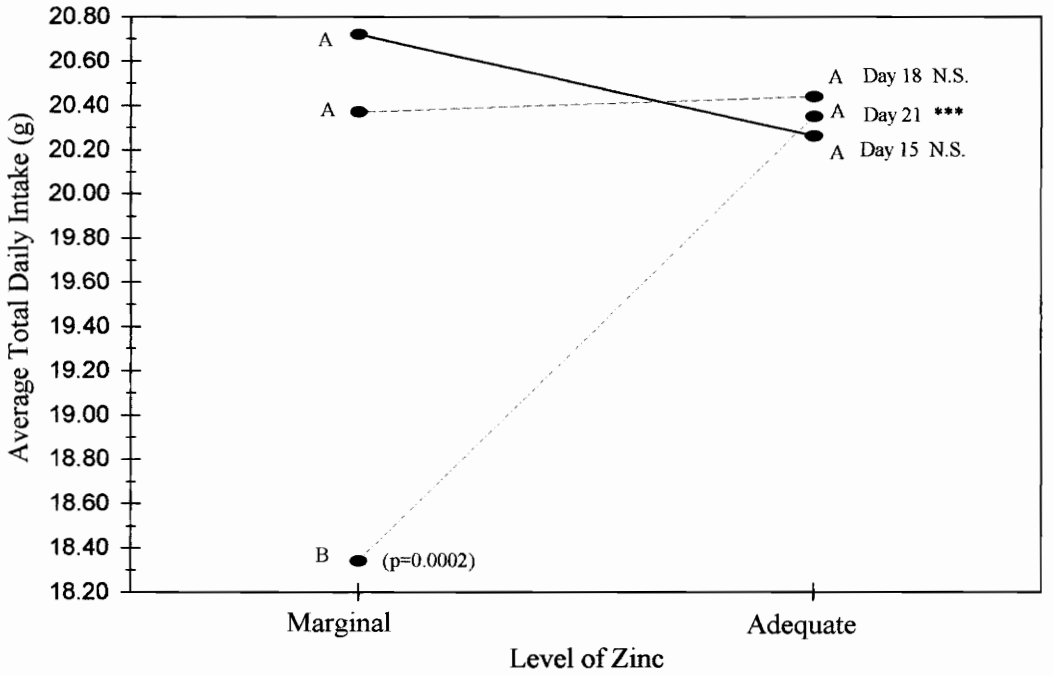

Figure 23 Zinc-Gestation Interactions for Total Daily Intake- Gestation Effect.

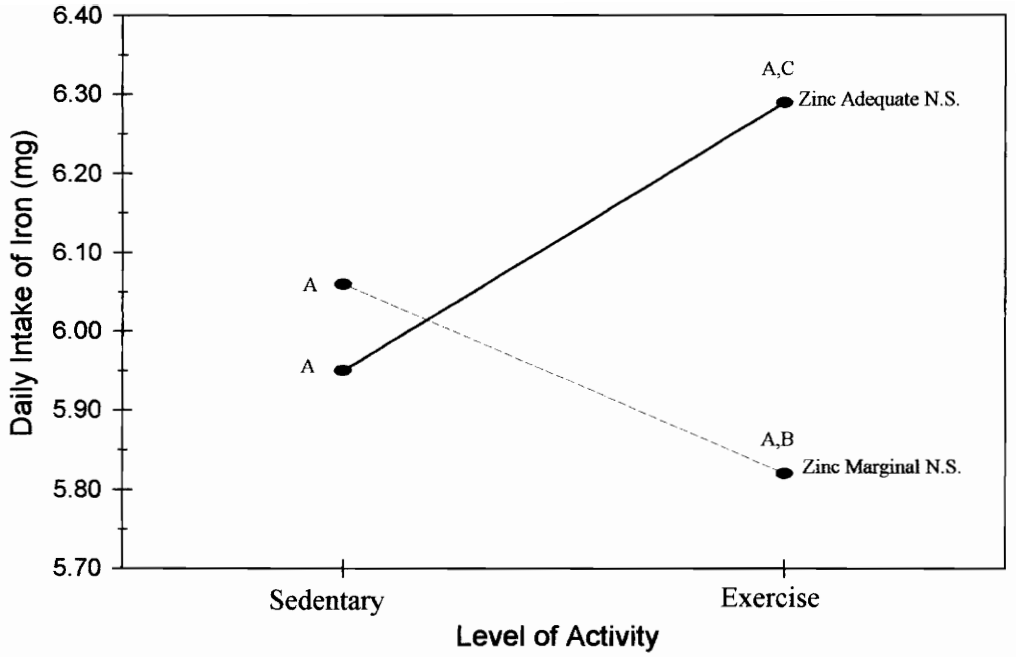

Figure 24 Zinc-Exercise Interactions for Daily Intake of Iron- Zinc Effect.

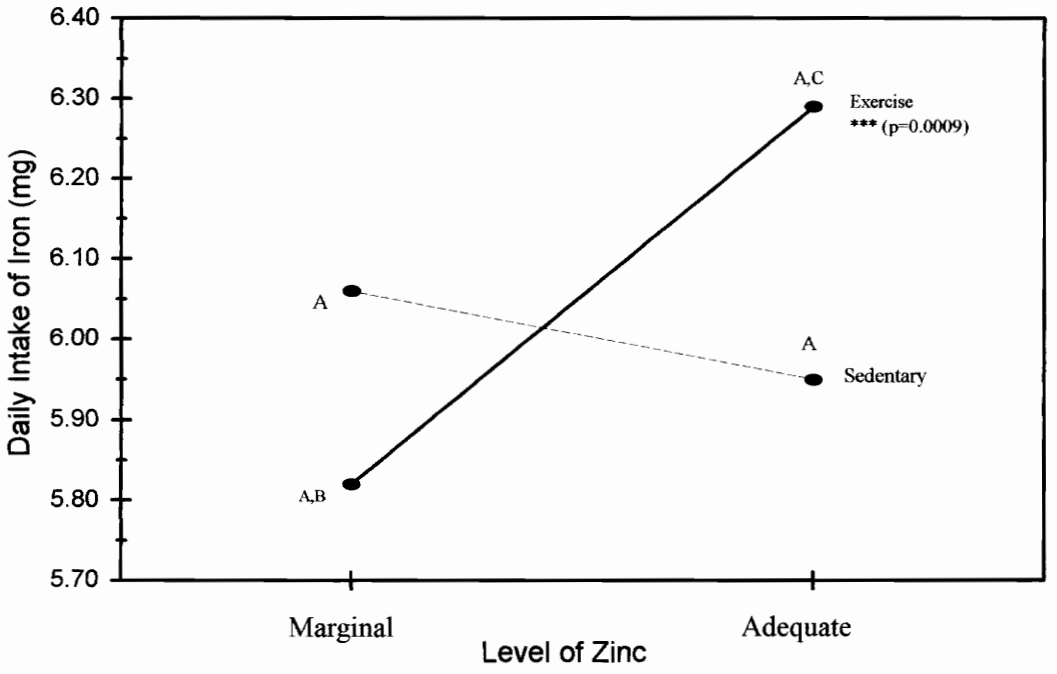

Figure 25 Zinc-Exercise Interactions for Daily Intake of Iron- Exercise Effect.

