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Zinc Metabolism in the Streptozotocin (STZ)-Diabetes

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ZINC METABOLISM IN THE STREPTOZOTOCIN (STZ)-DIABETES

A Dissertation Presented

by

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Dedicated

To my wife Tieying Xie and my son Steel Fu

To my parents

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ABSTRACT

ZINC METABOLISM IN THE STREPTOZOTOCIN (STZ)-DIABETES

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Hyperzincuria in diabetics has been regarded as the culprit depleting body zinc stores. Studies were designed to assess rates of ^{65}Zn absorption and retention as a possible compensation mechanism; to assess zinc concentrations and distribution among body compartments; and to assess the kinetics of ^{65}Zn metabolism in STZ-diabetic rats.

The rates of ^{65}Zn absorption and retention were not significantly different between STZ-diabetic and control rats. However, STZ-diabetic rats had significantly higher rates of ^{65}Zn absorption (16.88%) and retention (34.36%) when they were "Post-fasted" than when they were "Prior-fasted" (9.04% and 18.68% respectively). These differences were also present in control rats at a lesser degree (14.86% and 30.19% for "post-fasting" and 11.76% and 23.25% for "prior-fasting" respectively). This observation suggests that dietary pattern affects ^{65}Zn absorption and retention. The results indicate also that STZ-diabetic rats are capable of absorbing and retaining enough zinc to meet body needs.

The STZ-diabetic rats had higher zinc levels in all tissues analyzed when they were on diet adequate for zinc, with significant increases in liver, duodenum, pancreas and femur; they had significantly higher zinc levels in liver and muscle but decreased plasma zinc levels when fed the diet marginal for zinc; on zinc-supplemented diet, they had significantly higher zinc levels in liver, kidney, duodenum, muscle and femurs, but decreased plasma zinc levels. Plasma zinc levels, a common measure of zinc status, did not reflect dietary zinc intake of STZ-diabetics. Endogenous zinc secretion was not decreased as judged by measuring ^{65}Zn in feces and duodenum. Whole body mean zinc concentrations (WBMZC) were significantly higher in STZ-diabetic rats on all three

dietary zinc levels. This increased WBMZC in diabetes may be the result of both increased absorption and tissue catabolism during diabetes. One may conclude that to accommodate zinc from increased tissue catabolism, the liver functions to store and sequester zinc from the circulation; while the kidney is overloaded and secreted it. Hyperzincuria in diabetes in this regard is a salvage of increased body zinc level. The diabetic bone may have disorders in utilizing Zn during Zn deficiency as judged by its kinetics and decreased ^{65}Zn specificity. The whole body mean concentrations of copper and iron of STZ-diabetics were also increased, suggesting similar disorders.

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GLOSSARY

Abbreviation	Meaning
AAS	Atomic Absorption Spectrophotometry
FFDS	Fat Free Dry Substance
GLM	General Linear Model
Prior-Fast	Rats were fasted overnight and fed after administration of ^{65}Zn
Post-Fast	Rats were fasted after administration of ^{65}Zn
SEM	Standard Error of the Mean
ZD	Zinc deficient diet
ZC	Normal zinc diet
ZS	Zinc supplemented diet
STZ	Streptozotocin
STZ-D	STZ-diabetic rats
WBMZC	Whole Body Mean Zinc Concentration

CHAPTER 1
INTRODUCTION AND OBJECTIVES

Diabetes mellitus, particularly insulin-dependent diabetes mellitus (IDDM) is a chronic and potentially disabling disorder. The disease and its many complications are a major public health and clinical concern because of its impact on affected countries and national health cost (Rubin et al. 1994). Diabetics are frequently reported to have, among many other metabolic abnormalities, hyperzincuria, which is an increased excretion of Zn via urine (Canfield et al. 1984; Heise et al. 1988; Kiilerich et al. 1990; Raz and Havivi 1989; Fu 1991; Nakamura et al. 1991; Meltzer et al. 1962). The cause of the hyperzincuria is far from clear. Long-term hyperzincuria may lead to depletion of body Zn stores and development of a Zn deficiency. This may further aggravate the disease manifestation and cause other clinical problems such as stunted growth, delayed sexual maturation, decreased immune function and many other health problems in diabetics. At present, how these diabetics maintain their Zn status and compensate for the extra losses of Zn via urine is not understood.

The true status of Zn nutrition in diabetic subjects is unknown. Development of techniques for assessing Zn status in diabetes mellitus have been unsuccessful. The commonly used approaches to assessing Zn status are analysis of Zn concentrations in plasma or serum, blood components, and tissues like muscle and bones. Zn concentration in plasma or serum, the most frequently used measure in assessing Zn status, may not be a good indicator of Zn status. Plasma or serum Zn levels of diabetics were reported to be either decreased (Car et al. 1991; Rosner and Gorfien 1968; Melinkeri et al. 1990), or increased (Mateo et al. 1975; 1978; Nobles et al. 1986). Mononuclear concentrations of Zn were regarded as a more accurate measure of Zn status (Prasad 1988), but levels of leukocyte Zn in diabetes are also inconsistent (Raz and Havivi 1989; Kumar and Jaya Rao 1974; Pidduck et al. 1971; Raz and Havivi 1989; Pai et al. 1988). The same are also true

for assessing erythrocyte Zn (Sjogren et al. 1985; Raz and Havivi 1989; Rosner and Gorfien 1968) and muscle Zn levels (Failla and Kiser 1981; Levine et al. 1983).

The uncertainty of Zn status in diabetes has led studies to measure Zn absorption and endogenous secretion as the sources of Zn compensating for urinary losses (Heise et al. 1988; Johnson and Canfield 1985; Craft and Failla 1983; Kiilerich et al. 1990). The ability to absorb dietary Zn was not significantly altered and the total amount of Zn absorbed was increased in diabetic animals due to their increased food intake. The increased Zn absorption in STZ-diabetic animals may be due to their swollen intestine (Craft and Failla 1982; Gourley et al. 1983), stimulated digestive enzymes (Olson and Roger 1971), decreased lumen to mucosal flux of Zn (Ghishan and Greene 1983), and decreased intestinal secretion of endogenous Zn (Johnson and Canfield 1985). The only study on Zn absorption in human IDDMs (Kiilerich et al. 1990) reported a decreasing ^{65}Zn absorption, which is contradictory to all these animal studies mentioned above. Studies in this laboratory did not show an obvious benefit to human IDDMs from Zn supplementation (Fu, 1991; Cunningham et al. 1994), suggesting Zn status in diabetics is not significantly altered.

The present study tried to resolve these uncertainty on Zn metabolism in diabetes mellitus by three approaches: first, a study on the effect of feeding pattern on four-hour Zn absorption and retention in diabetes; second, a detailed analysis of tissue Zn distribution and concentrations under both Zn depletion and supplementation; third, kinetics of Zn metabolism in diabetes at the whole body and tissues levels with the following three objectives:

1. To assess ^{65}Zn absorption and retention as a mechanism of compensation for hyperzincuria in STZ-diabetic rats; to compare the effects of post-fasting and prior fasting on the rates of ^{65}Zn absorption and retention; To explore what other variables relate to ^{65}Zn absorption.
2. To assess the Zn status of diabetics by analyzing whole body and tissue Zn levels in STZ-diabetic rats and by studying the effect of diets which contain different amounts of Zn, e.g. Zn-depleted, normal and Zn-supplementation, on

tissue Zn concentration and distribution in STZ-diabetic rats in comparison with their controls;

3. To determine kinetics of ^{65}Zn metabolism, e.g. whole body retention of ^{65}Zn, turnover rate of ^{65}Zn, excretion of ^{65}Zn in urine and feces and ^{65}Zn retention at the tissue or organ levels within 12 days after ^{65}Zn administration in STZ-diabetic rats in comparison with that of their controls.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction to Diabetes Mellitus and Zinc

2.1.1 Diabetes Mellitus: A Disorder of Metabolism

Diabetes mellitus is a genetically-determined disorder characterized by disturbed metabolism of carbohydrate, fat, and protein caused by a relative or absolute insufficiency of insulin and/or varying degrees of insulin resistance (Fajans 1990; Olefsky and Molina 1991). The disease is chronic and potentially disabling in nature. It represents a major public health and clinical concern. People with diabetes mellitus are at increased risk in developing chronic complications which affect wide variety of body systems (Rubin et al. 1994).

There are two types of primary diabetes mellitus: Type 1 diabetes mellitus or insulin-dependent diabetes mellitus (IDDM) and Type 2 diabetes mellitus or non-insulin-dependent diabetes mellitus (NIDDM). The two types of diabetes mellitus are distinct entities, differing in etiology, pathophysiology and treatment.

IDDM is caused by β-cell destruction which results in insulin deficiency. Factors which lead to beta-cell destruction are not clearly understood (Lernmark 1991). Generally, genetics, autoimmunity, and environmental factors have all been considered to cause IDDM (Warram et al. 1987; Rossini et al. 1988; Oldstone et al. 1991). The degenerative changes noticed in all forms of diabetes appear to have a common etiology which involves hyperglycemia. The disturbances in metabolism due to insulin deficiency or insulin resistance during hyperglycemia in diabetes mellitus can be summarized as follows (Davidson 1991):

1. Substrate anabolism decelerates;
2. Tissue catabolism accelerates;
3. Levels of stress hormones increase (epinephrine, norepinephrine, glucagon, cortisol, and growth hormone);
4. Plasma glucose rises;

5. Plasma pH decreases because of fatty acid release and ketone body production.

The major complications of diabetes mellitus are listed in Table 2.1. The major causes for morbidity and mortality in patients with diabetes come from the long-term microvascular complications. The microvascular complications result in an increased incidence of heart disease and cerebrovascular episodes (stroke, hemorrhage, etc.) as well as peripheral vascular occlusion resulting in amputations. Diabetic retinopathy and nephropathy stemming from vascular changes eventually develop into blindness and renal failure respectively. Diabetes is the most common cause of blindness in the United States. Kidney transplants from renal failure due to diabetes are performed more frequently than for any other causes (Diabetes Surveillance 1980-1987). The mechanisms of the diabetic complications are thought to be due to the prolonged effect of hyperglycemia on polyol (such as sorbitol) formation and its subsequent accumulation in tissues which causes tissue damage (Kinoshita, et al. 1990) and/or glycosylation of important structural proteins, thereby affecting their functions. A recent hypothesis proposed that systematic hyperinsulinemia and hepatic hypoinsulinemia are both responsible for the vascular complications of the disease, but the hypothesis needs to be tested (Gwinup and Elias 1991).

Diabetes is one of the major public concerns due to its huge medical costs on both the patients and the nation. Approximately 1 in 500 children less than 18 years of age in the United States are affected by IDDM (Laporte et al. 1981). Diabetics (both IDDM and NIDDM) accounted for 4.5% of U. S. population in 1992, but accounted for 14.6% of total U. S. health care expenditure (105 billion), the per-capita expenditure for diabetes ($11,157) is four times that for the non-diabetics ($2604) (Rubin et al. (1994). Of all diabetes mellitus diagnosed, about 90% are NIDDM. The prevalence of NIDDM increased as much as 10-20 fold during the last half century, perhaps as a result of both increasing longevity and an increased incidence of obesity (Davidson and DiGirolamo 1991).

Table 2.1. Diabetic Complications

Eyes
Diabetic retinopathy
- Non-proliferative (background)
- Proliferative

Cataracts
- Subcapsular (snowflake)
- Nuclear (senile)

Nervous system
Peripheral neuropathy
- Distal, symmetric sensory loss
- Motor neuropathy
- Foot drop, wrist drop
- Mononeuropathy multiplex (diabetic amyotrophy)

Cranial neuropathy
- Cranial nerve III, IV, VI, VII

Autonomic neuropathy
- Gastrointestinal neuropathy
- Resting tachycardia
- Loss of sweating
- Postural neuropathy
- Gastroparesis
- Diabetic diarrhea
- Urinary bladder atony
- Impotence (may be also secondary to pelvic vascular disease)

Bones and joints
Diabetic cheirarthropathy
Dupuytren's contracture
Charcot's joint

Kidney
Intracapillary glomerulosclerosis
- Disfuse
- Nodular

Infection
- Pyelonephritis
- Perinephric abscess
- Renal papillary necrosis
- Renal tubular necrosis

Following dye studies
- (urogram, arteriogram)

Skin
Diabetic dermopathy (skin spots)
Necrobiosis lipodica diabeticorum
Candidiasis
Foot and leg ulcers
Neurotropic
- Ischemia

Cardiovascular system
Heart disease
Myocardial infarction
Cardiomyopathy
Gangrene of the feet
Ischemic ulcers
Osteomyelitis

Unusual infections
Emphysematous cholecystitis
Malignant externa
Necrotizing fasciitis
Necrotizing myositis
Mucor meningitis

* From Greenspan F. S. and Forsham P. H. (eds): Basis & Clinical Endocrinology, 2nd ed. 1986; East Norwalk, Connecticut: Appleton-Century-Crofts. P. 523-574.

2.1.2 Zn: An Essential Nutrient

Zn is indispensable for all forms of life and critical to transmission of genetic messages, differentiation, development, growth, and the ultimate preservation of species. It is such an essential element that no disorders are known to be associated with its excessive accumulation (Vallee and Falchuk 1993). However, its antagonistic effect on Cu, and the many side effects through inappropriate uses can not be ignored (Cunnane 1988). Zn deficiency has been frequently reported in both developing countries as well as developed countries (Sandstead 1991) and is manifested in variety of clinical disorders (Table 2.2).

The major function of Zn is as a co-factor in many enzymes (Girchev and Tzachev 1988). Zn has been found in all six categories of enzymes; a total 160 enzymes from different species are reported to have Zn as a component (Galdes and Vallee 1983). Functions of Zn in these enzymes include catalytic, structural, regulatory, and non-catalytic (neither involved directly in catalysis nor essential for structural function) functions involved in DNA, RNA and protein synthesis (Falchuk 1988; Hurley 1981).

Normal secretion and function of several hormones, including insulin may depend on a normal Zn status. Deficiencies of Zn influence growth hormone, somatomedin, and glycosaminoglycan formation, resulting in growth impairment (Bolze et al. 1987). Zn is involved in insulin synthesis and secretion (Kirchgessner and Roth 1983). Zn deficiency causes hypogonadism, thereby decreasing the ability of testes to secrete sex-hormones (Abbasi, et al. 1980; Bunce, 1988). During Zn deficiency, plasma levels and functioning of some other hormones, such as prolactin, thyroid hormone, corticosteroids are also affected (Hambidge et al. 1986). It has been demonstrated that Zn is directly involved in the binding of some hormones to their plasma membrane receptors and subsequently alters signal transduction across plasma membranes (Anonymous 1991). Zn also affects the structure of hormone receptors (Vallee and Falchuk (1993).

Zn improves wound healing (Henkin 1974) and vision (Smith et al. 1974). A recent hypothesis suggests that aging process may be due to an intracellular Zn deficiency, which affects some of the "overly-vulnerable" Zn enzymes, resulting in accumulation of

useless (or toxic) materials, altered production of essential proteins, neoplastic changes and cell death. Zn ions exert an inhibitory effect on the functional components of the cell membranes (Chvapil, 1976; Bettger and O'Dell 1993). Zn affects the functional activity of macrophages, polymorphonuclear leukocytes and possibly some other cells. Zn is thus able to relieve the inflammatory reaction of the system (Chvapil 1976). A recent study demonstrated that the concentration of red-cell membrane Zn correlated well with osmotic frangibility during Zn depletion and repletion (Jahanning and O'Dell 1989).

Zn deficiency has marked effects on almost all components of the immune system: decreased antigen response, decreased cell-mediated response, and decreased performance of normal cell function (Fraker et al. 1987). Zn deficiencies in animals increase susceptibility to a number of bacterial, viral, and parasitic challenges (Keen 1990). Peripheral blood neutrophils from patients with acrodermatitis enteropathica (a genetic disorder of Zn metabolism) were defective in their chemotactic activity, and this defect could be reversed successfully by treating the patients with Zn.

Zn deficiency influences sexual maturation, fetal growth and development, and labor (Girchev and Tzachev 1988; Apgar 1992). Taste and smell (Henkin et al. 1974), as well as brain and peripheral nerve function (Prohaska 1987; O'Dell et al. 1990) are all inhibited by Zn deficiency.

Zn status is altered by wide varieties of diseases. Decreased Zn status has been reported in gastrointestinal diseases (McClain et al. 1988), liver disease (Sullivan and Burch 1976), cancer (Schrauzer 1977), diabetes mellitus (Kiilerich et al. 1990), sickle cell anemia (Prasad 1986), renal disease (Prasad 1986), and cystic fibrosis (Halsted and Smith 1970). It has been hypothesized that Zn has a universe role in cellular function (Burnet 1982). Metabolic abnormalities in Zn Deficiency are listed in Table 2.3.

Table 2.2 Clinical Features of Zn Deficiency

Skin and hair lesions
Loss hair, Alopecia,
Skin dry and hard
Scaly, cracking, fissures and thickening
Bleeding
Abnormal feather (birds)
Inflammation
Oesophageal parakeratosis
Reduced feeding consumption and utilization of food
Catabolism
Growth retardation
Increased urinary nitrogen and sulphur
Impaired reproduction
Reduced testicle size
Decreased libido
Lower spermatogenesis
Retarded sexual maturity
Skeletal malformation
Impaired immune responses
Impaired wound healing
Behavioral disturbance
Lethargy
Reduced learning ability
Emotional stability
Anemia
Hepatosplenomegaly
Night blindness
Impaired taste (hypogeusia)
Aging

Modified from Solomon, 1988.