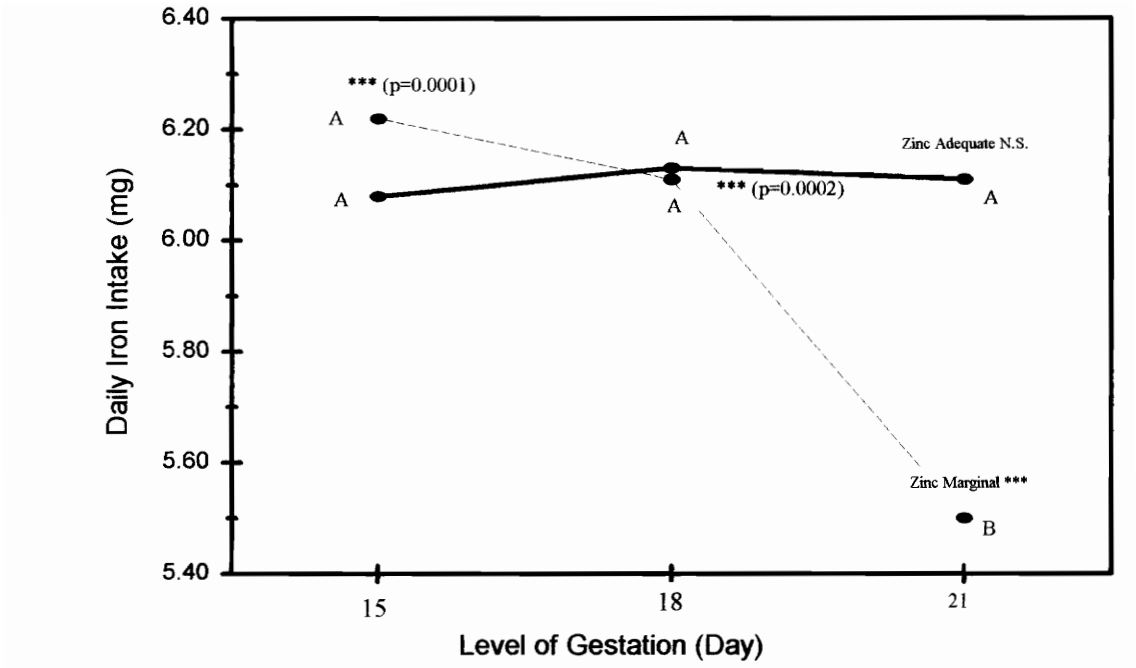

Figure 26. Zinc-Gestation Interactions for Daily Intake of Iron- Zinc Effect.

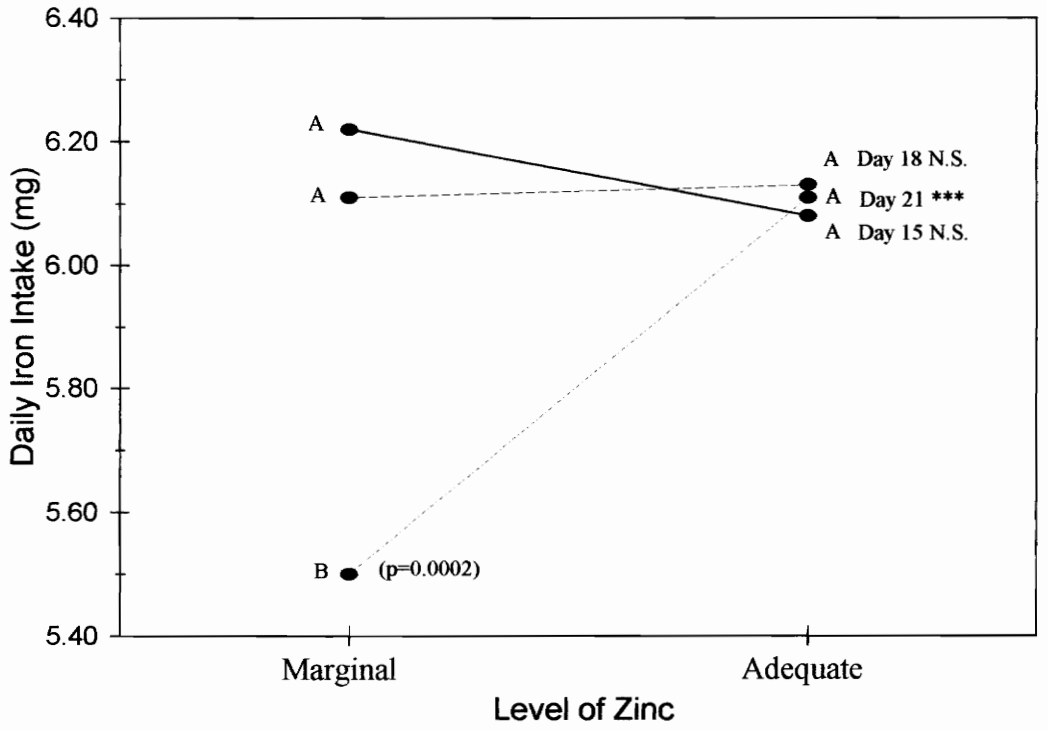

Figure 27 Zinc-Gestation Interactions for Daily Intake of Iron- Gestation Effect.

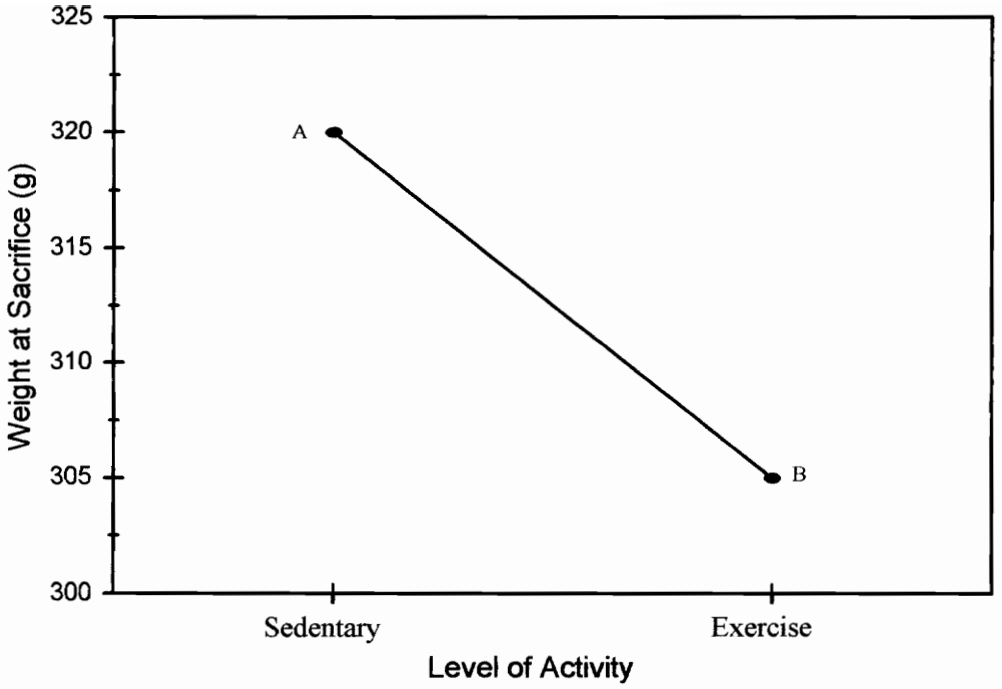

Figure 28 Exercise Analysis for Dam Weight at Sacrifice.