Table 2.3 Metabolic Abnormalities in Zn Deficiency

Decreased circulating Zn
Decreased albumin synthesis
Decreased serum alkaline phosphatase activity
Decreased carboxypeptidase activity (intraduodenal)
Decreased alcohol dehydrogenase (retina, testis)
Decreased RNA polymerase activity (liver)
Decreased nucleoside phosphorylase activity (lymphocytes)
Elevated serum RNase activity
Increased serum ammonia
Impaired glucose metabolism
Decreased insulin response
Disordered prostaglandin sensitivity
Decreased testosterone level
Decreased white cell chemotaxis
Impaired T-lymphocyte function
Decreased serum thymic factor
Decreased resistance to infections
Increased resistance to autoimmune disease
Decreased intestinal disaccharidase activity
Elevated 3-hydroxyglutamyl coenzyme A reductase
Abnormalities of retinal pigments
Decreased collagen synthesis
Decreased platelet function

Modified from Solomons, 1988.

2.2 Zinc and Diabetes Mellitus

2.2.1 Introduction

A history of relationship between Zn and diabetes mellitus traces back more than 60 years. The discovery that crystalline insulin contained considerable quantities of Zn (Scott 1934) stimulated many investigations into the physiological role of Zn in insulin synthesis, storage, secretion and peripheral metabolism, and ultimately its role in diabetes mellitus. As early as 1937, Hove (Hove et al. 1937) noted a small difference in glucose tolerance in Zn deprived rats compared with normal animals. Vikbladh in early 1951 tried to assess Zn status by determining serum Zn levels in insulin-treated diabetics. Tarui (1963) reported hyperzincuria was a prominent feature of experimental diabetes. Since these early experiments, Zn metabolism and assessment of Zn status in diabetes mellitus have been under active study. The possible links between Zn and diabetes mellitus are listed in Table 2.4.

Table 2.4 Possible Links between Zn and Diabetes Mellitus

Links	Mechanisms
Zn and insulin	
Insulin synthesis,	Insulin from most species contains Zn
Insulin storage	Pancreas contains large amount of Zn
Insulin secretion	Co-secretion of Zn and insulin from pancreas Zn deficiency decreases insulin secretion
Insulin degradation	Zn deficiency increases insulin degradation
Zn and proinsulin	Proinsulin contains 5 moles of Zn, it is soluble Zn may also be involved in the conversion of proinsulin to insulin
Zn and glucose tolerance	Zn deficiency results in glucose intolerance May involve insulin resistance, decreased tissue sensitivity to insulin
Zn and insulin-like activity	Zn deficiency may decrease secretion of insulin-like substance
Hyperzincuria	Extra loss of Zn from urine in diabetes

2.2.2 Roles of Zn in Insulin Metabolism and Glucose Tolerance

2.2.2.1 Zn and Insulin Synthesis in the Islets of Langerhans

Several lines of evidence suggest that Zn is essential for insulin metabolism but its direct roles are still speculative. It is likely that Zn facilitates the processing and packaging of insulin (Cunnane 1988a). Insulin is synthesized at the ribosome as a single-chain polypeptide, preproinsulin. Removal of the N-terminal leader sequence yields proinsulin, which aggregates into hexamers with two Zn atoms per hexamer connected to the six histidines. Proinsulin is a highly soluble molecule itself, and its solubility is primarily enhanced by the weak binding of up to 30 atoms of Zn per hexamer. Removal of the connecting peptide and exposure of non-polar residues of proinsulin dramatically decrease both its Zn binding capacity and solubility. As a consequence, insulin precipitates and is packaged into the beta cell storage vesicles as the two Zn hexamers ready for releasing by appropriate stimulus (Maske 1957; Vallee 1959; Chvapil 1976; Yoshinaga and Ogawa 1975). The study by Epand et. al. (1984) showed that stripping pancreatic granules of Zn may induce diabetes. They found that the diabetogenicity of Zn chelators was correlated with their ability to sequester Zn. The diabetogenicity of alloxan, a commonly used drug to induce diabetes in animals, is also correlated to its ability to chelate pancreatic Zn ions (Maske 1957; Lowry et al. 1954).

However, it has also been shown that Zn is not an obligatory component of insulin synthesis. Cultured islet cells have been found to synthesize insulin in Zn-depleted media for 9 days (Howell et al. 1978; Hoftiezer et. al. 1985). Moreover, the fact that the active form of circulating insulin is a Zn-free monomer (Blundell et. al. 1972; Goldman and Carpenter 1974) is also contradictory to the indispensable role of Zn in insulin metabolism..

At the whole body level, whether or not insulin metabolism is affected by dietary Zn intake is unclear. Pancreatic Zn concentration may be influenced by dietary intake (Southon et al. 1988; Williams and Mills 1970) and Zn deficient diets have also been associated with a decreased granulation in the beta-cells (Boquist and Lernmark 1969). While Zn supplementation increases insulin content and granulation of the pancreatic

islets; it also decreased the abnormally high insulin-secretion response to glucose by pancreatic islet cells from ob/ob mouse (Begin-Heick et al. 1982). However, other studies (Huber and Gershoff 1973) found no significant effect of high levels of dietary Zn (1200 ppm) on pancreatic insulin content compared with low level of Zn (1 ppm).

2.2.2.2 Zn and Insulin Secretion

It should be pointed out that the pancreas contains a large amount of Zn, but only a small percentage of Zn is associated with insulin (Figlewicz, et al. 1984). A concurrent change in Zn and insulin contents within the islets of Langerhan suggests Zn may be involved in insulin secretion (Grosby and Schmid-Formby 1985). Very high concentration of Zn in the media stimulates in vitro insulin secretion from pancreas (Flgelwicz et al. 1981). When the pancreas was derived from Zn deficient rats, the effect of glucose on insulin release was markedly diminished (Huber and Gershoff 1973). Serum insulin in lambs was decreased when they were fed on a Zn deficient diet but normal in lambs fed on diets either marginal or adequate for Zn (Droke et. al. 1993), suggesting under normal conditions, serum insulin level may not be affected. However, it was also observed that animals fed Zn depleted diet had decreased levels of serum insulin and insulin-like growth factor-1 (Dorup et al. 1991).

Formby Grosby and Schmid-Formby (1985) showed that extra-granular Zn efflux from the islets of Langerhans was sensitive to secretagogues and cellularly-active agents but was not related quantitatively to insulin secretion. Pancreatic islets have been shown to secrete less Zn when stimulated by glucose, but when stimulated by stronger secretagogues, the secretion ratios of Zn to insulin gradually increased. This increase in Zn secretion was sustained over a long time period (Figlewicz et al. 1984; Florence et al. 1984).

Southon, et al. (1988) reported that genetically diabetic mice, 4-5 weeks of age, fed a diet low in Zn for 28 days had higher levels of fasting-blood-glucose and liver glycogen, but their fasting blood insulin level was unaffected. These authors suggested

that this may be an adverse effect of decreased Zn-intake on glucose utilization in the genetically diabetic mice, which may precede any significant plasma insulin change.

2.2.2.3 Zn and Proinsulin

Zn, as an indispensable component of proinsulin, is thus associated with insulin metabolism. The conversion of proinsulin to insulin in vitro is decreased in a Zn-deficient media (Howell et al. 1978; Grant et al. 1972) suggesting that the conversion of proinsulin to insulin is a Zn-dependent process. Zn also increases proinsulin stability and decreases insulin solubility (Gold and Grodsky 1984). Zn may affect the conversion process of proinsulin to insulin through its effect on some enzymes which may be involved in the process. As an example, it is known that carboxypeptidase B loses about 50% of its activity in Zn-deficient rats (Kirchgessner and Roth 1983; Steiner et al. 1974). However, serum proinsulin concentration has also been shown not to be influenced by Zn deficiency in some other studies (Howell et al. 1978).

2.2.2.4 Zn and Glucose Intolerance

Zn deficiency may impair glucose tolerance. Hove et al. (1937) reported differences in glucose tolerance curves between Zn-deficient and ad libitum-fed control rat after oral-glucose dosing. Using intraperitoneal glucose injection, Quartman et al. (1966) found decreased glucose tolerance of Zn-deficient rats compared with pair-fed control animals after an extended experimental period and an overnight fast. This has also been seen in other studies using Chinese hamsters (Boquist and Lernmark 1969; Hueber and Gershoff 1973). In human beings with dwarfism and symptoms of Zn deficiency, decreased glucose tolerance was also observed (Sandstead et al. 1967).

The relationship between Zn deficiency and glucose intolerance may be underlined by the observation that Zn deficiency may blunt insulin secretion. Kirchgessner and Roth (1983) observed that Zn-deficient animals lack the second phase of insulin secretion in response to glucose injection. However, Reeves and O'Dell (1983) concluded that the

diminished insulin synthesis and secretion do not adequately explain the observed effect of Zn deficiency on glucose metabolism.

Several explanations have been proposed to account for Zn deficiency affecting glucose intolerance. The effect of Zn on glucose tolerance in some of the studies appears to be related to the route of glucose administration. A significant increase in plasma glucose occurred in the Zn-deficient animals when glucose was injected intraperitoneally, however, no difference was found in the rate of metabolism of orally administered glucose between Zn-deficient and Zn-supplied rats (Hendricks and Mahoney 1972). On the other hand, Quartman and Florence (1972) did not find any effect of Zn deficiency on glucose tolerance when glucose was administered intraperitoneally. They attributed this lack of effect to the pattern of food intake. The difference in results noted between oral glucose dosing and intraperitoneal or intravenous glucose injection might be due to a greater stimulation of insulin secretion in response to oral glucose (Fasel et al. 1970). However, Brown, et al. (1972) did not find any effect of Zn deficiency on glucose tolerance after oral administration , suggesting there may be other factors involved.

The rate of insulin degradation increased in the Zn-deficient rats as they became much more resistant to hypoglycemic coma than Zn-repleted rats (Hendricks and Mahoney 1972; Quartman et al. 1966). These observations are supported by the observation that higher Zn levels in cultural media increase insulin binding to liver plasma membranes and subsequently decrease insulin degradation (Arquilla et al. 1978).

Zn deficiency may induce an insulin resistance in peripheral tissue similar to that noted in some cases of diabetes mellitus (Baer et al. 1985; Reeves and O'Dell 1983). Fat cells require insulin for the uptake of glucose. The considerable decline in glucose utilization by fat cells in Zn-deficient meal-fed rats is most readily explained by one of two mechanisms: either circulating insulin levels are low or the response of fat cells to insulin is decreased. The study by Park et al. (1989) clearly showed that rats with pure Zn deficiency displayed a considerable glucose intolerance, but the effect is not due to lower level of circulating insulin rather to peripheral resistance to insulin action, since these animals had normal levels of serum glucagon, and slightly elevated serum insulin. Since

Zn-deficient rats manifested an increased corticosterone as well as increased plasma glucose, corticosterone has been hypothesized to take a part in the impaired glucose tolerance (Park. et al. 1989).

2.2.2.5 Zn and Insulin-Like Substance

Sera from both animals and humans contain insulin-like substances which possess high specific biological activity but which differ biochemically from insulin. It was observed that sera from Zn-deficient animals had an increased insulin-like activity, which may be a compensatory mechanism to decreased insulin secretion in response to a glucose load (Kirchgessner and Roth 1983). Droke et al. (1993) found that serum insulin-like growth factor-1 was decreased in Zn deficient animals while this decrease was not seen in animals fed on marginally Zn deficient and adequate Zn diets.

Zn itself possesses a stimulatory effect similar to insulin on carbohydrate metabolism (May and Contoreggi 1982). Concentrations of Zn between 250 and 1000 uM have been found to stimulate 3-O-methylglucose transport and glucose metabolism to CO_2 and other intermediates; and Zn ions stimulate the pentose phosphate cycle and inhibit lipolysis (May and Contoreggi 1982). The effect of Zn ions on glucose oxidation and lipolysis was inhibited by extracellular catalase, suggesting this effect of Zn may related to hyperoxide generation, as well as the direct effect of Zn ions on intracellular metabolism. Coulston and Dandona (1980), who used the rate of lipogenesis by rat epididymal adipocytes as an index of the biological potency of insulin, found that Zn had a potent stimulatory effect on lipogenesis in vitro. This effect was similar to and independent of insulin, and Zn was found to have an additive effect when incubated together with insulin in the in vitro medium (Coulston and Dandona 1980). A recent report (Thorne and Lockwood 1991) indicates that Zn inhibits proteolysis, which is also one of the effects of insulin. These effects of Zn were unique since other cations, such as Co^{+2}, Mn^{+2}, Fe^{+2}, and Cr^{+3}, have no effect.

2.2.2.6 Hyperglycemic and Glucosuria Action of Zn

Some studies have shown that Zn exhibits both hyperglycemic and glycosuric effects, which may explain the significantly increased plasma glucose levels in rats tested Zn either intraperitoneally or orally (Etzel and Cousins 1983). The increase in serum glucose after administration of Zn was prompt, within 15 minutes, and it returned to normal within 4 hours. Significant depletion of hepatic glycogen suggests it is a glycogenolytic process. Adrenalectomy completely eliminated the hyperglycemic effect of Zn, and adrenergic blockade was also effective in preventing the hyperglycemic effect, suggesting the effect depends on an intact adrenal gland (Etzel and Cousins, 1983). As early as 1918, it was noted that when animals such as cats and dogs dosed with Zn salts in the form of malate or acetate, significantly greater amounts of sugar was excreted in the urine (Salant and Wise 1918). This effect of Zn has not been reported since then, however.

The above review summarizes the available findings concerning the relationship between Zn and insulin, glucose metabolism and the possible links between Zn and diabetes mellitus. Although data are inconsistent, the influence of Zn on insulin and glucose metabolism can not be excluded. It is likely that normal Zn nutrition is an important factor to ensure normal metabolism of insulin and glucose, however, the mechanisms are still not clear.

2.2.3 Alterations of Zn Metabolism in Diabetes Mellitus

2.2.3.1 Experimental Studies

2.2.3.1.1 Increased Urinary Zn Excretion

Urinary Zn excretion increases dramatically as animals develop diabetes (Tarui, 1963). Both pancreatomized dogs and alloxan-treated rabbits exhibited a 5 to 8 fold increase in urinary Zn after a latent period of 2-3 days. A transient increase in urinary Zn excretion occurred within 24 hours after treating rabbits with alloxan and a permanent hyperzincuria began 2 to 3 days later (Tarui, 1963). Ikeda and Kotake (1986) observed that Zn was substantially excreted as its complex with xanthurenic acid (XA). Insulin added to the vascular perfusate directly inhibits excretion of Zn by the in vitro perfused dog kidney (Vander et al. 1983). Acute infusion of glucagon increases Zn excretion in the same manner (Victery et al. 1982), suggesting that the hormonal imbalance present in diabetes mellitus may be responsible for hyperzincuria. Similarly, Lau and Failla (1984) found that diabetic rats excreted 3.4-. 5.0- and 4.9-times more Zn, Cu and Fe respectively than the control rats. Insulin treatment of diabetic rats significantly reduced the quantity of the micronutrients in their urine. The study also showed that the enhanced urinary output was not associated with reduction in plasma, liver and kidney concentrations of these metals. Decreased plasma insulin levels appear to be a major determinant factor for hyperzincuria (Vander et al. 1983). The same authors (Vander et al. 1983) observed that infusion of insulin alone caused decreased urinary Zn excretion and a physiological level of insulin exerts an inhibitory effect on Zn excretion. Insulin has also been shown to prevent the increase in Zn excretion caused by somatostatin (Vander et al. 1983). The above studies indicate that hyperzincuria in diabetic subjects is the natural result of insulin deficiency.

Based upon a recent understanding of diabetic nephropathy, diabetic hyperzincuria may be related to alteration of kidney structure and function. The diabetic kidney could be leaky to Zn and other metals due to hypertrophy and hyperfiltration. Zn may also be excreted as conjugated with albumin or alpha-globulin, since these components are also present early in the diabetic urine (Marshall 1991).

2.2.3.1.2 Uncertainty in Plasma Zn Level

The fact that hyperzincuria occurs in diabetes raised concern for a possible Zn deficiency in diabetics and studies have been undertaken to assess Zn status in diabetes. The commonly used approach is to assess Zn levels in plasma or serum. Plasma or serum Zn levels were elevated in streptozotocin-induced diabetic rats (Burke and Fenton 1989; Failla and Kiser 1981; Failla and Gardell 1985). Serum Zn was decreased in the genetically diabetic mice and ob/ob mice (Levine et al. 1983; Begin-Heick et al. 1982), but not in streptozotocin-induced diabetic mice (Levine et al. 1983). The two types of diabetes should differ in their etiology and plasma insulin level. These differences in the response of either plasma or serum Zn levels may indicate the two types of diabetes influence Zn metabolism by different mechanisms.

2.2.3.1.3 Alterations of Zn Concentrations in Tissue

While tissue Zn concentrations of diabetic animals are altered, but they may not necessarily be deficient. Measurement of tissue Zn levels of spontaneously diabetic BB Wistar rats with hyperphagia, hyperglycemia, glucosuria, polyuria, polydipsia and impaired weight gain (but without ketosis) showed that, besides of elevated urinary Zn, these animals had significantly increased Zn levels in plasma, liver, and kidney but no changes were found in duodenum, muscle, and spleen (Failla and Kiser 1981; Failla and Gardell 1985). By pair-feeding Zn to diabetic and control rats, it has been shown that hyperphagia of diabetic rats was not a factor for the accumulation of these metals. Failla and Kiser (1981) suggested that the hormonal imbalance influenced trace metal metabolism in the diabetic rats.