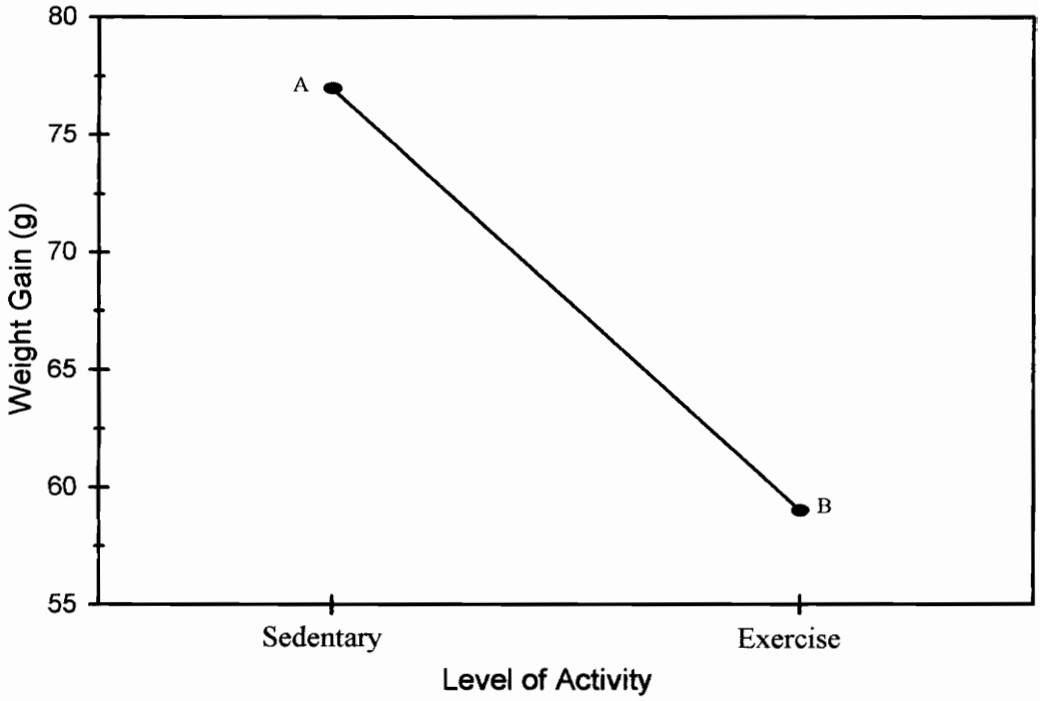

Figure 29 Exercise Analysis for Total Weight Gain.

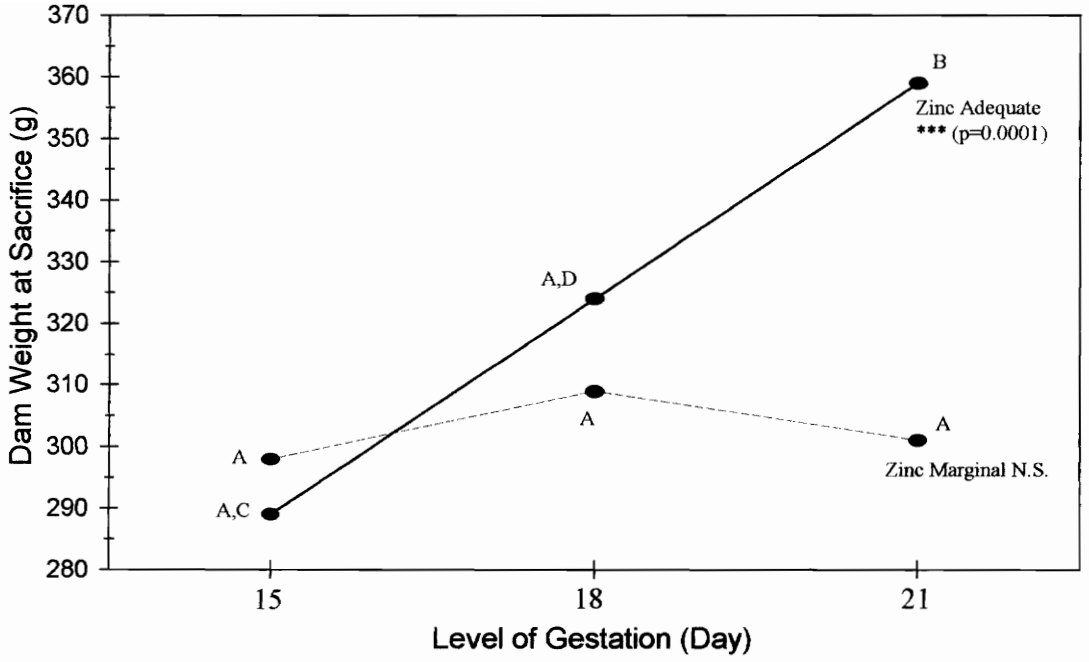

Figure 30 Zinc-Gestation Interactions for Dam Weight at Sacrifice- Zinc Effect.

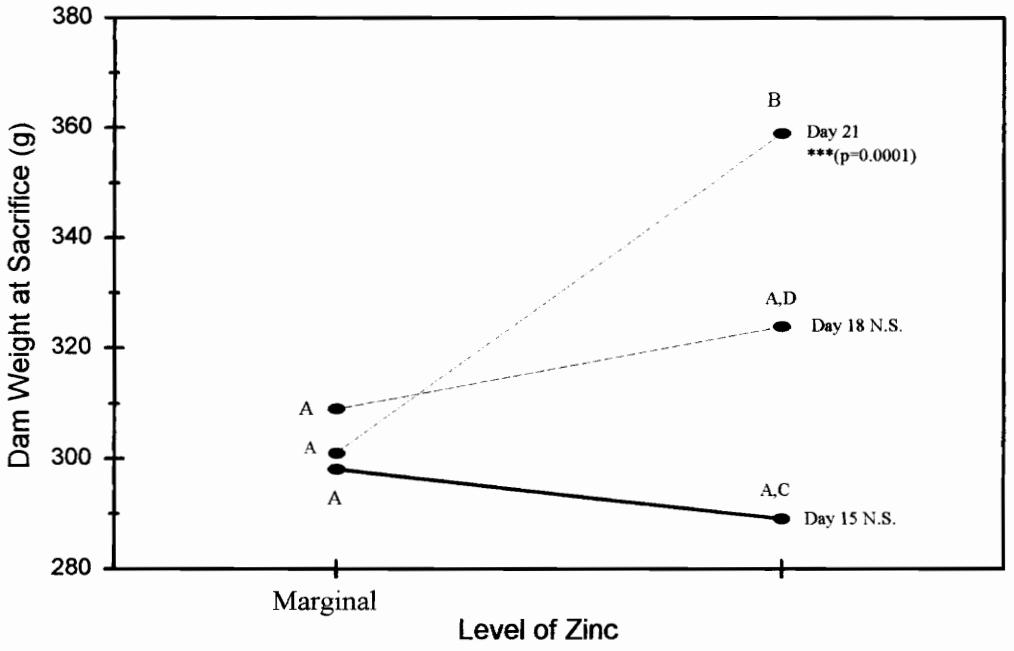

Figure 31 Zinc-Gestation Interactions for Dam Weight at Sacrifice- Gestation Effect.

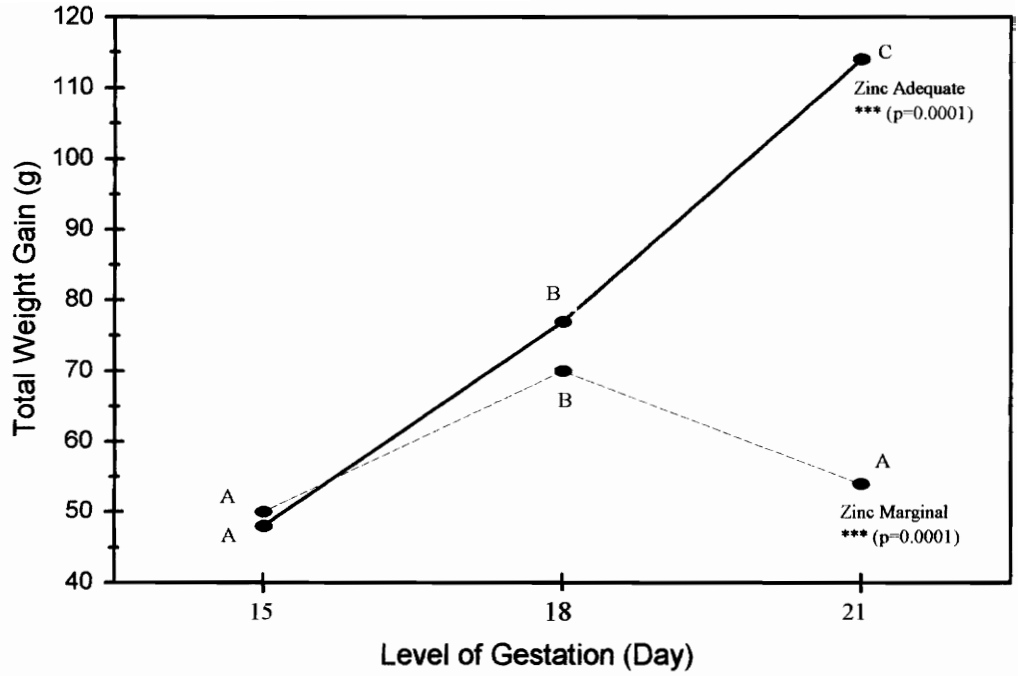

Figure 32 Zinc-Gestation Interactions for Total Weight Gain- Zinc Effect.