The differences between the spontaneous and chemically induced diabetes noted previously are also apparent in terms of the concentrations of Zn found in tissues. While Zn concentrations in femurs and serum were decreased in the genetically diabetic mice, no difference in serum and tissue Zn concentrations was noted in the streptozotocin-induced diabetic mice (Levine et al. 1983). However, Southon et al. (1988) reported that the genetically diabetic mice that consumed a marginal Zn depleted diet for 28 days from 4-5

weeks of age exhibited only a minimal effect on the Zn status as indicated by growth rate, food intake, and femur and pancreatic Zn concentrations. Donaldson et al. (1988) found that decreased femur Zn content in the genetically diabetic mice disappeared when the data were expressed on a dry ash basis, suggesting that low femur Zn concentration may reflect a generalized depletion rather than a specific depletion of Zn in the genetically diabetic mice.

Diet composition was observed to be associated with tissue Zn levels in STZ-diabetic rats (Johnson and Evans 1984). They found that duodenal Zn concentration was higher in diabetic rats when they were fed "high" mineral diets compared with rats fed "low" mineral diet. Femur Zn was also higher in diabetic rats fed a "high" protein rather than a "low" protein diet. However, hepatic and kidney Zn levels seemed to be not influenced by these factors.

Increased Zn levels in liver and kidney of diabetic animals were a characteristic finding in the diabetic rats (Failla and Kiser 1983). The Zn in the livers and kidneys of diabetic animals are mostly in the form of Zn-metallothionein in the soluble fraction of the cells. This event may represent a biological adaptation process by sequestrating Zn in response to stress. Hallmans and Lithner (1980) applied acute heat-trauma to diabetic rats and showed that alterations in Zn metabolism were greater in the diabetic rats when compared with controls. It was also suggested that there was a sequestration of serum Zn by the liver and an increased Zn absorption from gastrointestinal tract occurred in diabetes (Hallmans and Lithner 1980). Burke et al. (1988) reported that the membrane bound Zn did not seem to be affected although the lipid profile of the db/db mice was altered.

2.2.3.1.4 Altered Zn Absorption As One of the Compensation Mechanisms for Urinary Zn Losses in Diabetic Animals

Craft and Failla (1982) reported that absorption of Zn by diabetic animals were increased two fold, which may explain the increased urinary Zn losses. They (Craft and Failla 1983) subsequently observed that Zn absorption by diabetic animals was the same as that of normal controls per 20 cm duodenal loop by an in-situ, ligated-loop technique, but

Zn absorption per gram of dry mucosa was decreased by 45-53%. The overall absorption of Zn was increased due to a 50% increase in mucosal mass. In experimental diabetes, a number of intestinal brush border hydrolases and transport systems have been shown to be increased (Olson and Rogers 1971; Younoszai and Schedl 1972; Caspary et al. 1972). There was a considerable increase in the total membrane volume from the diabetic rats, although the ratio of lipids to protein was found to be similar (Gourley et al. 1983). These data suggest that one of the mechanisms of diabetic animals to maintain Zn balance is through hypertrophy of their mucosal tissue to augment the absorption of Zn.

Intestinal Zn transport measured by a single pass perfusion technique indicates that the relative lumen to mucosal flux of Zn was significantly decreased in all segments of gut from diabetic rats (Ghishan and Greene 1983), as was intestinal excretion of endogenous Zn (Johnson and Canfield 1985). Net absorption of Zn from jejunal segments was also decreased in streptozotocin-induced diabetic rats, and treatment with insulin had no effect on net Zn absorption (Song and Mooradian 1988). It is debatable whether Zn absorption of this segment represents total Zn absorption in rats, since studies have shown that the major site of Zn absorption may shift with Zn status (Schwarz and Kirchgessner 1975).

2.2.3.1.5 Increased Zn Turnover in Diabetic Animals

Whole body retention of ^{65}Zn in carcass and in carcass+liver was lower in alloxan-diabetic rats than in controls 50 hours after the injection. The total Zn concentrations of various tissues (liver, pancreas, spleen, kidney, heart and muscle) of the diabetic rats, however, were higher than in the control rats either expressed on fresh weight or dry weight basis (Hallmans et al. 1984). Since Zn levels in several tissues of these rats were not decreased, even in the presence of hyperzincuria, a faster turnover rate of Zn in diabetes may be implied.

2.2.3.1.6 Zn Deficiency in Diabetic Pregnancy

Fetuses from diabetic rats have lower liver Zn concentrations and a higher frequency of malformations than fetuses from control dams (Uriu-Hare et al. 1989;

Eriksson, 1984). The similarities between malformations seen in fetuses from diabetic rats and those from purely Zn deficient rats suggest that the teratogenic effect of diabetes may be partially due to a Zn deficiency (Uriu-Hare et al. 1989; Eriksson 1984). These studies also showed that diabetes during pregnancy can amplify the teratogenic effects of mild maternal Zn deficiency and the maternal diabetic condition also alters the transport of Zn and Cu across diabetic placentas and/or alters Zn and Cu retention of fetuses of the diabetic mothers (Uriu-Hare et al. 1989). Adequate Zn intake significantly improved fetal growth, high Zn-diets further improved fetal length and weight, but, supplementation of Zn did not decrease the frequencies of malformation (Uriu-Hare et al. 1989).

2.2.3.1.7 The Effect of Zn Supplementation in Diabetic Animals

Begin-Heick et al. (1982) found that levels of Zn in plasma and tissues were significantly lower in diabetic ob/ob mice when compared to lean controls. Consequently, Zn supplementation (1000 ppm) restored plasma Zn to normal and even elevated Zn in some tissues. Moreover, Zn supplementation was found to increase the insulin content and granulation of the pancreatic islets. Zn supplementation also decreased the abnormally high insulin secretory response of the ob/ob mouse islets to glucose. The existence of Zn deficiency in diabetes mellitus may be inferred from these data and the benefit of Zn supplementation may be seen. Levine et al. (1983) showed that Zn supplementation had a positive effect on femur Zn levels but had no significant effect on serum, liver and kidney Zn levels. Zn supplementation seems to have little influence on Zn status in the genetically diabetic mice. As regard to the femur Zn content, Donaldson et al. (1988) found that lower femur Zn may not be a specific depletion, but reflected a generalized decrease in bone mineral content.

Summary: Animal studies indicate hyperzincuria is a prominent feature of diabetes mellitus. However, even in the presence of greater loss of Zn from urine, there is generally no specific depletion of Zn in the diabetic animals. Zn absorption in diabetic animals may be increased because of the hypertrophy of the small intestine and the stimulation of a number of enzymes involved in absorption. Zn secretion back into the intestine may be

deceased in order to compensate for the increased loss of Zn from urine. Turnover of Zn in diabetic animals may be increased due to the hyperzincuria and increased absorption of the metal. Some mechanisms may exist in the diabetic animals to regulate their altered Zn metabolism. The above review also indicates a lack of data on the effect of Zn deficiency and insulin treatment on Zn status during diabetes mellitus. Zn supplementation may be beneficial as shown in some of the studies.

2.2.3.2 Human Studies

2.2.3.2.1 Evidence of Zn Deficiency in Diabetes Mellitus

Hyperzincuria is a persistent phenomena observed in diabetic subjects (Kiilerich et al. 1990; Raz and Havivi 1989; Fu 1991). Long term hyperzincuria may deplete their body Zn stores (Kiilerich et al. 1990). Diabetic patients suffer from persistent ulcers of the lower extremities and animal experiments suggest that diabetes decreases wound-healing and the histopathology is similar to Zn deficiency (Engel et al. 1981). This similarity is further reinforced when it is noted that Zn supplementation completely restores normal wound healing in diabetics . The decrease in the activity of Zn-dependent thymic hormone has been observed in diabetics and in vitro addition of Zn to diabetic plasma samples restore the hormone (Mocchegiani et al. 1989). More recently, Zn absorption by diabetics has been observed to be depressed, providing evidence of possible Zn deficiency exists in diabetic subjects (Kiilerich et al. 1990)

2.2.3.2.2 Hyperzincuria in Human Diabetes Mellitus

Daily urinary Zn excretion in both IDDM (Nakamura et al. 199; Fu 1991) and NIDDM (Meltzer et al. 1962; Kinlaw et al. 1983) subjects was 2 to 5 times higher than that of their non-diabetic controls . It appears that diabetics selectively excrete more Zn than other minerals, such as lead, cadmium, molybdenum, tin, nickel, bismuth, and cobalt (Meltzer et al. 1962). Moreover, the hyperzincuria seems to be a persistent phenomenon in the diabetic subjects (Tarui 1963). In some studies, females seemed to excrete more urinary Zn than males (Mateo et al. 1975).

The cause of excessive urinary Zn loss in diabetics, however, remains obscure. Studies have indicated the increased urine volume was not the cause of hyperzincuria (Brun et al. 1988; Heise et al. 1988; Tarui 1963; Fu 1991), so polyuria is not an cause for the observation. Urinary Zn excretion tended to decrease as control of diabetes improved (Tarui 1963), treatment of subjects with insulin even for a short duration has been observed to cause Zn excretion to subside (Viberti et al. 1979). Urinary Zn excretion was reported to be correlated to urinary protein loss (Heise et al. 1988; Aronoff et al. 1981). Further, urinary protein excretion correlated significantly with duration of diabetes and with diabetic complications (Aronoff et al. 1981). The cause of hyperzincuria due to damage of kidneys may be implied, however, no significant correlation has been found between hyperzincuria and microalbuminuria (Brun et al. 1988), suggesting that many other factors are involved.

Some studies found that urinary Zn excretion was related to glycemic controls (namely glycosylated hemoglobin, fasting plasma glucose and urinary glucose levels) of the diabetic subjects (Canfield et al. 1984, Fu 1991). Lower concentrations of urinary glucose were found to be significantly related to lower levels of urinary Zn excretion (Kiilerich 1985, Pidduck et al. 1970). These observations suggest that the cause of the hyperzincuria in diabetes mellitus is due to excretion of glucose. However, some other studies also reported that glycemic control was not correlated to urinary Zn excretion (Heise 1988), and an inverse correlation was also reported (Sjogren et al. 1986).

Glycosylation of blood proteins in diabetes was implicated as one of the causes for hyperzincuria in diabetes mellitus (Dolhofer and Wieland 1980). Glycosylation of serum proteins interfere with their Zn binding capacity, thus more circulating Zn may be chelated by small amino acids and peptides and secreted subsequently via urine (Canfield et al. 1984). Glycosylation of serum albumin was found to correlate strongly ($r=0.88$) with the percentage of HbA1 (Dolhofer and Wieland 1980). Furthermore, urinary excretion of glycosylated amino acids and glycosylated peptides in diabetes was reported to be much greater (Brownlee et al. 1975). Thus it is possible that Zn may be excreted as albumin-Zn

or alpha-globulin-Zn, as these proteins could be leaked through hyperfiltration by the hypertrophied kidney.

Diabetes mellitus results in a number of other humoral changes, which may be possible causes of hyperzincuria. Blood glucagon is increased in diabetes mellitus (Pidduck et al. 1970). Glucagon infusion significantly increased Zn excretion without increasing the ultrafiltration rate (Victery et al. 1981). Hyperzincuria in diabetics may be related to their reduced absorption of Zn in the renal tubules as has been reported (Krausova et al. 1990).

2.2.4 Zn Status of Human Diabetic Subjects

Despite the presence of hyperzincuria, Zn status, as assessed by plasma Zn, blood cell Zn and even muscle Zn in human diabetic subjects are inconsistent among studies. This may reflect the inaccuracy of the inadequacy of methodologies used to assess Zn status and cast doubt on the validity of the present approach to assess Zn status during diabetes mellitus (Raz and Havivi 1989; Abdulla 1983; Hambidge et al. 1988; Lifschitz and Henkin 1971). It also points out the need for more reliable approach to evaluate the Zn status of diabetic subjects.

2.2.4.1 Uncertainty of Plasma or Serum Zn Concentrations

Plasma Zn levels of diabetics were reported to be either higher (Mateo et al. 1975; 1978; Nobles et al. 1986) or lower (Car et al. 1991; Rosner and Gorfien 1968; Melinkeri et al. 1990), or similar to those of their study controls (Pidduck et al. 1970; McNair et al. 1981; Nakamura et al. 1991). Low serum Zn was also found in some patients with NIDDM (Niewoehner et al. 1986; Melinkeri et al. 1990). The differences in plasma or serum Zn levels in diabetic subjects among studies can not be easily explained.

Glycemic control seemed not to be correlated to plasma Zn levels in some of the studies (Canfield et al. 1984; Hayes et al. 1987; Mocchegiani et al. 1989; Niewoehner et al. 1986). However, duration of diabetes and diabetic complications seemed to associated

with lower plasma Zn (Nobels et al. 1986; Walter et al. 1991), suggesting that duration and severity of diabetes are possible risk factors in developing Zn deficiency.

Higher plasma Zn levels accompanying higher blood-glucose concentrations have been reported (Canfield et al. 1984; Heise et al. 1988). Higher plasma-Zn levels which occurred in the diabetic subjects may reflect a catabolic state rather than a better Zn status (Fell et al. 1973; Heise et al. 1988). Canfield et al. (1982) reported that higher plasma Zn levels in diabetic children and young adults were inversely correlated with age and duration of diabetes. High plasma-Zn accompanied with high insulin level in the NIDDM patients has been reported to reflect either a deficiency of insulin or a chronic hypersecretion of insulin in diabetic patients (Mateo et al. 1978).

Results of plasma Zn levels in diabetic subjects varied widely from different studies (Chooi et al. 1976; Chooi et al. 1976; Kumar et al. 1974). Methodology employed among studies may be one source of the confusion. Different determination methods could account for 25% of the differences in plasma Zn levels (Wilkins et al. 1972). It is known that serum Zn undergoes a circadian variation (Lifschitz and Henkin, 1971; Hetland and Brubakk, 1973), which may also contribute to the above differences. These may make the results among different studies incomparable. However, within the same studies the same method was used to determine the Zn level in plasma or serum, thus it is also unlikely methodology could play any significant role within each individual study.

Age and sex may be other factors contributing to differences reported in concentrations of plasma Zn in diabetics. There has been reported that age was significantly and negatively correlated with plasma Zn level (Lindeman et al. 1971). Females may have a lower plasma Zn level than males (Melchior et al. 1989; Sjogren et al. 1986; Kiilerich 1985; Nobles et al. 1986). The subjects recruited in each study may contribute to the difference on the reported values due to age and sex differences, however, it can not be confirmed.

2.2.4.2 No Evidence of Lower Erythrocyte Zn Concentration in Human Diabetics

A recent study has observed that erythrocyte Zn level of IDDM was shown to be somewhat lower, but not significantly due to the small sample size (Fu 1991). However, other studies did not find any difference between IDDM and their controls (Sjogren et al. 1985; Raz and Havivi 1989; Rosner and Gorfien 1968). The significance of using erythrocyte Zn as an indicator of Zn status in diabetes may deserve further study.

2.2.4.3 Uncertainty of Leukocyte Zn Concentration in Human Diabetics

Leukocyte Zn has been shown to be sensitive to changes in Zn status (Prasad 1991; Prasad 1988; Rabbani et al. 1987), and provided a basis for using leukocyte Zn level as an indicator of Zn status. A significantly decreased leukocyte Zn level was observed in some studies (Kumar and Jaya Rao, 1974; Pidduck et al. 1971; Raz and Havivi 1989; Pai et al. 1988), and Pai et al. (1988) found that diabetic subjects had significantly lower Zn levels in plasma, lymphocytes, granulocytes and platelets. However, a recent study shows that no significant difference in leukocyte Zn levels either at baseline or after a month of Zn supplementation between diabetics and the controls (Fu 1991). Raz and Havivi (1989) also observed that NIDDM did not have decreased leukocyte Zn level when compared with their non-diabetic control subjects.

2.2.4.4 No Change in Muscle Zn Concentration in Human Diabetics

A single determination of muscle Zn level showed no significant difference between diabetics and their non-diabetic controls (Sjogren et al. 1985). These values were 243 ng/mg fat free dry substance (FFDS) in the IDDMs and 258 ng in their controls ($P > 0.05$). It has been reported that depletion of up to 30% of body Zn content in rats did not alter the muscle Zn content, even if Zn levels in other tissues such as liver, bone and testes were significantly decreased (Jackson et al. 1982). These studies may suggest muscle Zn is not sensitive to Zn depletion.

2.2.4.5 The Effect of Zn Supplementation in Human Diabetics

Normal subjects with Zn deficiency respond positively to Zn supplementation (Wilkins and Dreosti 1972; Weismann and Hayer 1985). In a recent study 50 mg Zn daily in the form of Zn gluconate was given as a supplement to IDDM and no significant different responses were found between diabetics and their non-diabetic controls (Fu 1991). Niewoehner et al. (1986) supplemented 220 mg Zn sulfate to 9 NIDDMs with low serum Zn levels and significantly increased their serum Zn from 63 ug/100 ml to 110 ug/100 ml, suggesting that extremely large doses of Zn are effective in increasing serum Zn in Zn deficient diabetic subjects. Recent studies indicate a beneficial effect of Zn supplementation in diabetics with thyroid failure (Arreola et al. 1990) and gonadal disfunction (Arreola et al. 1991). These studies may suggest Zn deficiency may be present in a subset of diabetic subjects.