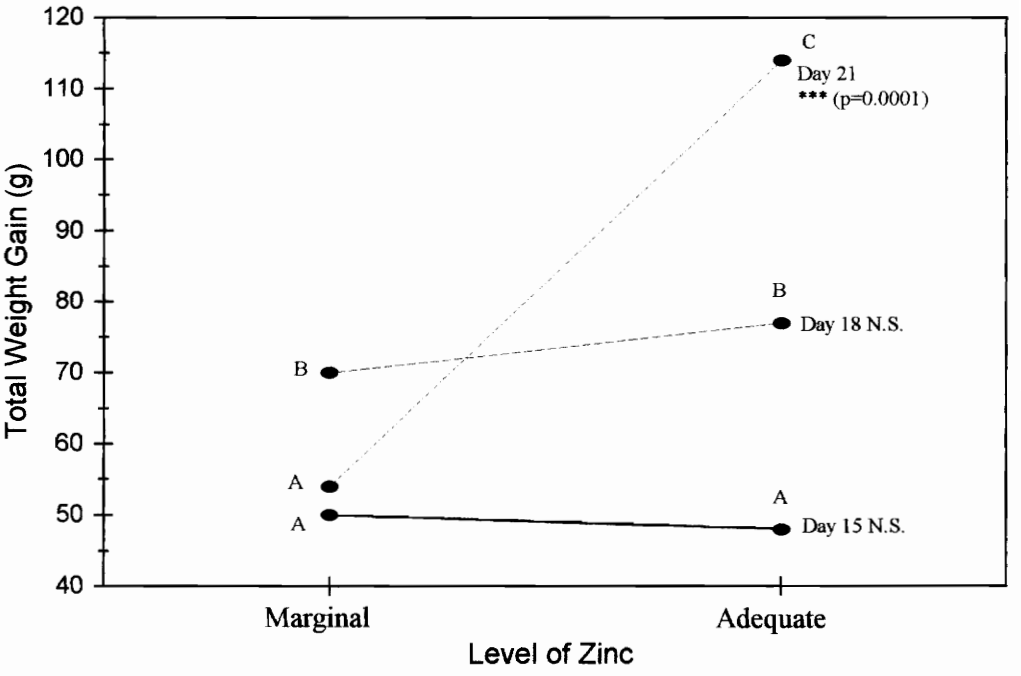

Figure 33 Zinc-Gestation Interactions for Total Weight Gain- Gestation Effect.

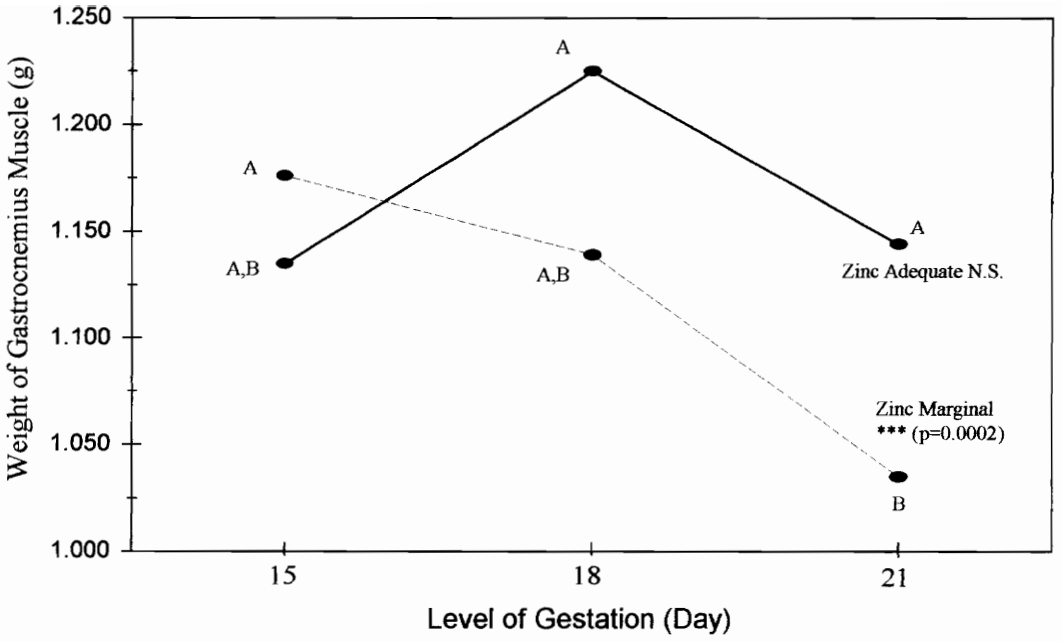

Figure 34 Zinc-Gestation Interactions for Weight of Gastrocnemius Muscle- Zinc Effect.

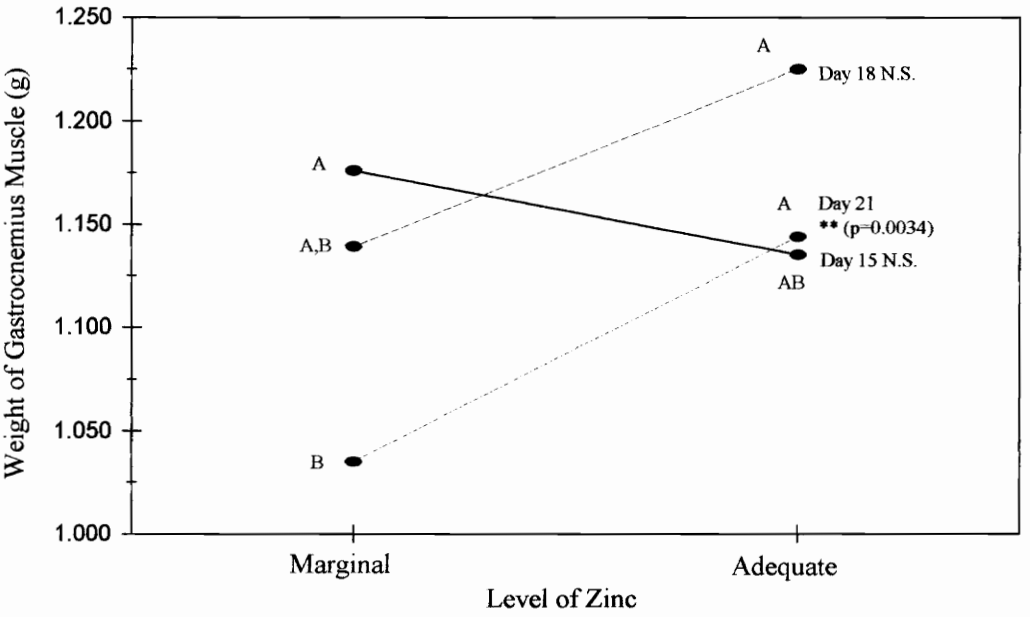

Figure 35 Zinc-Gestation Interactions for Weight of Dam Gastrocnemius Muscle-Gestation Effect.