2.2.4.6 Uncertainty in Zn Absorption in Human Diabetics

Individuals with diabetes were thought to have an increased rate of Zn absorption rates to compensate for Zn loss since no obvious signs of Zn deficiency were observed (Heise et al. 1988). However, data from Zn absorption in NIDDM patients revealed a decreased response to an oral Zn tolerance test (Kinlaw et al. 1983), as indicated by a delayed increase in plasma Zn levels within 4 hours of the test. By using the whole-body-count radioisotope Zn technique, Kiilerich et al. (1990) revealed absorption of ^{65}Zn tended to be lower in IDDM, but not significantly so. They hypothesized that intracellular Zn depletion with time in insulin-dependent diabetics may occur. However, the decreased retention of ^{65}Zn may be a result of higher urinary Zn output and a faster turnover of body Zn.

2.2.4.7 Higher Zn Intakes by Human Diabetics

Zn intake by individuals with diabetes did not differ from their controls. (Hayes and Kohrs 1987; and Heise et al. 1988; Fu, 1991). The reported values (mg/d) were 12.2 versus 11.2; 8.5 versus 8.5 and 9.66 vs 6.69 mg/day in the diabetic subjects and in non-

diabetic controls respectively in the above three studies. Zn intake was significantly higher for IDDMs in one of the studies (Fu 1991). The recommended dietary Zn intake is 15 mg/day for males and 12 mg/day for females (National Research Council 1989). The daily Zn intakes of adults in the United States, as estimated from duplicated food composites of self-selected diets, were in the range of 6-12 mg/day (Holden et al. 1979; Kinard et al. 1989; Kant et al. 1989). It seems that the Zn intakes of diabetic subjects are adequate when compared with these figures.

Summary: Urinary Zn increased one to three fold in diabetic subjects as compared with their non-diabetic controls. A single determination of plasma or serum Zn, even cell Zn and muscle Zn level has not been able to show the existence of Zn deficiency in diabetic subjects. Other parameters such as response to Zn supplementation, plasma protein Zn saturation, distribution of Zn in plasma proteins reveals existence of a possible Zn deficiency. Dietary intake of Zn between diabetic subjects and their controls is not different. However, ^{65}Zn retention and Zn absorption may be impaired in diabetic subjects. Whether or not there is a depletion of Zn store in the diabetics could not be confirmed. It is apparent that a kinetic approach may be needed to determination the alteration of Zn metabolism in diabetes mellitus.

2.2.5 The Value of A Simple Determination of Zn Levels in Plasma and Blood Components in Assessing Zn Status in Diabetes Mellitus

The fact that Zn balance can be maintained by minimal intake of 1.7 mg/day (King 1990) suggests that the homeostasis of Zn in the human body is strictly controlled, presumably by absorption and excretion. Unlike other nutrients, when the intake is insufficient, stores or functional reserves are mobilized. A functional store or reserves for Zn has been unidentified (Golden 1989). The response of the human body to Zn deficiency is to adjust the growth rate and tissue catabolism (King 1990). Thus, Zn levels in plasma and other functional parameters are rarely affected. There is no single parameter believed

to reflect Zn status, despite attempts that have been made (Thompson 1991; Aggett 1991).

2.2.5.1 Plasma Zn

Plasma Zn is the most frequent used parameter in assessing Zn status. However, caution should be taken when one explains the changes in plasma Zn levels. Plasma Zn is easily influenced by dietary, physiological, and pharmacological factors (Abdulla 1983; Fu 1991), age and sex (Lindeman et al. 1971), diurnal variation (Hetland and Brubakk 1973; Henkin and Lifschitz 1971) and length of time between blood drawn and separation (English and Hambidge 1988). Plasma Zn concentrations may fall when plasma volume expands inappropriately (Tuttle et al. 1983) and stress (Disilvestro and Cousins 1984). However, it is believed in both simple human and animal experimental Zn deficiency, plasma Zn concentrations did fall (Thompson 1991), suggesting plasma Zn level may be reflective in Zn deficiency.

2.2.5.2 Plasma Total, Albumin- and Macroglobulin-Bound Zn

Attempts to improve the accuracy of plasma Zn assays leads to determination of Zn distribution between plasma albumin-bound Zn and macroglobulin-bound Zn with the mathematical addition of the two equal to total Zn (Giroux 1975). Elevated plasma total Zn does not seem to produce a concurrent increase in plasma albumin-bound Zn, and the distribution of albumin-bound Zn and macroglobulin-bound Zn within plasma remained similar before and after Zn supplementation (Fu 1991). Similar results was reported by Heise et al. (1988), in which, both albumin-bound Zn and macroglobulin-bound Zn were higher in the IDDMs. There is a big range of normal macroglobulin-bound Zn level, from 2% to 40% (Parisi and Vallee 1970; Kiilerich and Christiansen 1984; Giroux 1975; Giroux et al. 1976). A report that decreased plasma macroglobulin Zn occurred in a group of patients with chronic renal failure, thus an increased plasma albumin-bound Zn may be implied (Kiilerich and Christiansen 1986). It was reported that albumin Zn was significantly correlated with total plasma or serum Zn (Kiilerich and Christiansen 1984),

however, alpha-macroglobulin Zn did not correlate with alpha-macroglobulin concentrations, suggesting that not all alpha-macroglobulin in human serum are Zn metalloproteins (Kiilerich and Christiansen 1984). It has also been reported that serum albumin could have more than 16 available binding sites, with only a limited number of sites occupied by Zn in most of the situations (Hambidge 1988). Therefore, decreases or increases in this fraction may not be necessarily related to Zn status. It, therefore, does not seem to be a better indicator of Zn status over simple plasma Zn.

2.2.5.3 Plasma Protein Zn Binding Capacity

It was demonstrated that plasma protein Zn binding capacity decreased with an increase in Zn intake and the pattern of change in plasma protein Zn binding capacity in IDDMs was different from that of the controls (Fu, 1991). This suggests that the parameter may have some value in assessing Zn status in the diabetes. Zn binding capacity of plasma has been shown to be sensitive in experiments with rats before and after injection of Zn (Roth and Kirchgessner 1980) and in pregnant women (Argemi et al. 1988). This parameter, thus, may need exploring.

2.2.5.4 Erythrocyte Zn level

Erythrocytes have a longer turnover time compared to serum proteins (About 30-40 days, Thompson 1991), so their Zn level does not appear to change. Erythrocyte Zn level is not influenced by age and sex factors (Lindeman et al. 1971), or current dietary Zn intake (Fu 1991). Erythrocyte Zn has been shown not to be related to any other Zn parameters (Fu 1991). A possible explanation for this may be that erythrocyte Zn is not related to the present Zn status, namely Zn intake, plasma Zn, or even leukocytes Zn level. It has been shown that depletion of Zn for up to 9 weeks did not change the value of erythrocyte Zn in the humans (Baer and King 1984). However, erythrocyte Zn is reduced in diabetes mellitus (Fu, 1991).

2.2.5.5 Leukocyte Zn level

Leukocyte Zn concentration has been suggested to be a sensitive measure in assessing Zn status (Prasad 1988; Jones et al. 1981). However, several sub-sets of white blood cells each have different half life and content of Zn (Aggett 1991). The result of mononuclear cell Zn determination is, therefore, dependent on the purity of the sample separated. This has been remained a problem. There have been reports that the Zn content of blood cellular components are not sensitive indicators of Zn deficiency in rats (Milne et al. 1985). In our data, leukocyte Zn has also been demonstrated to be increased rapidly after Zn supplementation, but no difference was detected between the diabetics and the control (Fu 1991).

CHAPTER 3

A SINGLE POINT FOUR-HOUR STUDY ON ^{65}ZN ABSORPTION AND RETENTION WITH TISSUE ZN AND COPPER ANALYSIS

3.1 Introduction

Hyperzincuria, an increased urinary Zn excretion in diabetes mellitus, could deplete body Zn stores in diabetic subjects (Kiilerich et. al., 1990). However, human subjects with diabetes mellitus did not benefit from Zn supplementation (Fu 1991). The altered Zn status of diabetic humans and animals has not been confirmed from the available studies (Engle et. al. 1981; Kumar and Jaya Rao 1974; Mocchegiani et al. 1989; Pidduck et al. 1971; Raz and Havivi 1989). Should diabetics maintain a normal Zn status despite their hyperzincuria, compensation mechanisms or alteration of metabolism must be present in diabetics.

A kinetic study has identified several sites of regulation of Zn metabolism: absorption, excretion, muscle turnover, bone storage, endogenous secretion, and erythrocyte exchange (Wastney et al. 1986). Increased Zn absorption in diabetes mellitus may be one of the compensation mechanisms. Craft and Failla (1983) reported that the Zn absorption of the diabetic animals had an overall increase in Zn absorption due to an increase in mucosal mass, which may compensate for the increased urinary Zn losses. Olson and Rogers (1971) reported that intestinal brush border hydrolases and transport systems was increased in experimental diabetic rats. There was also a considerable increase in the total membrane volume in the diabetic intestine to augment absorption of Zn (Caspary et al. 1972; Gourley et al. 1983; Younoszai and Schedl 1972). These data suggest that diabetic animals may maintain Zn balance through hypertrophy of their mucosal tissue to enhance their Zn absorption. Other possible compensation mechanisms for Zn loss via urine in diabetics are through decreasing mucosal to lumen flux of Zn (Ghishan and Greene 1983), and depressing intestinal excretion of endogenous Zn (Johnson and Canfield 1985).

However, the study on human diabetics indicated an decreased Zn absorption in diabetes (Kiilerich et. al. 1990). Song and Mooradian (1988) also reported that net Zn

absorption of the jejunal segments was decreased in rats with streptozotocin-induced diabetes. The difference among the above mentioned studies is most probably due to the study designs. It is reported that fasting may alter Zn absorption in diabetic animals (Quarterman and Morrison 1981). Sian et al. (1993) also indicated that absorption of Zn in normal human subjects was associated with whether or not a meal was administered at the time of absorption studied. It is therefore of interest to test whether fasting or feeding pattern in diabetic animals may alter Zn absorption.

The present study is thus designed to test 1). whether Zn absorption and retention are altered in diabetes; 2). whether altered Zn absorption and retention are related to dietary feeding patterns; 3). the associations between Zn absorption and retention with other easily measured variables, such as Zn concentrations in plasma, urine or tissues.

3.2 Materials and Methods

3.2.1 Study Animals and the Induction of Diabetes Mellitus

Male Wistar rats weighing 40-60 gm (Charles River Laboratories, Wilmington, MA) were housed individually in suspended stainless steel cages and maintained at a room temperature of 20-23° C. All rats were fed a semi-purified diet containing 30 mg Zn/kg diet (Table 3.1). They had free access to food and distilled water throughout the study. Food intake and body weight gains of animals were measured twice weekly . The experimental protocol was approved by the University of Massachusetts at Amherst Animal Use and Care Administrative Advisory Committee, and animals were cared for in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals. After two weeks of adaptation, animals were randomly assigned to the test and control groups. Diabetes in the test group was induced by a single injection of Streptozotocin in cold sterilized saline solution at a dose of 100 mg/kg body weight. Blood glucose levels were measured with a commercial monitoring device (One Touch, complete blood glucose monitoring kit, Lifescan Inc., Johnson and Johnson Company. Milpitas, CA 95035). The increases in blood glucose levels and appearance of polyuria were used as indicators of diabetes.

Table 3.1 Composition of Animal Diet

Ingredient	%
Casein- High Nitrogen (85% Protein)	20.00
DL-Methionine	0.30
Cornstarch	15.00
Sucrose	50.00
Celufil-Non-Nutritive Bulk	5.00
Corn Oil	5.00
AIN-76 Mineral Mixture	3.50
AIN- 76 A Vitamin Mixture	1.00
Choline Bitartrate	0.20
Zn	30.0 mg/kg
Cu	6.0 mg/kg

Detailed composition of vitamin and salt mixtures see: 10664 AIN mineral mixture 76- American Institute of Nutrition. American Institute of Nutrition, Ltr. Communication, March 15, 1977; J. Nutr. 1977; 107.

3.2.2 Single Point Four Hour Study on ^{65}Zn Absorption and Retention Rates

On the day of the absorption study, food was removed overnight (12 hours) for about 10 rats from both the STZ-diabetic and control groups, they were fed after ^{65}Zn loading. This group was designated as the "prior-fasting" treatment. For the "post-fasting" treatment, food was removed after administration of ^{65}Zn and such held until the rats were sacrificed four hours later. Rats in both diabetic and control groups were randomly chosen and given about 50 µl ^{65}Zn (containing 1 µCi radioactivity) by mouth using a 50 ul pipette. Another 50 ul water was given to clear the mouth and ensure all the radioisotope entered the stomach. Radioactivity was counted immediately after ^{65}Zn dosing using a small plastic box (4" x 2" x 2") directly under the detector, which kept the animal under counting in a relative geometric shape. Each animal was counted for 5 minutes and the counts were recorded for total area under the peak. Rats were then put back into the metabolic cage for 4 hours, allowed free to access drinking water with and without food depending on the group they were in. Urine was collected for four hours for later measurement

radioactivity. No attempt was made to collect feces during this study because of the irregular nature of rodent defecation pattern within a short period of time.

Four hours later after the ingestion of radioisotope ^{65}Zn, rats were killed under anesthesia (by injection of 0.5 ml 0.3% Sodium pentobarbital). The peritoneal cavity was opened, blood was drawn by cardiac puncture into 7-ml heparinized vacuum tubes. The entire gastrointestinal tract (GI) was taken out and cleared off all contents. The cleared GI tract and blood in the vaccutainers were then put back into the peritoneal cavity, the whole animal was then counted twice, once without the GI tract (absorption) and once with (retention). Urine samples were also counted by the same technique as whole body counting, the results were used for calculation of ^{65}Zn absorption.

Whole body radioactivity was counted by a Na-I crystal 2 π detector equipped with a Cabarran multiple channel analyzer (Series 35). During whole body counting, the following parameters were used: Output voltage 850 V, Vertical range 1048 K, memory 1/4, ADC gain 2048, Scan 31-47 λ, count time 300 second.

The following formulas were used to calculate ^{65}Zn absorption and ^{65}Zn retention:

^{65}Zn absorption (%) = 100 X (Carcass Count + urine count)/Whole Body count.

^{65}Zn retention (%) = 100 X (Carcass Count + GI Tract Count)/Whole Body Count

Whole Body Count = Count at 0 time after ^{65}Zn loading

3.2.3 Tissue and Organ ^{65}Zn Radioactivity Counting

Liver, kidney, testis, spleen, heart, femur, muscle (those muscles around the femurs) were taken after whole body radioisotope counting. Blood samples were separated by centrifugation at 1380 g for 15 minutes into red blood cell clot and plasma. Radioactivity of heart, kidney, blood, spleen, liver, and testes was counted by a scintillation counter (Gamma 4000 Counting System, Gamma Counting Spectrometer, Beckman Instrument Inc.) for 10 minutes. The same amount (50 μl) of ^{65}Zn solution was used as standard. All tissue samples were then kept frozen after ^{65}Zn counting for Zn, Cu and Fe analysis. Decay of Radioactivity of all samples was adjusted by the counts of their standards.

3.2.4 Organ and Tissue Zn Determination

Femurs was cleared by removing all visible tissues and ligaments; kidney, heart, testis, and spleen were collected after washing in saline and blotting with Kimwipe paper. All organs and tissues underwent wet-digestion in whole except liver, a portion of which was used for digestion. Samples were put into an 150 ml beaker, and to each was added 10 ml concentrated nitric acid followed by heating on a hot plate at 350° C. After the first phase of digestion, about 2 ml 70% of perchloric acid was added and heating continued. A clear layer of residue remained at the bottom of the beaker after acid was totally evaporated. Ten milliliter 5% diluted nitric acid was used to dissolve the contents which were then transferred into a 15 ml conic plastic tube (Sigma, Co.). for Zn and Cu analysis by Atomic Absorption Spectrophotometer (AAS, Model 2380, Perkin-Elmer) methods. Plasma Zn and Cu concentrations were analyzed after direct dilution with double distilled water and analyzed by AAS method under same conditions.

3.2.5 Statistical Analysis of Data

Statistical analysis of data was done with a computer software SAS (SAS Institute Inc. Cary, NC). A student T-Test was used to test for difference for between groups. A correlation procedure was used to analyze the relationship of variables.

3.3 Results

3.3.1 Body Weight and Food Intake

The initial body weight and food intake were similar for the 14 diabetic and 12 control rats before STZ-diabetes was induced (Figure 3.1). The body weight growth of the STZ-diabetic rats (n=14) decreased after injection of STZ, while their food intake increased significantly (Figure 3.2). A 28% weight loss was noted when compared with the control rats (12) on day 30. STZ-diabetic rats had a 16% increased in food intake on day 31, which was significantly higher than that of their controls ($P<0.0001$).

3.3.2 Blood Glucose

About half of rats died within the first 5 days of injection of STZ. Diabetic rats who were alive had some signs of inactivity and sleepiness, otherwise remained relatively normal. STZ-diabetic rats manifested polyuria, polydipsia, and polyphagia. Blood glucose levels were determined on day 2 and 6 after injection of STZ. Blood glucose levels of diabetic rats were significantly higher (300-310 mg/100 ml) when compared to the controls (71-76 mg/100 ml) (Figure 3.3).