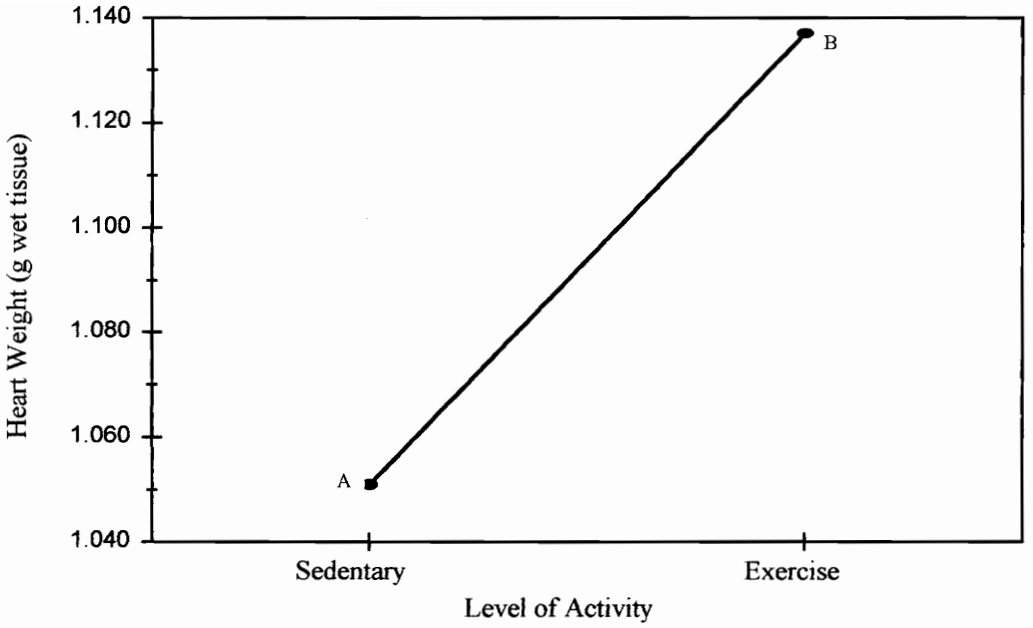

Figure 36 Exercise Analysis for Dam Heart Weight.

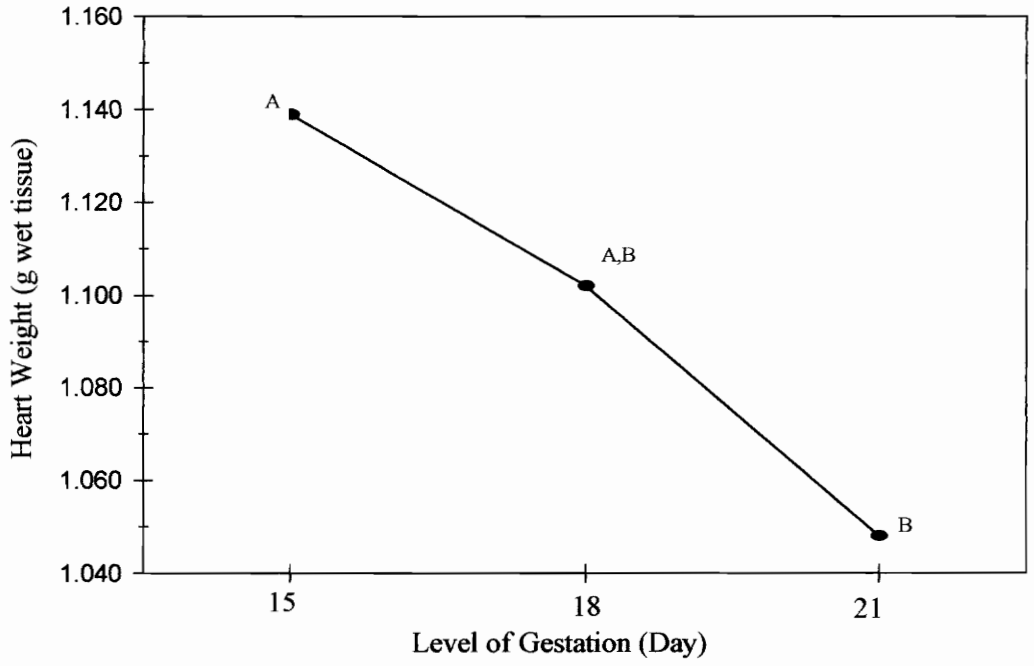

Figure 37 Gestation Analysis for Dam Heart Weight.

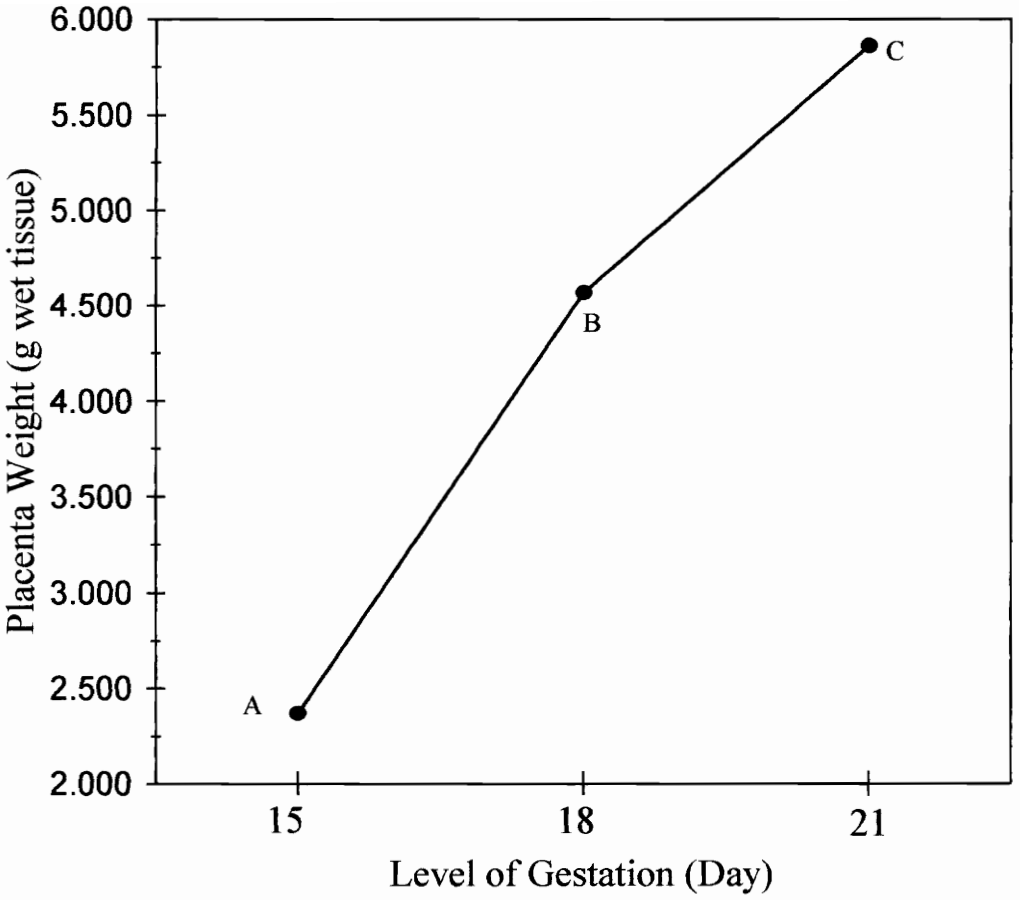

Figure 38 Gestation Analysis for Placenta Weight.