3.3.3 Single Point 4-Hours ^{65}Zn Absorption and Retention Rates

Absorption and retention of ^{65}Zn were measured 4 hours after the injection of ^{65}Zn. The results are presented in Figure 3.4 and 3.5. There was no significant difference between STZ-diabetic rats and their controls in either ^{65}Zn absorption or ^{65}Zn retention (figure 3.4). The two different dietary treatments, namely prior fasting and post fasting, produced different effects on ^{65}Zn absorption and retention in STZ-diabetic rats as well as in the control rats as seen Figure 3.5. STZ-diabetic animals that were on post-fasting showed a significantly higher absorption and retention than those who were on prior fasting ($P<0.05$). Control animals showed a significant difference in ^{65}Zn retention, but not ^{65}Zn absorption. This indicates that the dietary pattern at the time of the study significantly influenced the ^{65}Zn absorption and retention, and diabetic animals responded differently from their control counterparts to food availability. The fact that Zn absorption was unaffected in the control group suggests that the absorption may indeed be the site where diabetes exerts its influence on the time of feeding phenomena.

The overall values of ^{65}Zn absorption and ^{65}Zn retention were not different significantly between STZ-diabetic animals and control animals ($P=0.50$). However, If divided according to their feeding patterns, ^{65}Zn absorption and retention were lower in STZ-diabetic animals than their controls when they were "prior-fasted". Again the differences were not significant. If they were "post-fasted", the STZ-diabetic animals had a higher ^{65}Zn absorption and retention rate compared with their controls, but the difference was not significant.

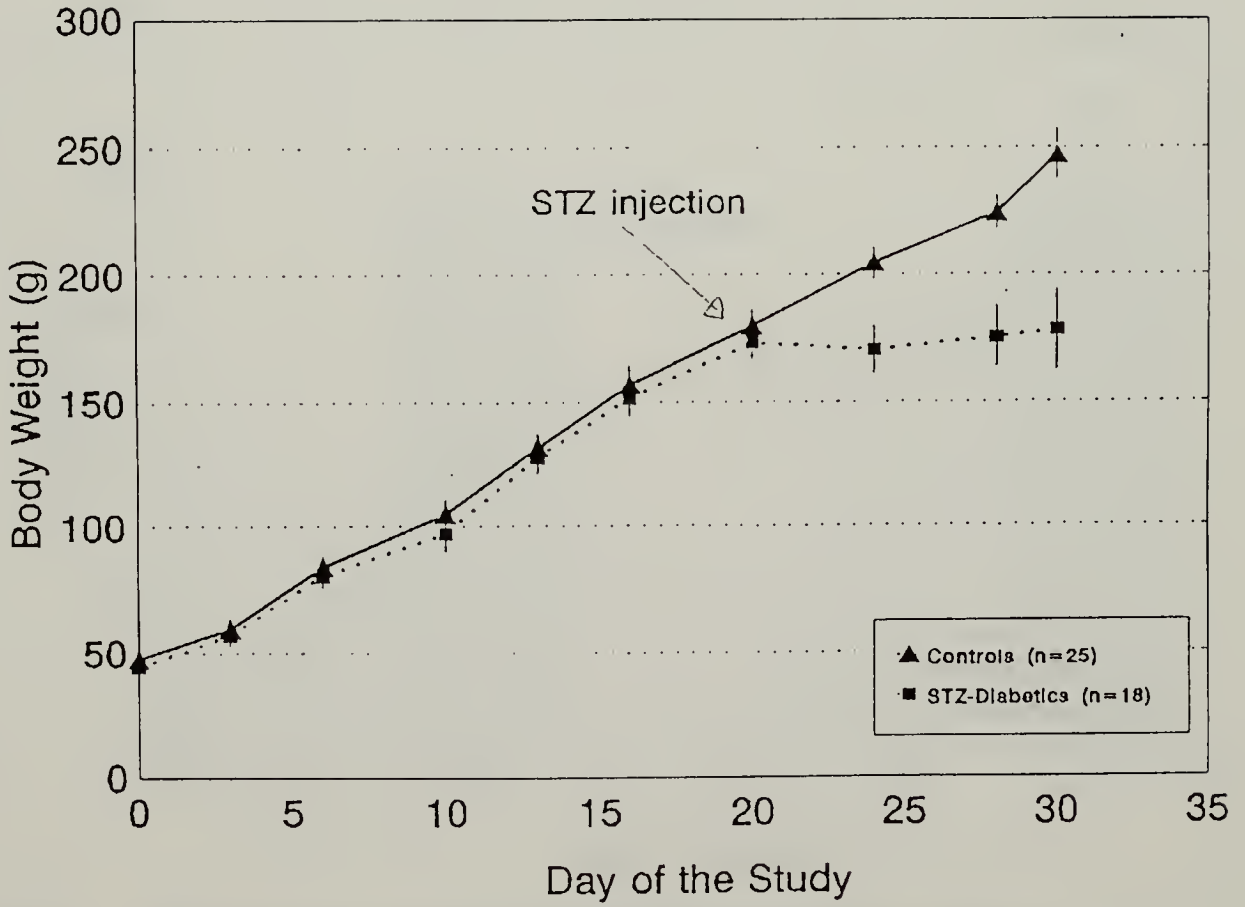

Figure 3.1 Body Weight of STZ-Diabetic Rats and Control Rats

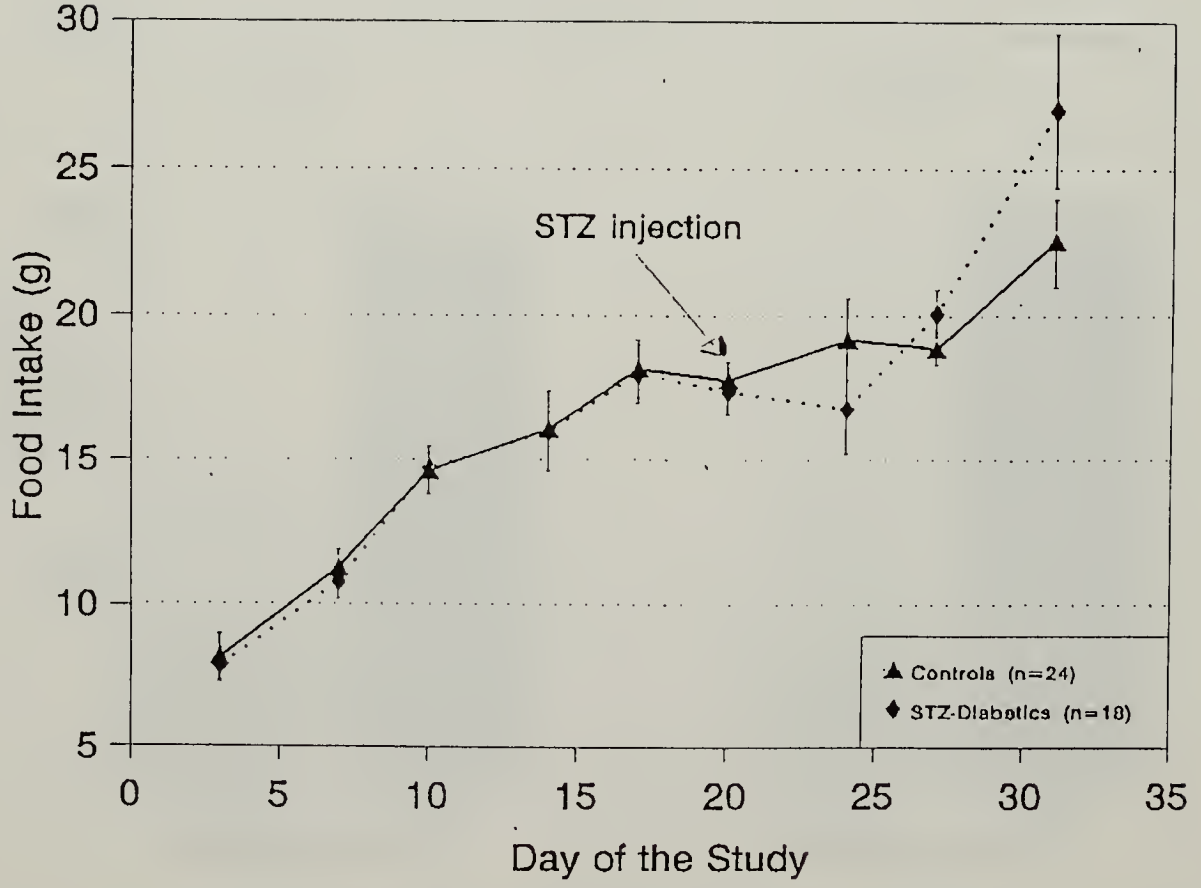

Figure 3.2 Food Intake of STZ-Diabetic and Control Rats

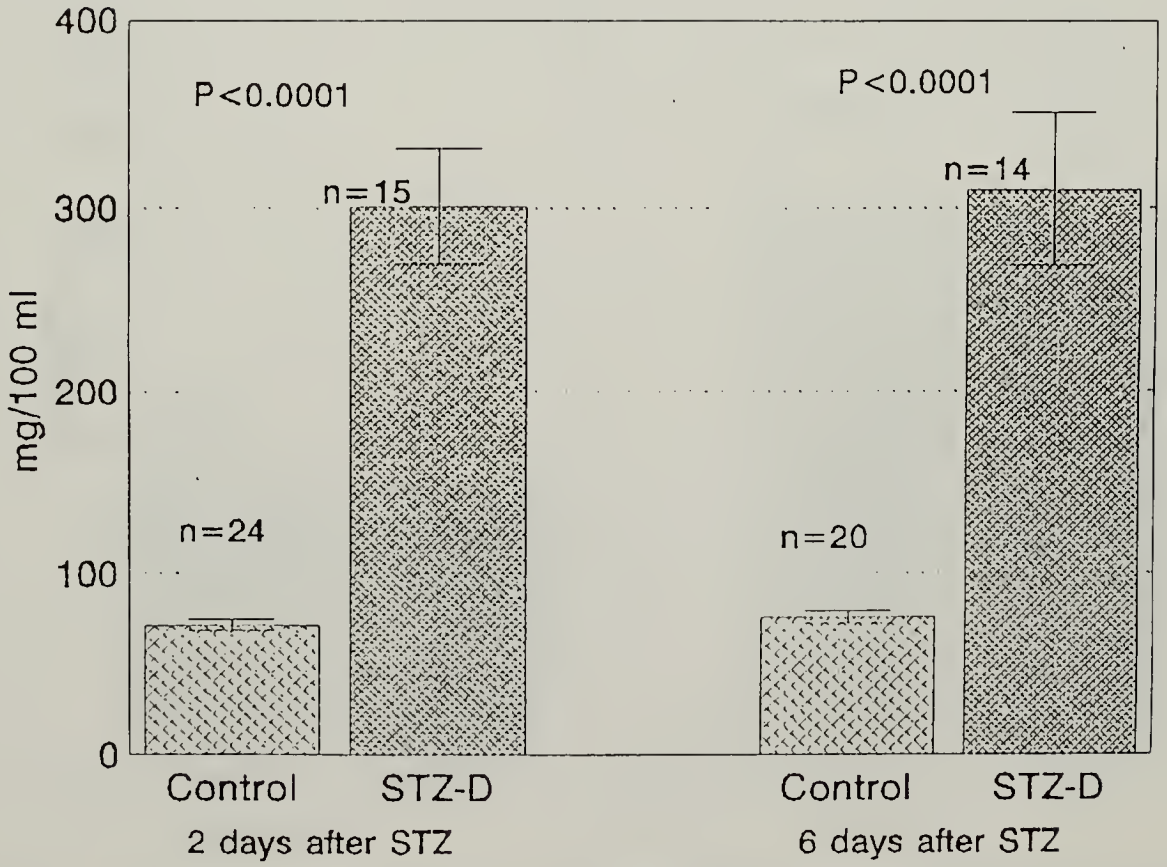

Figure 3.3 Blood Glucose Levels of STZ-Diabetic and Control Rats 2 and 6 days after STZ Injection

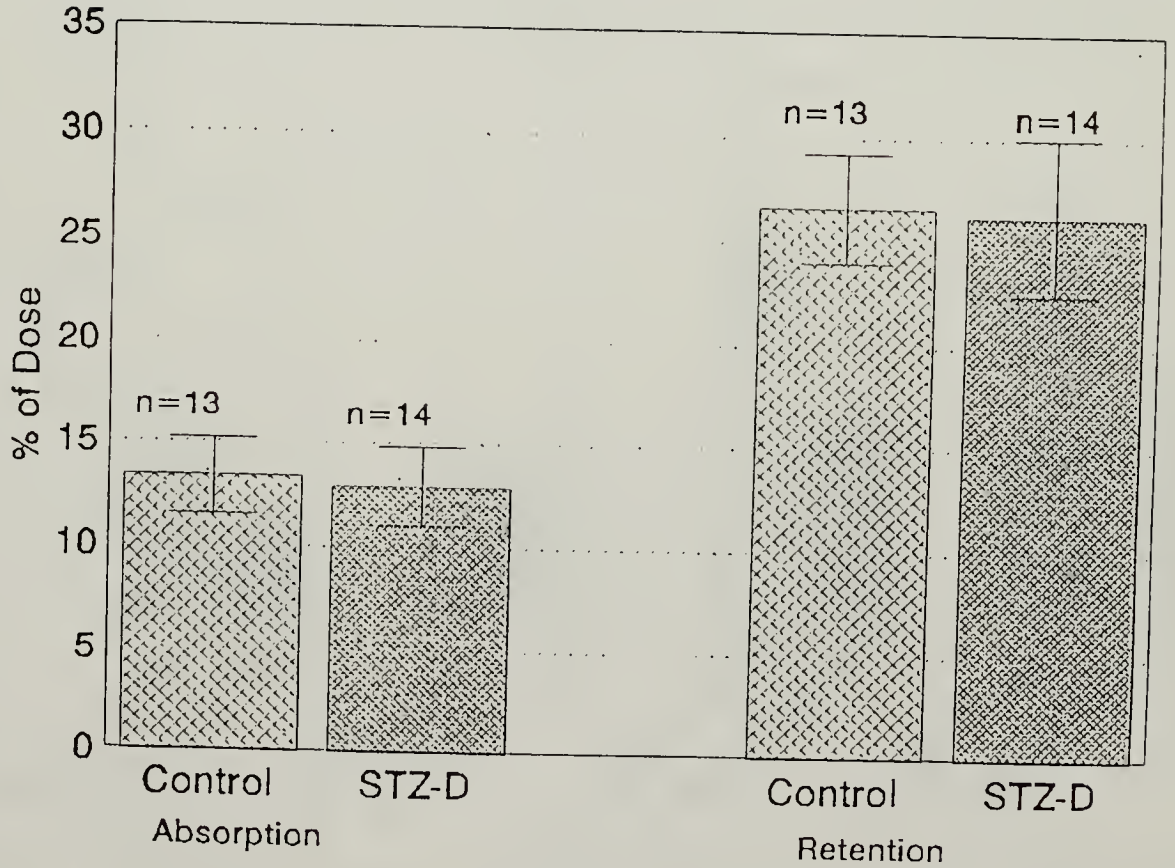

Figure 3.4 65-Zn Absorption and Retention in STZ-Diabetic and Control Rats 4 Hours after STZ Injection

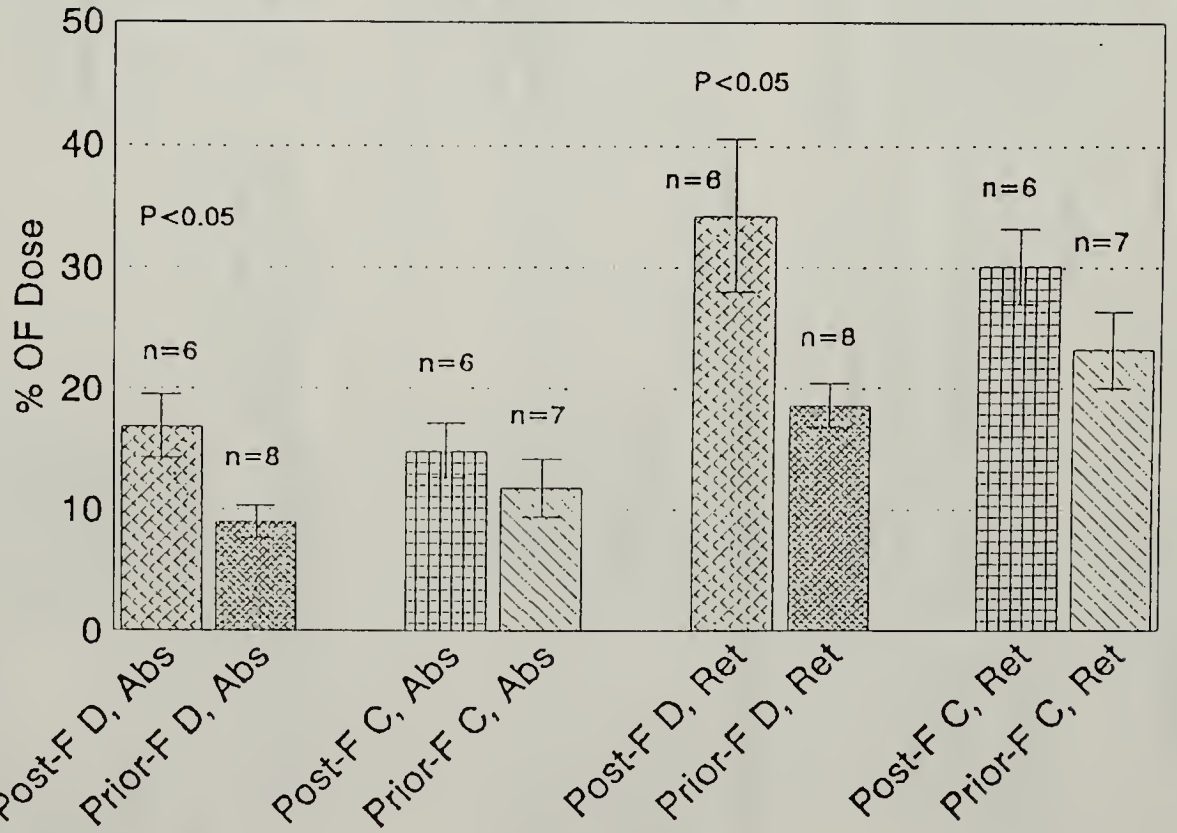

Figure 3.5 65-Zn Absorption (Abs) and Retention (Ret) of STZ-Diabetic Rats (D) and Control Rats (C) According to Feeding Patterns

3.3.4 Plasma ^{65}Zn Activity

Table 3.2 presents data of plasma ^{65}Zn (Counts/10 minutes/ml). STZ-diabetic rats had a higher plasma ^{65}Zn count either with or without an overnight fasting treatment than their controls. However, the difference was not significant ($P>0.05$).

3.3.5 Urine ^{65}Zn Activity

Urine ^{65}Zn counting results are present in Table 3.3. STZ-diabetic rats had significantly ($P<0.01$) increased their urine ^{65}Zn excretion when animals were "prior-fasted". Notice also the substantially increased urine ^{65}Zn counts when STZ-diabetic rats were "prior-fasted" when compared with "post-fasted".

Table 3.2 **Plasma ^{65}Zn Counts of STZ-Diabetic and Control Rats**
(Count/ml/minutes)

	STZ-Diabetics	Control
Post Fasting	11.2±3.7 (6)	9.6±2.5 (6)
Prior Fasting	4.3±1.4 (5)	2.8±2.1 (5)
Average	8.1+2.3 (11)	6.2+1.9 (11)

Table 3.3 **Urine ^{65}Zn Counts of STZ-Diabetic and Control Rats**
(Total Count/minutes)

	STZ-Diabetics	Control
Post Fasting	390±86 (6)	277±19 (6)
Prior Fasting	3176±397 (6)**	208±23 (6)**
Average	1982±443 (12)*	240±17 (12)*

* Significance at $P<0.01$ level between STZ-diabetic rats and their controls
** Significance at $P<0.0001$ level between STZ-diabetic rats and their controls.

3.3.6 Plasma Zn Levels of STZ-Diabetic Rats and Their Controls

Data for Zn in plasma are presented in Table 3.4. It is of interest to note that the overall values for plasma Zn concentration is not different significantly. However, if the animals were divided according to prior fasting and post fasting treatment, significant differences were noted in these two groups of animals. Both STZ-diabetic and control rats had significantly lower plasma Zn concentrations if they were on a prior fasting ($P<0.01$). On post fast, STZ-diabetic rats had a significantly lower plasma Zn concentration compared with their control rats ($P<0.05$).

Table 3.4 Plasma Zn (mg/L) of STZ-Diabetic and Control Rats

	STZ-Diabetics	Control
Post-Fasting	1.05±0.06 (6)##*	1.38±0.05 (6)##*
Prior-Fasting	0.77±0.05(5)##	0.68±0.03 (7)##
Overall Means	0.92±0.06(11)	1.00±0.10 (13)

* Statistical significance at $P<0.05$ level between STZ-diabetic and controls.
Statistical Significance at $P<0.01$ between two modes of dietary pattern.

3.3.7 Four-Hour Urine Zn Excretion

STZ-diabetic animals had significantly higher urinary Zn excretion which is the same as noted for human diabetes and consistent with all previous studies. However, in this study, urine Zn concentrations of STZ-diabetic rats was lower than their controls. The increased urinary Zn excretion in STZ-diabetic animals in this study was obviously due to their higher urinary volume (Table 3.5). This suggests that Zn loss may be due to diuresis in diabetes.

Table 3.5 Four Hour Urine Zn Excretion of STZ-Diabetic Rats in Comparison with Their Controls

	STZ diabetes (n=14)	Controls (n=14)
Urine Zn Concentration (mg/L)	0.78±0.15**	1.92±0.16**
Total Urinary Zn (mg/4 hours)	0.019±0.003**	0.009± 0.001**

** Significant level of $P<0.01$.

3.3.8 Organ and Tissue ^{65}Zn Counts

Radioactive ^{65}Zn counts of liver, heart, kidney, spleen and testes are presented in Table 3.6. Generally speaking, liver had the highest number of counts among these organs or tissues selected. Next greatest number of counts was found in the kidney. Testes had the lowest counting rate among these organs. There were no significant differences either between STZ-diabetic group and control group, or between the two modes of dietary pattern, possibly due to the short time span in this study.

Table 3.6 **Some Organ or Tissue ^{65}Zn Activity** (Count/Min/Gram)

	STZ-Diabetics	Control	STZ-D+Control
Post Fasting			
Liver	1587±263 (6)	1018±186 (5)	1329±182 (11)
Heart	24±11 (6)	26±5 (5)	25±6 (11)
Kidney	286±68 (6)	172±63 (5)	235±48 (11)
Spleen	83±18 (5)	66±18 (4)	75±13 (9)
Testes	23±6 (6)	11±5 (5)	17±4 (11)
Prior Fasting			
Liver	1151±187 (8)	905±208 (7)	1036±138 (15)
Heart	25±6 (8)	20±7 (7)	23±4 (15)
Kidney	246±43 (8)	195±41 (7)	222±30 (15)
Spleen	62±14 (8)	40±11 (7)	52±9 (15)
Testes	21±9 (8)	17±7 (7)	19±6 (15)
Average of STZ-Diabetic and Control rats			
Liver	1338±160 (14)	952±139 (12)	
Heart	25±5 (14)	22±4 (12)	
Kidney	263±30 (14)	185±48 (12)	
Spleen	70±11 (13)	50±10 (11)	
Testes	22±5 (14)	15±4 (12)	

3.3.9 Zn Concentrations in Selected Organs and Tissues

STZ-diabetic rats had significantly higher femur Zn concentrations than that found for their controls ($P<0.0001$). However, the total Zn content of femur was significantly lower in the STZ-Diabetic rats than in the control rats due to the decreased weight of the diabetic femurs. There was no significant difference in Zn concentrations in any other organ or tissue between the STZ-diabetic and control groups (Table 3.7).

Table 3.7 Zn Concentration of Selected Organs in STZ-Diabetic Rats and Their Controls (μg/g wet tissue, Mean+SEM)

Organs or Tissues	STZ Diabetes	Controls
Femur	62.220±1.748 (13)***	49.106±1.260 (14)***
Femur (total, ug)	24.408±0.363 (13)*	25.493±0.342 (14)*
Muscle	9.492±1.604 (12)	9.387±1.509 (12)
Heart	23.180±4.90 (14)	20.891±2.278 (13)
Kidney	14.764±1.395 (14)	15.661±1.576 (14)
Liver	17.017±1.468 (14)	17.976±1.542 (13)
Spleen	32.565±8.273 (12)	18.981±2.597 (12)
Testis	18.095±1.478 (14)	16.006±1.028 (14)

Numbers in () represent numbers of animal used.
*** Difference is significant at level of $P<0.0001$
* Difference is significant at level of $P<0.05$.

3.3.10 Organ and Tissue ^{65}Zn Specific Activity

When specific activities of ^{65}Zn in some tissue and organs were calculated (Table 3.8.). It was noted that STZ-diabetic rats had high specific activities of ^{65}Zn in all tissues examined. The difference between STZ-diabetic rats and their controls in kidney and urine ^{65}Zn specific activity were highly significant ($P<0.05$).

Table 3.8 Specific Activity of ^{65}Zn of Some Organs or Tissues (Counts/min/ug Zn)

Organs	STZ-Diabetics		Controls	
	No.	Mean±SEM	No.	Mean±SEM
Heart	14	1.4±0.4	12	0.99±0.26
Kidney	14	16.3±2.70*	13	9.4±1.7*
Liver	14	69.4±9.45	12	44.2±8.6
Spleen	12	3.6±1.3	11	2.7±0.6
Testis	14	1.2±0.3	13	0.9±0.3
Plasma	10	8.8±4.6	13	6.2±2.9
Urine	14	104.3±23.3***	13	26.7±1.7***

* Statistical significance at $P<0.05$ level between the two groups.
*** Statistical significance at $P<0.0001$ level between the two groups.

3.3.11 Correlations of ^{65}Zn Absorption and ^{65}Zn Retention with Various Variables

Correlations of ^{65}Zn absorption and ^{65}Zn retention with various variables were calculated in order to find out which variable or variables may be associated with changes in ^{65}Zn absorption and ^{65}Zn retention in STZ-diabetic and control rats. Such variables include plasma, urine and organ Zn concentration, ^{65}Zn count and ^{65}Zn specificity of various organs and tissues. These correlations are summarized in Table 3.9.

Body weight did not correlate with either ^{65}Zn absorption and ^{65}Zn retention. Food intake may influence both ^{65}Zn absorption and ^{65}Zn retention in STZ-diabetic rats, since their correlation coefficients were very significant. We have seen frequently that mode of feeding influences ^{65}Zn absorption and retention, but these correlations were not significant in control rats. Blood glucose was significantly higher in STZ-diabetic rats that in control rats as shown in Table 3.2 , however, both STZ-diabetic rats and controls rats did not show a significant correlation between their blood glucose levels and either with ^{65}Zn absorption, or ^{65}Zn retention.

Plasma ^{65}Zn was correlated significantly with ^{65}Zn absorption, but not ^{65}Zn retention in STZ-diabetic rats. While in control rats, plasma ^{65}Zn was significantly correlated with ^{65}Zn retention, but not with ^{65}Zn absorption. It seems plasma ^{65}Zn had a different relationship with ^{65}Zn absorption and ^{65}Zn retention. This relation was also different in STZ-diabetic rats than in control rats.

Plasma Zn level was significantly correlated with ^{65}Zn absorption and ^{65}Zn retention in control rats only. These relationships were absent in STZ-diabetic rats.

Both urine total ^{65}Zn count and urine ^{65}Zn count/3 ml urine were correlated significantly with both ^{65}Zn absorption and ^{65}Zn retention in STZ-diabetic rats. However, no such correlations existed in the control rats. Similarly, urine Zn excretion, either in terms of total excretion or concentration was also correlated with ^{65}Zn absorption and ^{65}Zn retention in only STZ-diabetic rats but not in control rats.

Table 3.9 Correlation of ^{65}Zn Absorption and ^{65}Zn Retention with Various Variables

Variables	^{65}Zn Absorption			^{65}Zn Retention		
	STZ-D	Control	Pooled	STZ-D	Controls	Pooled
Body Weight	--	--	--	--	--	--
Food Intake	++	--	--	++	--	--
Blood Glucose	--	--	--	--	--	--
Plasma ^{65}Zn	+	--	++	--	+	--
Plasma Zn	--	+	++	--	+++	++
Urine ^{65}Zn						
Total	++	--	++	++	--	++
Count/3 ml	++	--	++	++	--	++
Urine Zn						
Total	--	--	--	+	--	+
ug/ml	++	--	--	++	--	--
Liver ^{65}Zn	++	++	++	++	++	++
Kidney ^{65}Zn	+	+	++	--	--	--
Heart ^{65}Zn	--	--	+	--	--	--
Spleen ^{65}Zn	--	--	++	--	--	+
Testis ^{65}Zn	--	--	--	--	--	--
Liver Zn	--	--	+	--	--	+
Kidney Zn	++	--	++	++	--	++
Spleen Zn	++	--	+	++	--	+
Heart Zn	+	--	++	+	--	++
Muscle Zn	--	--	--	--	--	--
Femur Zn	--	+	--	--	--	--
Testis	--	--	--	--	--	--
Zn Specificity						
Liver	--	--	--	--	--	--
Kidney	--	--	--	--	--	--
Spleen	--	++	--	--	++	--
Heart	--	--	--	--	--	--
Testis	--	--	--	--	--	--
Plasma	--	--	--	--	--	+
Urine	++	--	++	+	--	++

-- No significant correlation

+, ++, +++ Significant Correlations at $P<0.05$, $P<0.01$ and $P<0.001$ level.

Among organ ^{65}Zn activities, liver was the only organ that its ^{65}Zn count was correlated significantly with both ^{65}Zn absorption and ^{65}Zn retention in both STZ-diabetic rats and control rats. Kidney ^{65}Zn count was only correlated with ^{65}Zn absorption, but not with ^{65}Zn retention in both STZ-diabetic rats and control rats. ^{65}Zn count of heart, spleen and testis in both STZ-diabetic and control rats were not correlated with either ^{65}Zn absorption and ^{65}Zn retention.

Zn concentrations of kidney, spleen, and heart correlated significantly with both ^{65}Zn absorption and ^{65}Zn retention in STZ-diabetic rats, but not in control rats.

Muscle and femur cold Zn concentration did not correlate with either ^{65}Zn absorption and ^{65}Zn retention in both STZ-diabetic and control rats.

Except for spleen, ^{65}Zn specificity of various organs, did not correlate with either ^{65}Zn absorption and ^{65}Zn retention in both STZ-diabetic and control rats. Spleen ^{65}Zn specificity was significantly correlated with both ^{65}Zn absorption and ^{65}Zn retention in control rats. These correlations were not present in STZ-diabetic rats. Urine ^{65}Zn specificity was correlated significantly with both ^{65}Zn absorption and ^{65}Zn retention in STZ-diabetic rats, but these relations were absent in control rats.

3.4 Discussion

3.4.1 The Establishment of the STZ-Diabetic Animal Models

The diabetic animal model was established by peritoneal injection of streptozotocin (STZ) at a single dose of 100 mg/kg. After introduction of STZ, these animals manifested polyphagia, polydipsia, and polyuria, all of which are typical of diabetes. Their urinary volume increased dramatically within 48 hours.

The STZ-diabetic animals had a significantly increased food intake and at the same time lost body weight (See Figure 3.1 and 3.2).

About one third of animals died after injection of STZ within five days, but the others diabetic rats alive remained in diabetic condition without insulin treatment. The STZ-diabetic animals had a significantly increased blood glucose level as assessed at 2 and

6 days after STZ introduction. It may be concluded that the STZ-diabetic model was well established.

3.4.2 No Significant Difference in the Rates of ^{65}Zn Absorption and Retention between STZ-Diabetic Rats and Controls

One of the objectives of this study was to find out whether STZ-diabetic rats had a different ^{65}Zn absorption and retention rate. The present study observed that the normal rats had an apparent ^{65}Zn absorption rate of 11-15% and ^{65}Zn retention rate of 23-31% of lavage dose within the four hours after ^{65}Zn dosing. These number are within the range of other studies. Cotzias et al. (1962) found that normal mouse on a normal Zn intake had a retention rate of 22% within 4.5 hours of ^{65}Zn loading. However, the Zn-loaded animals absorbed only 7% of the injected dose in that study. Coppen and Davies (1987) found that the true percentage absorption of ^{65}Zn was within the range of 68%-86%, which is substantially higher than the result of the present study.

Human subjects generally had an absorption rate of 30% (Cousins, 1985). Sandstrom (1989) reported that humans absorbed 20-25% of Zn from meals providing 3-5 mg of Zn and that the absorption rate increased up to 30-40% as the meal providing lesser amount of Zn. And even higher absorption rate (52%) and retention rate (49%) have also been reported (Payton et al. 1982). These results may reflect the differences in the study design and the instruments used, and the results may subsequently not be comparable directly from each other.

Craft and Failla (1983) observed an increased ^{65}Zn absorption in STZ-diabetic rats (34% vs 32% in controls, $P>0.05$), also they found that STZ-diabetic rats had a threefold apparent Zn absorption per 100 g body weight than their controls using dietary balance technique.

The results of Johnson and Canfield (1985) indicated that STZ-diabetic rats absorbed more Zn compared with that of control rats when consumed equivalent amount of Zn; they also found that STZ-diabetic rats excreted generally a lower percentage of

absorbed Zn via their feces. These results lead to the conclusion that the ability to absorb Zn to meet the body requirement is preserved in STZ-diabetic rats.

The present study observed that the percentages of ^{65}Zn absorption and retention were not significantly different between the STZ-diabetic rats and their controls. The results did not agree with the findings of lower ^{65}Zn absorption in human diabetics by Kiilerich et al. (1990). There is no ready explanation for this discrepancy between the results of the two studies, other than the different subjects used in the two studies. However, it may still be possible that the human diabetics could absorb more Zn if they increased their food intake.

The results of the present study agree with the results of Craft and Failla (1983). They determined Zn absorption of STZ-diabetic rats by feeding animals ^{65}Zn-labeled diet and reported a similar apparent ^{65}Zn absorption by STZ-diabetic rats. But the total absorbed ^{65}Zn per 100 g body weight was two times higher in STZ-diabetic rats than in control rats in their study. Due to the greater amount of food intakes of STZ-diabetic rats, the total amount of Zn absorbed and retained should be higher.