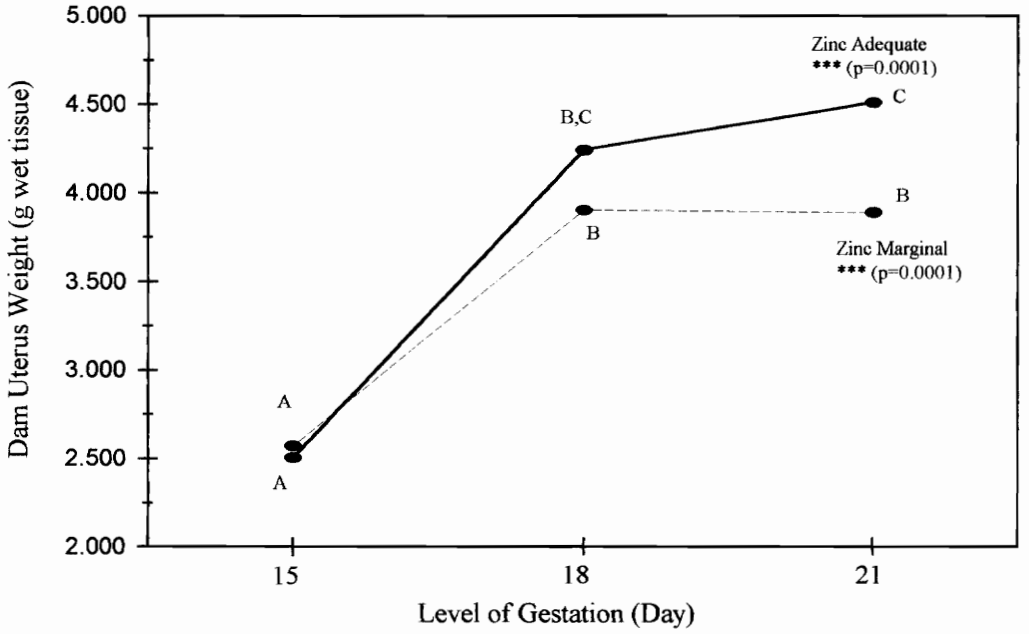

Figure 39 Zinc-Gestation Interactions for Dam Uterus Weight- Zinc Effect.

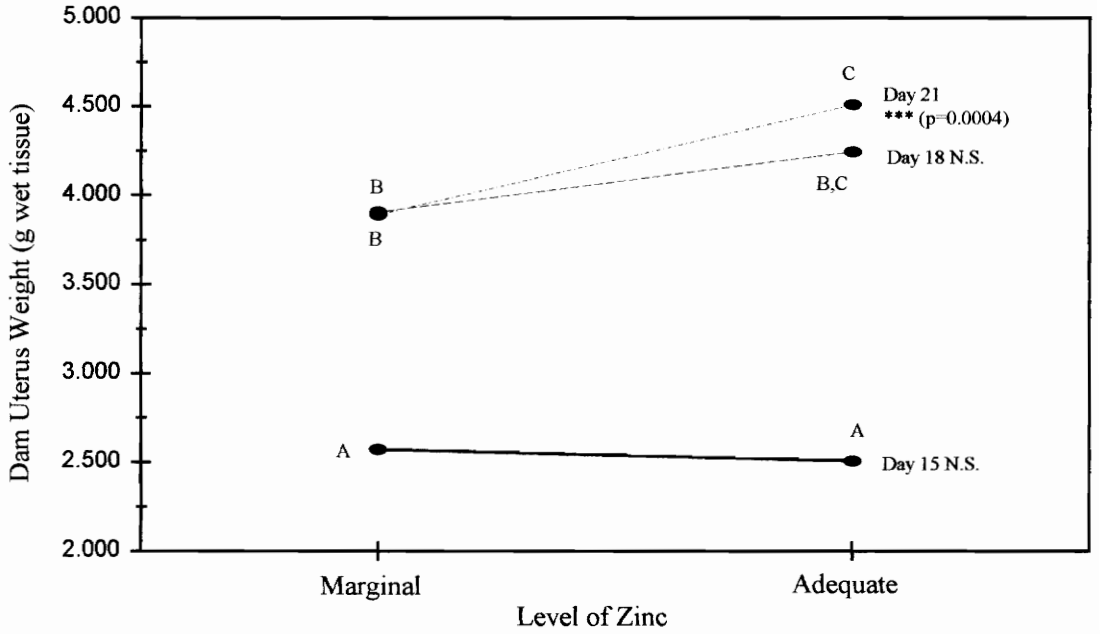

Figure 40 Zinc-Gestation Interactions for Dam Uterus Weight- Gestation Effect.

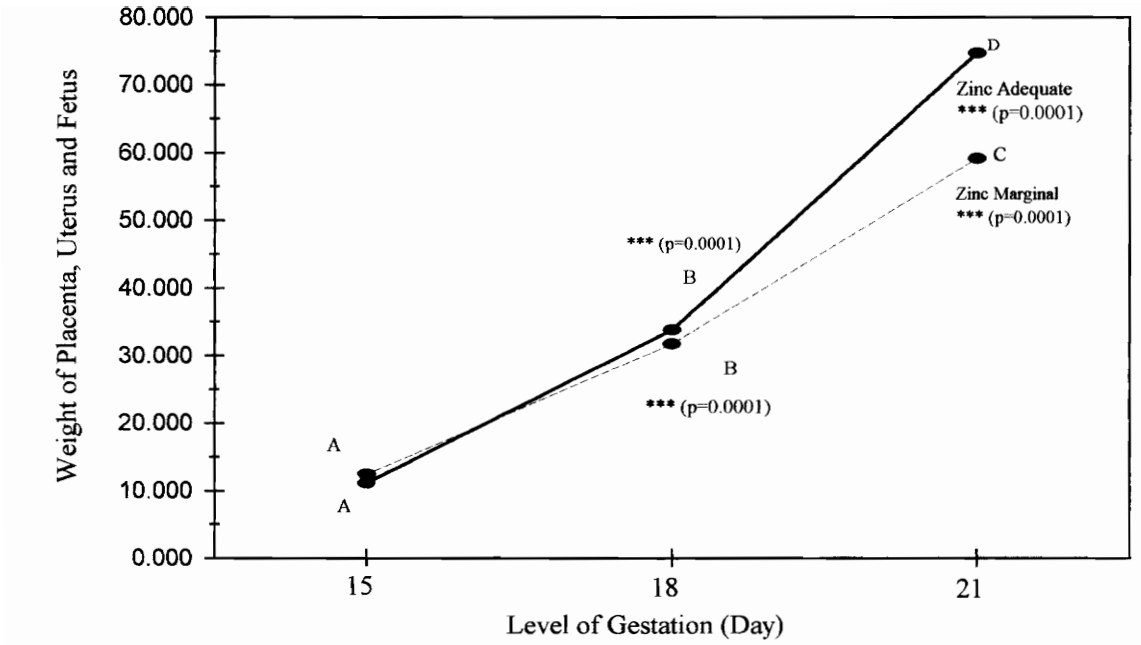

Figure 41 Zinc-Gestation Interactions for the Total Weight of the Placenta, Uterus and Fetus- Zinc Effect.

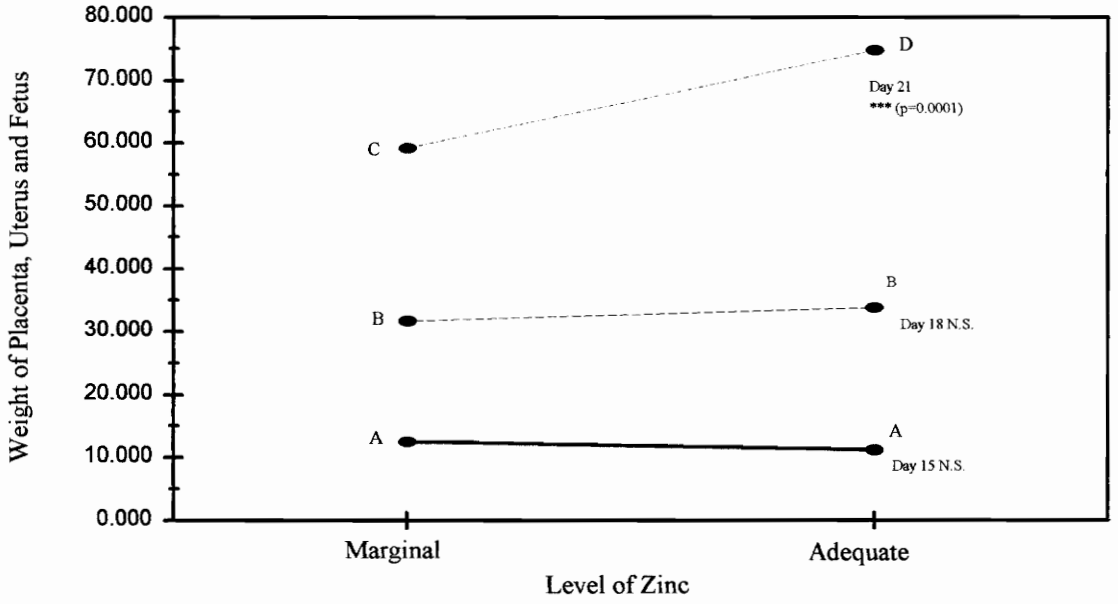

Figure 42 Zinc-Gestation Interactions for the Total Weight of the Placenta, Uterus and Fetus- Gestation Effect.