The increased Zn absorption in diabetes mellitus may also be possible from some other reasons: 1). Diabetic animals including humans have an enlarged mucosal mass (Craft and Failla, 1983; Gourley et al. 1983; Miller, et al. 1977); 2). Diabetic subjects including both animals and humans have a depressed intestinal excretion of endogenous Zn (Johnson and Canfield, 1985); 3). A number of intestinal brush border hydrolases and transport systems are stimulated in diabetic animals including humans (Olson and Rogers, 1971; Younoszai and Schedl 1972; Caspary et al. 1972).

Zn absorption is regulated tightly by both body requirement, dietary intake and by regulating excretion of Zn through both feces and urine (Vallee and Falchuk 1993; Giugliano and Millward 1984). These same mechanisms of Zn homeostasis was also operating in STZ-diabetics in the present study.

3.4.3 The Significance of the Finding that Feeding After ^{65}Zn Loading Affects ^{65}Zn Absorption and Retention

A significant finding of the present study is the finding that the feeding pattern had a significant influence on ^{65}Zn absorption and retention in the STZ-diabetic rats. STZ-diabetics increased significantly ^{65}Zn absorption and retention rates when they were "post-fasted" in comparison with those that were "prior-fasted" ($P<0.05$). But, this phenomenon was much less obvious in the control rats. These results suggest that some unidentified alteration in Zn metabolism existed in diabetes.

Sian et al. (1993) noted that the absorption of exogenous Zn competed with endogenous Zn that has been secreted into the lumen of the gastrointestinal tract in response to a meal. Sian et al. (1993) were working with non-diabetic animals and the observation made here with non-diabetics also shown a lesser but similar trend in ^{65}Zn retention, but not ^{65}Zn absorption. It seems that the same mechanism may be at work, and this mechanism may be exaggerated by diabetics.

Another possible explanation is that continued feeding may further exhaust insulin secretion in STZ-diabetic animals, thus, decreasing the efficiency of absorption of Zn. In contrast, a nil food intake may significantly spare insulin secretion, thus more metal was absorbed because it could gain entry to the mucosa.

The increased ^{65}Zn absorption in STZ-diabetic rats during "post-fasting" in the present study may indicate that the STZ-diabetic rats possess the ability to alter their Zn absorption rate to meet their body requirement for Zn. The significance of the results may be two aspects: one is for the future study design that feeding pattern can alter the results significantly; another is that the ability of Zn absorption in the diabetics can vary to meet the body requirement for Zn.

Along with altered ^{65}Zn absorption and retention, plasma Zn concentrations and urine ^{65}Zn excretion were also different when "prior-fasting" and "post-fasting" modes were considered. This suggests that the feeding condition of the animals may play an important role in regulating Zn uptake. These data may help explain some of the previous contradictory results for the effects of diabetes on Zn metabolism.

3.4.4 Factors Associated with Zn Absorption and Retention

The rates of ^{65}Zn absorption and retention were not correlated with body weight, suggesting body growth is not a factor in Zn absorption. Blood glucose was also not associated with rates of ^{65}Zn absorption and retention, indicating severity of hyperglycemia does not affect Zn absorption directly.

Food intake (of day 31, two days before the absorption study started) was correlated with both ^{65}Zn absorption and ^{65}Zn retention in STZ-diabetic rats, but this correlation was absent in control rats. It may suggest the polyphagia in STZ-diabetic rats played a role. It is generally agreed that the greater amount of Zn absorbed in diabetics is due to their greater amount of food intake, rather than their rates of Zn absorption (Craft and Failla 1984; Johnson and Canfield 1985).

Although the correlation of ^{65}Zn absorption and retention with plasma ^{65}Zn level was significant when values of both diabetic and control were combined. It became insignificant if they were analyzed separately.

The same holds true for the correlation of ^{65}Zn absorption and retention with plasma Zn levels. This may suggest that the absorbed Zn may directly go into some tissue deposit after absorption, and plasma Zn levels are tightly regulated within a small range.

The significant correlation of ^{65}Zn absorption and retention with urine ^{65}Zn counts in both the diabetic and control groups may indicate that urinary excretion of Zn may be a regulatory mechanism of Zn homeostasis, which helps control the amount of Zn in the body Zn pool. It may also indicate that hyperzincuria in diabetes may be a result of this homeostatic regulation of body Zn metabolism.

A significant correlation of ^{65}Zn absorption and retention was noted with urine ^{65}Zn specificity in the STZ-diabetic rats, but not in their controls. The data, combined with very significant correlation of ^{65}Zn absorption and retention with urine Zn concentration, may indicate that the more Zn is absorbed the more the urinary Zn loss. In other words, the STZ-diabetic rats may both absorb more and excrete more Zn at the same time.

The significant correlation of ^{65}Zn absorption and retention with Zn concentrations in liver, kidney, and heart in the STZ-diabetic group, but not in the control group, may

indicate that more Zn will go into these organs after absorption. This would be expected if these organs are the repository for Zn with a short half-life and play a regulatory role in Zn metabolism.

CHAPTER 4

TISSUE AND ORGAN CONCENTRATIONS, CONTENTS AND BODY COMPARTMENTAL DISTRIBUTIONS OF ZINC, COPPER AND IRON IN STZ-DIABETIC RATS

4.1 Introduction

The intent of this study was to assess Zn, Cu and Fe status of diabetes mellitus through analysis of concentrations of these elements in various tissues or organs. It has been suggested that subjects with diabetes mellitus who display long term hyperzincuria may deplete their body Zn stores (Kiilerich et al. 1990). Children with diabetes mellitus have been reported to have a low growth velocity, also suggesting a possible secondary Zn deficiency (Nakamura et al. 1991). A low concentration of plasma Zn in diabetics has been seen frequently in some diabetic studies (Chooi et al. 1976; Kiilerich et al. 1985; Kiilerich and Christiansen 1984; Kumar and Jaya Rao 1974; Melchior et al. 1989; Mocchegiani et al. 1989; Raz and Havivi 1989; Sjogren et al. 1986; Walter et al. 1991). However, higher levels of plasma or serum Zn have been reported also (Heise et al. 1988; Canfield et al. 1984). It is not known whether these alterations in plasma Zn concentration may reflect a Zn deficient status in the diabetic subject, since plasma Zn concentration may not reflect Zn status and is easily influenced by many other factors (Abdulla 1982; Hambidge et al. 1988; King 1990).

There are only a few studies which analyzed Zn, Cu and Fe contents of organs or tissues of STZ-diabetic animals (Failla and Kiser 1981; Johnson and Evans 1984; Levine et al. 1983). The results of Failla and Kiser (1981) showed that elevation of tissue Zn levels in kidney and liver, and no significant difference in muscle, intestine and spleen between the STZ-diabetic and control rats. Levine et al. (1983) found that STZ-diabetic rats had decreased levels of Zn in serum, liver and kidney, but elevated amount of Zn in femur in STZ-diabetic rats when compared with control rats. These results support an alteration of Zn metabolism in diabetes mellitus.

Johnson and Evans (1984) demonstrated that tissue Zn levels of STZ-diabetic rats were influenced by dietary protein and Zn levels. They found that duodenal Zn concentration was higher in STZ-diabetic rats when they were fed "high" mineral diets compared with rats fed "low" mineral diet, and femur Zn was higher in STZ-diabetic rats fed "high" protein diet than STZ-diabetic rats fed "low" protein diets. However, hepatic and kidney Zn levels seemed not to be influenced by these factors. Hallmans and Lithner (1980) applied acute heat-trauma to STZ-diabetic rats and showed that alterations in Zn metabolism were greater in the STZ-diabetic rats when compared with controls. These results suggest that there was a sequestration of serum Zn by the liver and kidney, and an increased Zn absorption from gastrointestinal tract in diabetes. However, whether or not Zn status in diabetes mellitus is compromised has remained to be determined.

It is expected that alteration of dietary Zn intake may affect tissue Zn concentrations. If diabetics have a metabolic Zn deficiency, then feeding a Zn-depleted diet should aggravate their Zn deficiency. On the other hand, Zn-supplementation may also elicit a beneficial effect if metabolic Zn deficiency existed in diabetes mellitus. To test this assumption a study was designed to assess Zn status of STZ-diabetic animals and control animals fed different dietary Zn levels.

4.2 Materials and Methods

4.2.1 Animals and Induction of STZ-Diabetes Mellitus

The animals employed, the method of diabetes induction and the maintenance of animals were the same as described in Chapter 3, section 3.2.1. Briefly, Wistar rats about 50 grams were randomly assigned into three dietary groups: marginal zinc deficient group (ZD), normal zinc group (ZC) and zinc supplemented group (ZS). After two weeks of acclimatization, half number of the animals were injected intraperitoneally with streptozotocin in a dose of 100 mg/kg body weight. Due to the lower body weight in the ZD group did not start inducting STZ-diabetes until one month later. The appearance of polyuria and polydipsia and polyphagia were used as criteria of diabetes. No attempt to

measure blood glucose since the previous experiment had shown that the hyperglycemia already occurred as soon as the appearance of these signs.

4.2.2 Preparation of Animal Diets with Three Levels of Zn

Three different diets, ZD, ZC and ZS, were prepared and their compositions are listed in Table 4.1. The ZC and ZS diets had the same composition as shown in Table 3.1, except that ZC diet contained 33 mg Zn/kg and ZS diet contained 75 mg Zn/kg. The composition for ZD diet is listed in Table 4.1. The Zn content of the ZD diet is 15 mg/kg.

Table 4.1 Composition of Marginal Zn-Deficient Animal Diet (%)

Ingredient	Percentage
Celufil (cellulose fiber and fillings)	3.0
Corn oil	10.0
Dextrose	68.3
Egg albumin (Spray dried)	15.0
Zn free salt mixture	3.7
Biotin	2 mg/kg
Vitamin Supplement	1 kg/100 lbs

For composition of Zinc free salt mixture see United States Biochemical, 26111 Miles Road, Cleveland, Ohio 44128.

4.2.3 Organ and Tissue Zn, Cu and Fe Determinations

Plasma, whole blood, liver, kidney, duodenum, pancreas, spleen, femur and muscle from both the STZ-diabetic rats and their controls on ZD, ZC and ZS diets were analyzed for their Zn, Cu and Fe concentrations. All organs or tissues underwent wet digestion using concentrated nitric acid and perchloric acid. Kidney, spleen and femur each were digested as a whole. Femurs was cleaned by removing all visible tissues and ligaments before digestion. Liver, pancreas, duodenum, muscle were sampled for digestion. One milliliter whole blood was used for digestion. Samples were put into an 150 ml beaker, to each was added 10 ml concentrated nitric acid. They were heated over a hot plate at 350-

500^0C. After the first phase of active reaction, about 2 ml perchloric acid was added to each. The acids were totally evaporated until a clear layer of residue remained at the bottom of the beaker. With some samples it was necessary to use more nitric acid and perchloric acid. Ten milliliters of 5% (v/v) diluted nitric acid was used to dissolve the residues. This solution was transferred into a 15 ml conic plastic tube for later AAS analysis of Zn, Cu, and Fe under standard conditions. Plasma was diluted 4 to 6 times for direct analysis by AAS method. Standards of Zn, Cu and Fe were prepared by dilution with 5% (v/v) nitric acid.

4.2.4 Statistical Analysis

Statistical analysis of data was done with SAS statistics software (SAS Institute Inc. Cary, NC, Version 6.09). A student T-Test was used to test difference between groups. A correlation procedure was used to analyze the correlation among variables. General Linear Model was used for multiple variant analysis and for multiple comparison. Regression analysis was performed as necessary. A $P \leq 0.05$ for all comparisons was considered significant.

Whole body Zn, Cu and Fe contents were calculated by adding up Zn, Cu and Fe contents of these organs or tissues analyzed. The contents of Zn, Cu and Fe of each organ or tissue were calculated by multiplying tissue or organ Zn, Cu and Fe concentrations with their organ weight. The total weight of plasma, blood clot, GI tract, pancreas, muscle and bone was based on the body weight of the animal by multiplying the following figures: plasma 0.026, blood clot 0.03, GI tract 0.0241, pancreas 0.0028, muscle 0.39, and bone 0.1 (Owen, 1964).

4.3 Results

4.3.1 The Establishment of Streptozotocin (STZ)-Induced Diabetes

STZ-diabetes was induced by injection intraperitoneally 100 mg/kg body weight of streptozotocin saline solution. The injection did not cause immediate death of animals, however, about 10% of animals fed on ZC diet died within 24 hour. No death occurred in animals fed on the ZS diet. About 10% of animals on ZC and ZS diets did not develop diabetes even when given a second injection of streptozotocin solution at the same dosage. In contrast to animals fed the ZC and ZS diets, 16 out of 20 Zn deficient animals died within 48 hours after STZ injection. Despite the severe condition, the Zn deficient animals alive in both diabetic and control groups barely maintained their body weight but remained the whole study.

The presence of diabetes in the three diabetic rat groups was evident by large amounts of urination, increased food intake and inactivity. Body weight initially decreased, but recovered about 3 days later. The smell of their urine was very fetid, their urine become muddy and turbid. The granules of their feces became larger and voluminous. No attempt was made to analyze their blood glucose concentration, since previous study had shown as soon as animals increased their urination, diabetes already existed and blood glucose level became abnormally high. Also the added stress of blood sampling was not considered acceptable.

4.3.2 The Effect of Zn Deficiency on Body Growth and Organ Weight of STZ-Diabetic Rats

Zn is essential for growth and development. Zn deficient diet (3 mg/kg of food) resulted in impaired growth and development, and barely maintained life in these animals. Increasing dietary Zn levels to 15 mg/kg, which is half the amount of normal diet, maintained body weight and improved appetite, but the impaired growth and appetite were not fully recovered. The final dietary Zn levels of the Zn deficient diet was therefore adjusted to 15 mg Zn/kg diet (ZD), actually a marginal Zn deficient diet. Normal diet had

a Zn level of 33 mg/kg (ZC). and the Zn supplement diet (ZS) was adjusted to contain 75 mg Zn/kg of diet.

Table 4.2 Body Weight and Weights of Liver, Kidney and Spleen of STZ-Diabetic and Control Rats

Diet	Zn Deficient	Normal	Zn Supplement
Body Weight C$$$@@@			
STZ-Diabetics	132+3 (5)**	206+15 (13)*	201+11(12)**
Control	208+16 (11)**	256+11 (12)*	262+9 (13)**
Liver Weight (g)b$$@@#			
STZ-Diabetics	7.7+0.6 (5)**	12.1+0.7 (13)	12.1+0.5 (12)
Control	12.4+1.0 (11)**	12.2+0.7 (12)	13.1+0.3 (13)
Liver Weight/Body Weight (%)c$$@@@			
STZ-Diabetics	5.8+0.1 (5)	5.8+0.2 (13)**	5.9+0.2 (12)**
Control	6.0+0.1 (11)	4.9+0.2 (12)**	5.1+0.1 (13)**
Kidney Weight (g)c$$$@@@##			
STZ-Diabetics	2.0+0.2 (5)	3.2+0.1 (13)***	3.2+0.2 (12)**
Control	2.1+0.1 (11)	2.2+0.1 (12)***	2.5+0.2 (13)**
Kidney Weight/Body Weight (%)c@@@			
STZ-Diabetics	1.6+0.1 (5)***	1.61+0.1 (13)***	1.57+0.1 (12)***
Control	1.0+0.03 (11)***	0.88+0.02 (12)***	0.95+0.1 (13)***
Spleen Weight (g)$			
STZ-Diabetics	0.2+0.03 (5)***	0.9+0.3 (13)	0.6+0.1 (12)*
Control	0.6+0.05 (11)***	0.8+0.03 (12)	0.8+0.02 (13)*
Spleen Weight/Body Weight (%)			
STZ-Diabetics	0.2+0.02 (5)**	0.4+0.1 (13)	0.3+0.02 (12)
Control	0.3+0.02 (11)**	0.3+0.01 (12)	0.3+0.01 (13)

*, **, ***: T-Test significant differences at P<0.05, P<0.01 and P<0.0001 between STZ-diabetic and control group; a, b, c: GLM model at P<0.05, P<0.01 and P<0.0001; $, $$, $$$: diet effect at P<0.05, P<0.01 and P<0.0001; @, @@, @@@: Diabetes effect at P<0.05, P<0.01 and P<0.0001; #, ##, ###: Diet and group interaction at P<0.05, P<0.01 and P<0.0001 levels.

The body weight of diabetic and control rats at sacrifice is presented in Table 4.2. STZ-diabetic rats had a significant decrease in their body weight compared with their controls at all the three dietary Zn levels ($P<0.01$). Animals fed on the ZD diet in both diabetic and control groups significantly decreased body weight than animals in the other two dietary groups ($P<0.0001$). Animals of both diabetic and control groups on ZS diets had similar body weight as they did on the NC diet, suggesting that increased dietary Zn level above normal does not further increase body growth. On the other hand, even marginal Zn deficiency could not maintain normal body growth of the growing animals.

The whole liver weight of animals in both groups was not different significantly when they fed the normal Zn diet and Zn-supplemented diet (Table 4.3). However, when on a diet marginal for Zn, liver weight was significantly decreased in STZ-diabetic rats when compared with their controls ($P<0.01$). Both dietary Zn levels and diabetes were the factors affecting liver weight as assessed by the GLM model. There was also a significant interaction between dietary Zn levels and diabetes, indicating that at the three dietary Zn levels, diabetes had a different effect on liver weight.