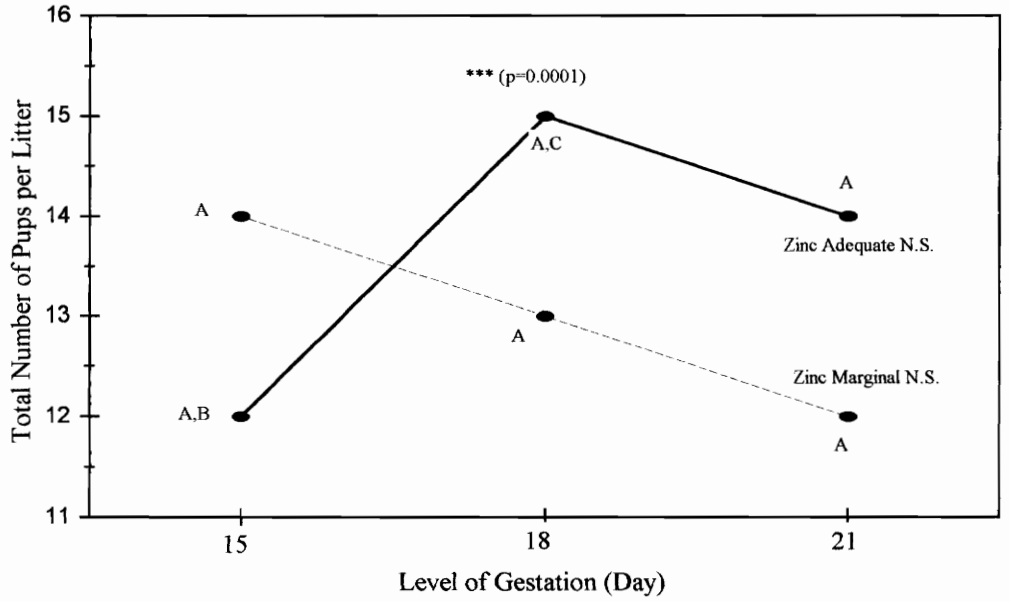

Figure 43 Zinc-Gestation Interactions for Total Number of Pups per Litter- Zinc Effect.

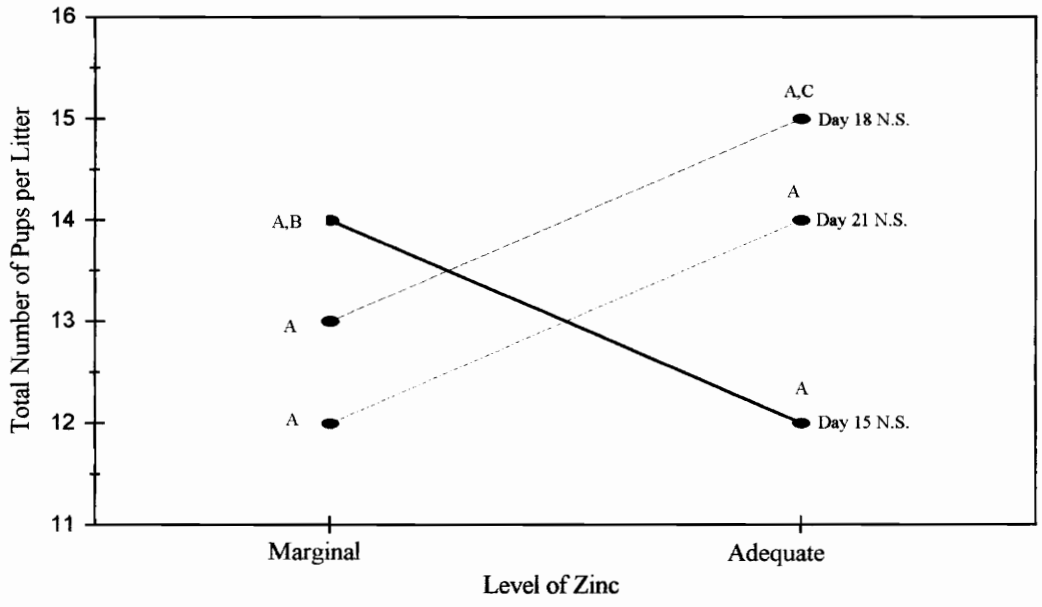

Figure 44 Zinc-Gestation Interactions for Total Number of Pups per Litter- Gestation Effect.

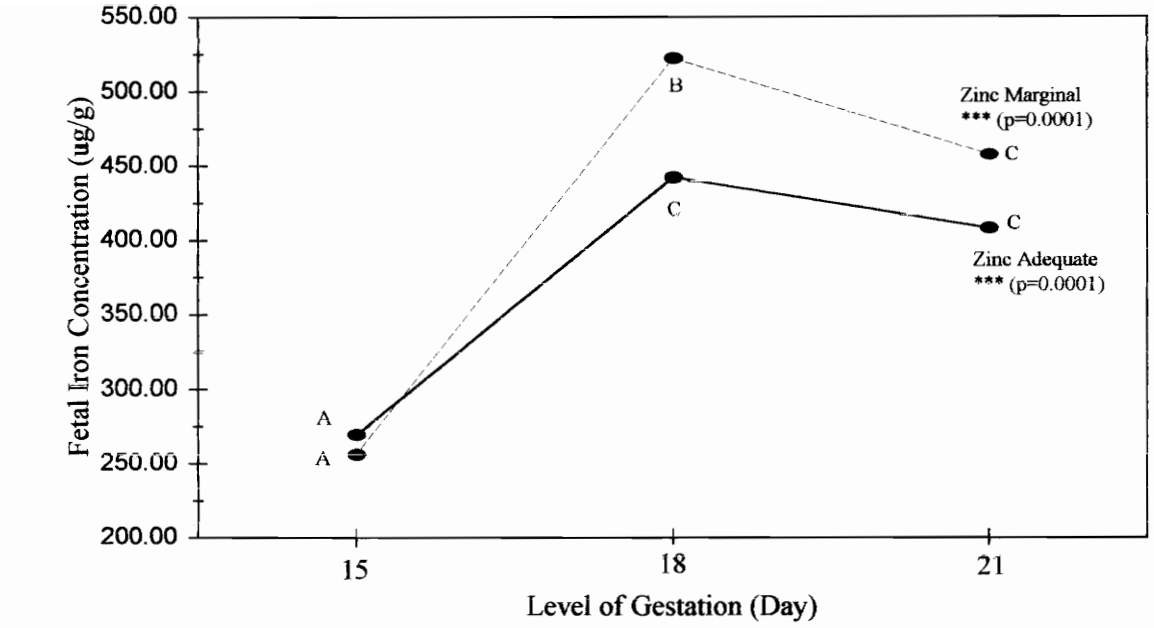

Figure 45 Zinc-Gestation Interactions for Fetal Iron Concentration- Zinc Effect.

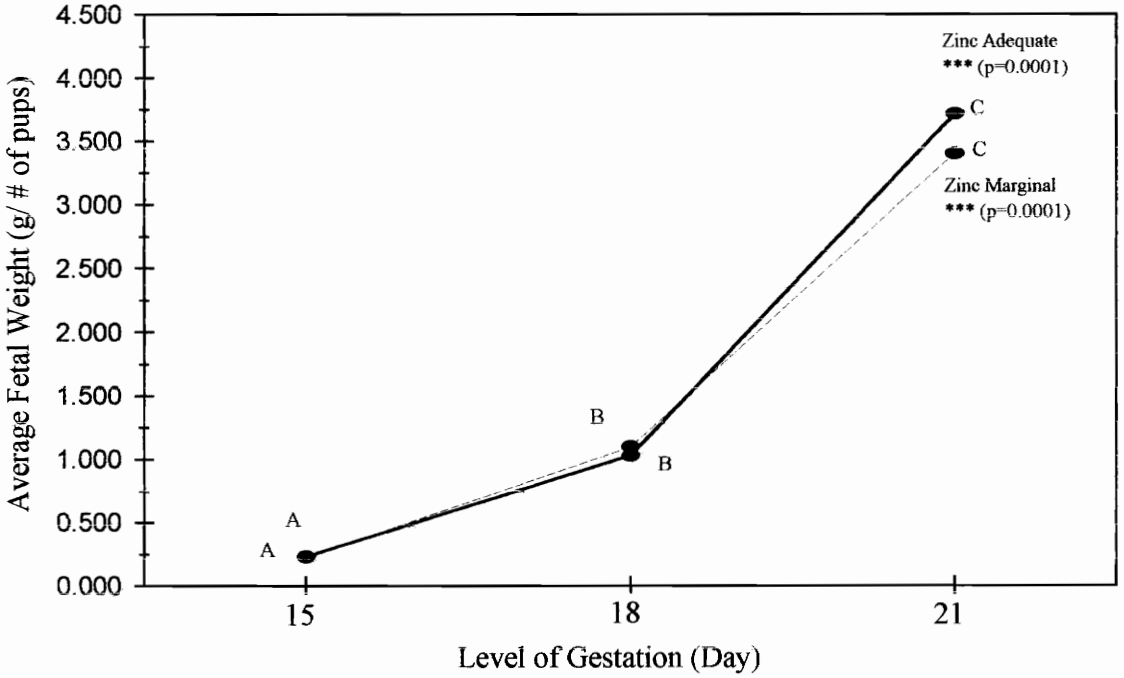

Figure 46 Zinc-Gestation Interactions for Average Fetal Weight- Zinc Effect.

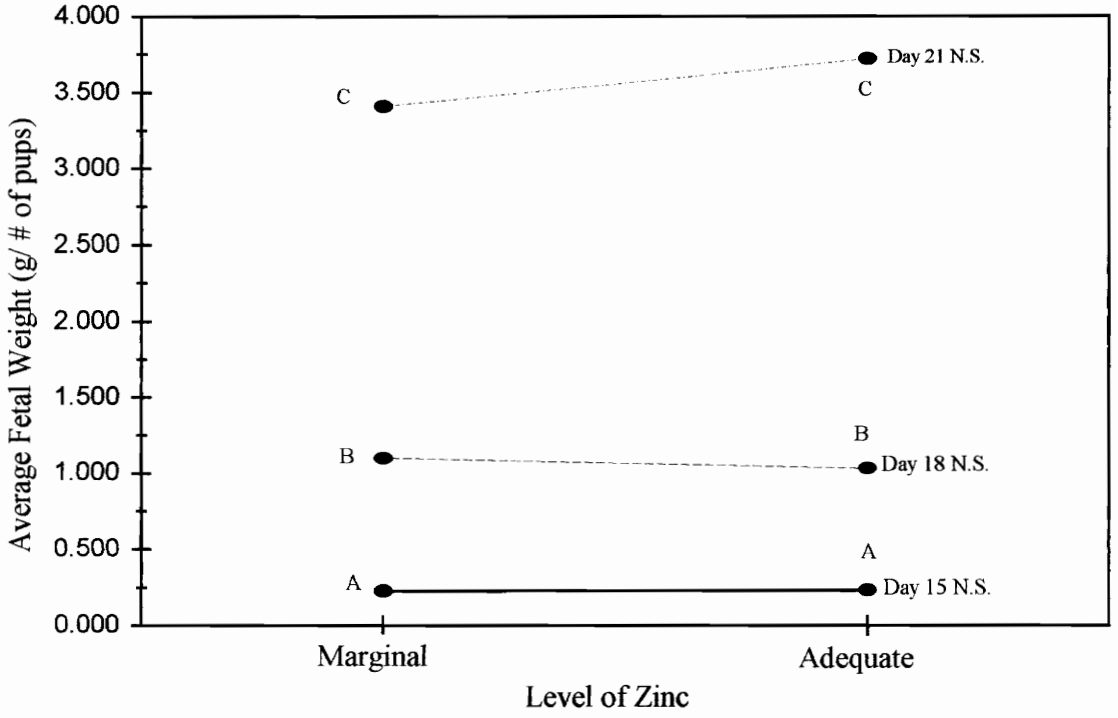

Figure 47 Zinc-Gestation Interactions for Average Fetal Weight- Gestation Effect.

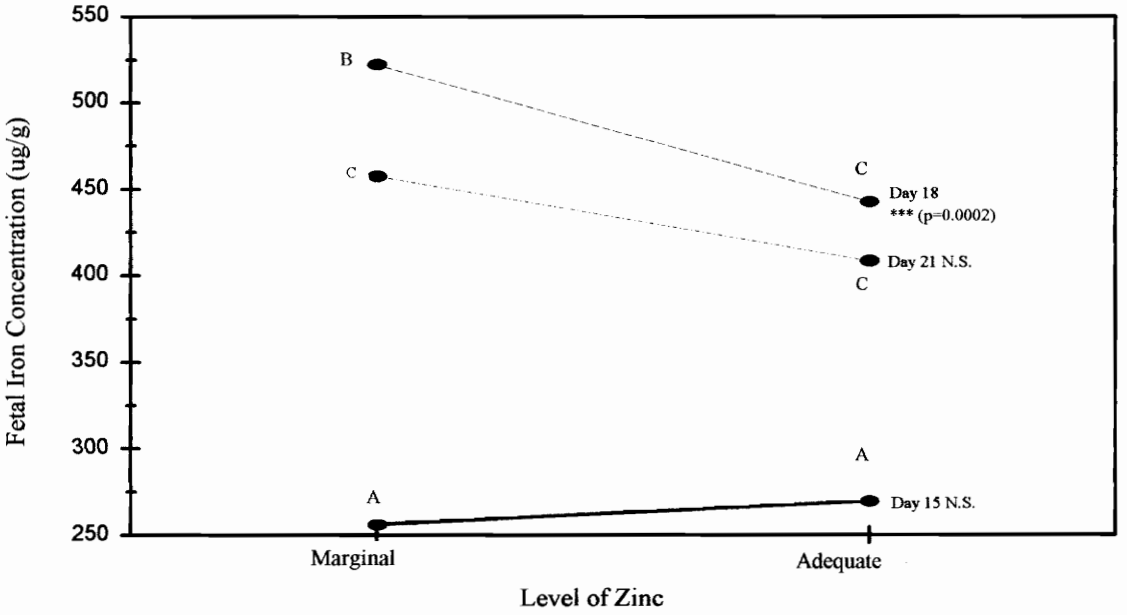

Figure 48 Zinc-Gestation Interactions for Fetal Iron Concentration- Gestation Effect.

VITA

Patricia Eileen Waters Young was born in Weisbaden, West Germany, January 4, 1961. She attended Caribou High School in Caribou, Maine and graduated from Patrick Henry High School, in Roanoke Virginia. She attended Virginia Tech and received a Bachelor of Science degree in Biology. She attended Roanoke Memorial Hospital's School of Medical Technology and is a Registered Medical Technologist. In addition to persuing a Master's degree, she completed a Coordinated Program in Dietetics at Virginia Tech and is a Registered Dietitian. She completed the requirements for a Master of Science degree in Human Nutrition and Foods, at Virginia Tech in 1997.

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