Control animals on the diet marginal for Zn had a largest liver relative to their body weight. But, when animals on the diets containing normal or increased amount of dietary Zn, the relative weights of livers to body weight of STZ-diabetic rats were significantly heavier than that of their controls ($P<0.01$). Both dietary Zn levels and diabetes were significant factors affecting the relative size of liver based on the GLM model.

STZ-diabetic rats fed on the diet marginal for Zn had decreased significantly kidney weight compared with STZ-diabetic rats fed on diets with adequate and supplemented Zn ($P<0.0001$). While dietary Zn levels did not affect kidney weight in control rats. In rats fed diets with adequate and supplemented Zn, STZ-diabetic rats had significantly larger kidney weight in comparison with their controls ($P<0.001$). GLM analysis indicated that both dietary Zn levels and diabetes were factors that significantly affect kidney size. There was also a significant interaction between dietary Zn levels and

diabetes ($P<0.01$), indicating diabetes had a different effect on kidney weight when their dietary Zn levels were different.

Since the body weight was significantly decreased, the kidney weight relative to body weight was not significantly affected by dietary Zn levels in STZ-diabetic rats. However, the relative size of diabetic kidneys was significantly larger than that of the controls at all the three levels of dietary Zn, suggesting diabetes affects kidney size. GLM analysis indicates that only diabetes was the significant factor that affect kidney size.

Diets containing normal and increased amount of Zn had no significant effect on both absolute or relative spleen weight in both STZ-diabetic and control groups. However, when fed on the diet marginal for Zn, STZ-diabetic rats had significantly decreased spleen weight either expressed in absolute weight or relative size to body weight in comparison with the controls ($P<0.0001$ and $P<0.01$ respectively).

4.3.3 Other Effects of Dietary Zn Deficiency

Besides impaired body growth and impaired appetite, other effects of Zn deficiency include physical inactivity, impaired hair growth and hair loss, skin impairment, and uncontrolled urination and feces (the food bottles of these Zn deficient animals were always contaminated with feces and urine). Their feces were sticky, thin and malodorous. The feces and urine of Zn deficient animals could not be collected due to the contamination of each other. The outlook of these Zn deficient animals clearly indicated the importance of Zn, which is essential for normal growth and development of all species.

4.3.4 The Effect of Diabetes and Dietary Zn Levels on Organ and Tissue Zn Concentrations

Zn concentrations of selected organs and tissues of both STZ-diabetic and control rats are presented in Table 4.3 and Figure 4.1- 4.9. Values are expressed as mean+SEM. Significance between two subject groups (diabetics and controls) and among three dietary groups are indicated.

Several features of the results are noteworthy. All STZ-diabetic rats on Zn diet had higher Zn concentrations in all the organs or tissues analyzed when compared with their controls, especially, liver and femur. On ZD diet, STZ-diabetic rats had lesser Zn levels than their controls in duodenum, pancreas, spleen, femur and plasma. Muscle and liver Zn concentration was significantly higher in diabetics than in control ($P<0.05$). Zn concentrations found in kidney, whole blood, and femur were not significantly different between the two groups. On the ZS diet, Zn concentrations of nearly all organs or tissues were also higher in diabetic than in controls, except for plasma Zn concentration which was significantly lower. The variances, in terms of standard error of means, in Zn concentrations of organs and tissues of STZ-diabetic rats were larger than in the controls, which may suggest that STZ-diabetic rats had a lesser control of their Zn metabolism in response to dietary Zn changes.

4.3.4.1 Plasma Zn Concentrations of STZ-Diabetic Rats

As seen from Table 4.3 and Figure 4.1 that STZ-diabetic rats had a 20% higher plasma Zn concentration than their controls on NC diet. However, the difference is not significant ($P>0.05$) due to a large variations of the data. The lowest level of plasma Zn concentration occurred in STZ-diabetic rats fed on the ZD diet. Plasma Zn concentrations of STZ-diabetic rats on the ZD and ZS diets were about 20%less than that of their controls ($P<0.05$). Analysis of variance indicated that diet and diabetes were not significant factors affecting plasma Zn concentration. A significant interaction between diets and diabetes, suggesting the effect of diabetes on plasma Zn concentration differed at different dietary Zn levels.

Table 4.3 Organ and Tissue Zn Concentrations of Diabetic and Control Rats

Dietary Groups	Zn Deficient	Normal	Zn Supplement
Plasma (mg/L)#			
Diabetic	0.909+0.088 (4)*	1.400+0.156 (10)	1.0568+0.053 (10)*
Control	1.209+0.09 (9)*	1.163+0.098 (10)	1.294+0.085 (12)*
Whole Blood (mg/L)a$			
Diabetic	3.867+0.214 (5)	3.414+0.209 (13)	3.451+0.144 (10)
Control	3.773+0.113 (11)	3.139+0.180 (10)	3.156+0.144 (14)
Liver (ug/g wet wt)c$@@			
Diabetic	27.018+0.969 (5)**	24.561+0.920 (13)**	23.108+0.643 (12)**
Control	21.011+0.387 (11)**	20.423+0.727 (11)**	20.371+0.364 (12)**
Kidney (ug/g wet wt)a@@			
Diabetic	18.511+0.305 (5)	19.131+0.666 (13)	19.636+0.510 (10)**
Control	18.122+0.483 (11)	17.699+0.336 (11)	17.530+0.246 (12)**
Duodenum (ug/g wet wt)c$$$###			
Diabetic	13.667+0.927 (5)	18.222+0.381 (13)*	20.245+0.476 (12)**
Control	15.923+0.250 (11)	16.836+0.275 (12)*	17.407+0.342 (12)**
Pancreas (ug/g wet wt)b$@#			
Diabetic	14.328+1.038 (5)	14.441+0.650 (15)*	13.879+0.308 (10)
Control	15.478+0.766 (10)	11.131+0.785 (8)*	11.850+0.592 (11)
Spleen (ug/g wet wt)a$$			
Diabetic	13.591+1.263 (5)	16.617+0.599 (13)	16.581+0.663 (14)
Control	15.930+0.285 (11)	16.288+0.083 (11)	16.859+0.342 (11)
Muscle (ug/g wet wt)c$$$@@@###			
Diabetic	16.035+2.062 (5)**	9.547+0.292 (12)	9.655+0.362 (13)**
Control	8.254+0.509 (11)**	9.106+0.474 (11)	8.453+0.207 (12)**
Femur (ug/g wet wt)a			
Diabetic	77.734+5.643 (5)	86.286+1.953 (13)*	91.978+2.385 (11)**
Control	81.299+5.322 (11)	77.775+2.562 (11)*	82.083+2.448 (12)**

*, **, ***: P<0.05, P<0.01, and P<0.0001 between diabetic and control groups from T-test. The following are from GLM: a.,b, c.: model significance at P<0.05, 0.01 and 0.0001 level; $, $$, $$$: Diet effect at P<0.05, 0.01 and 0.0001 level; #, ##, ###: Significant interaction between dietary Zn level and diabetes at P<0.05, 0.01 and 0.0001 levels. @, @@, @@@: Groups effect at P<0.05, 0.01 and 0.0001 levels. (number): number of rats.

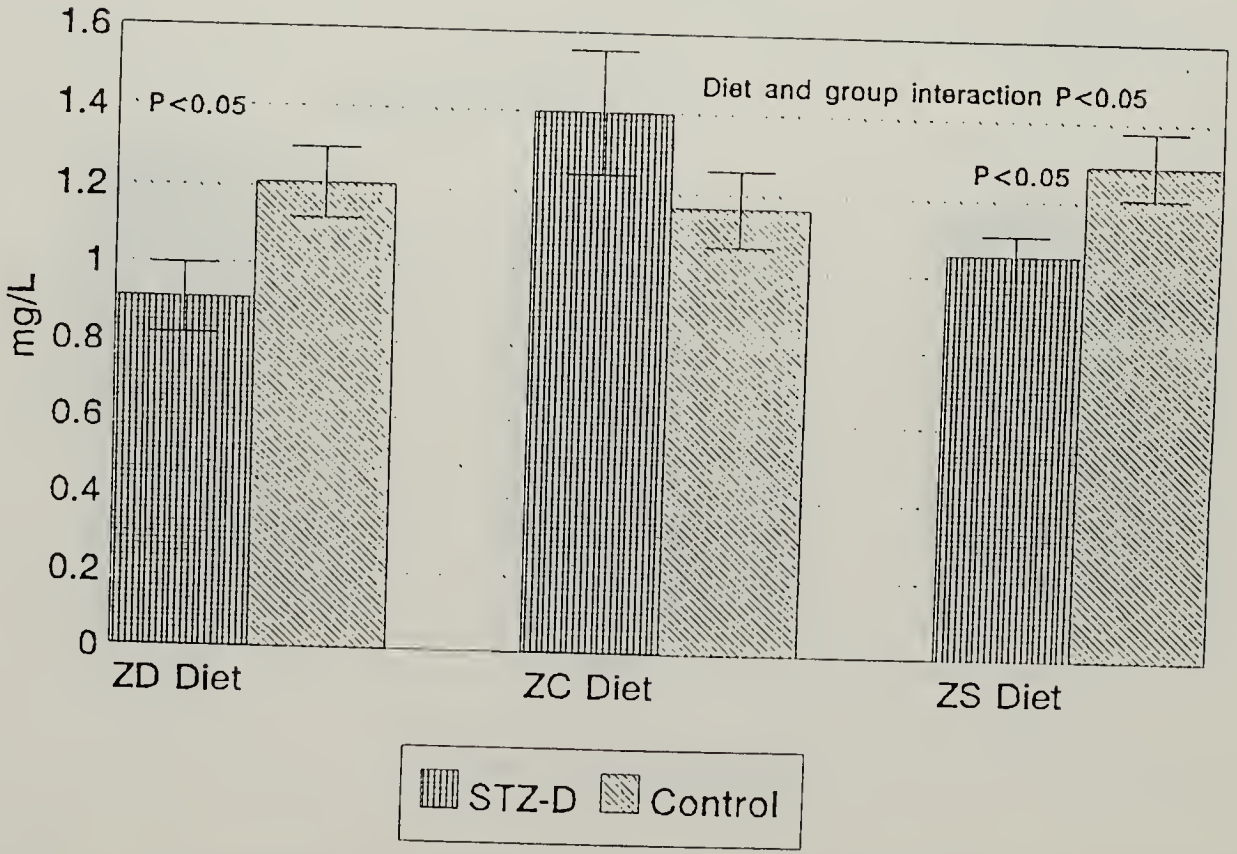

Figure 4.1 Comparison of Plasma Zn Concentrations between STZ-Diabetic and Control Rats Fed Three Levels of Zn

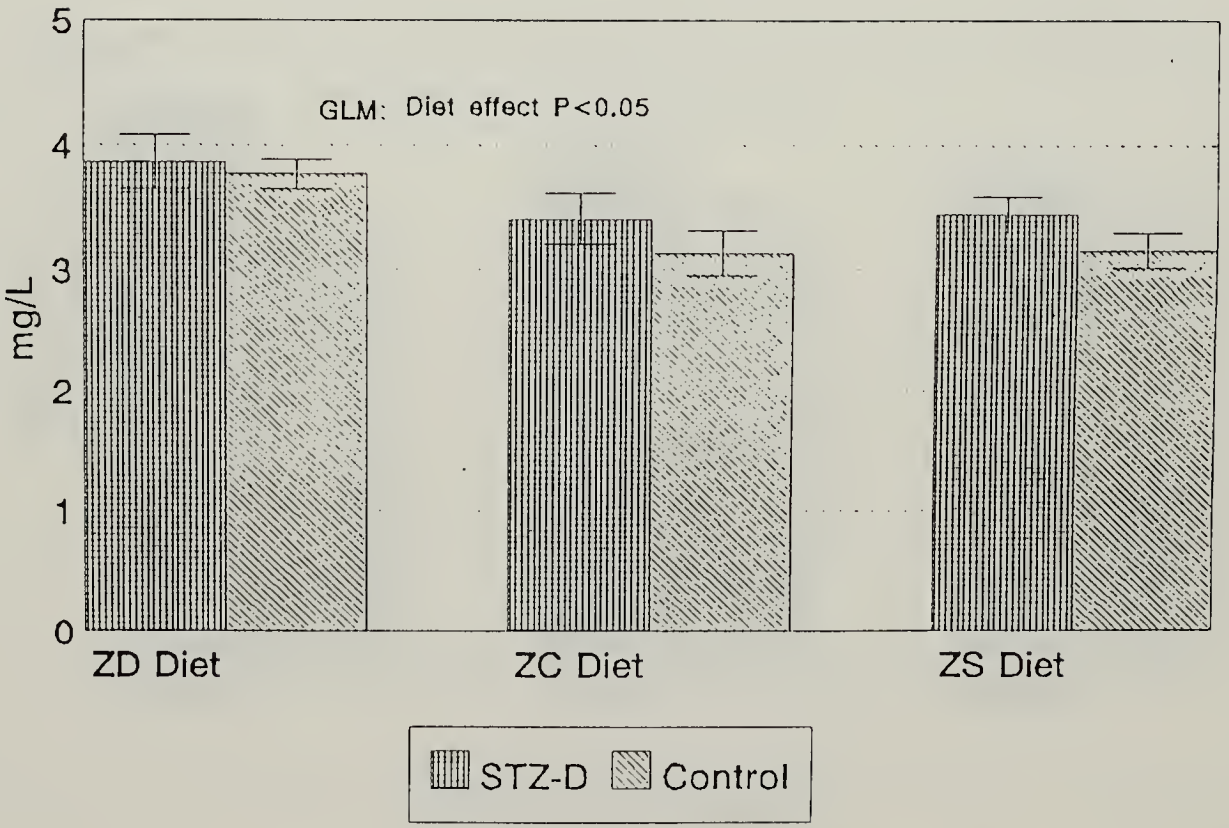

Figure 4.2 Comparison of Whole Blood Zn Concentrations between STZ-Diabetic and Control Rats Fed Three Levels of Zn

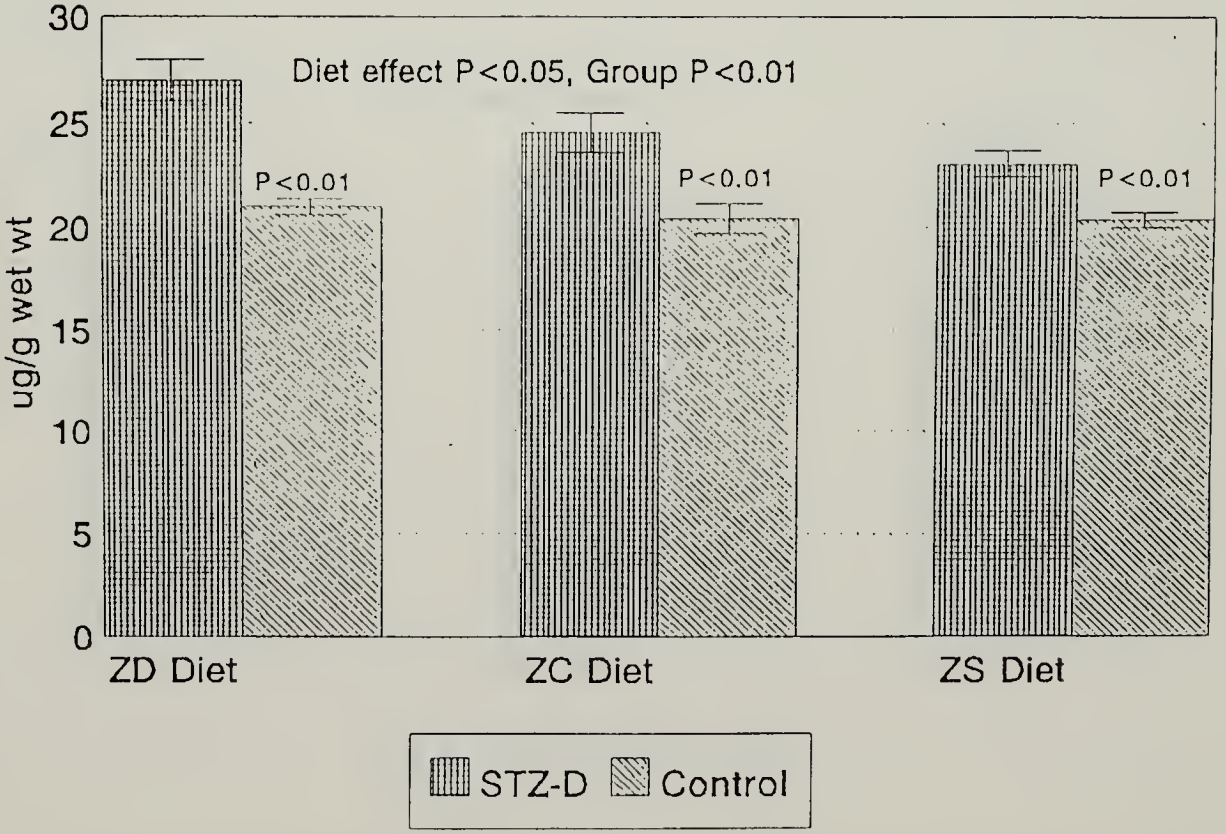

Figure 4.3 Comparison of Liver Zinc Concentrations between STZ-Diabetic and Control Rats Fed Three Levels of Zn

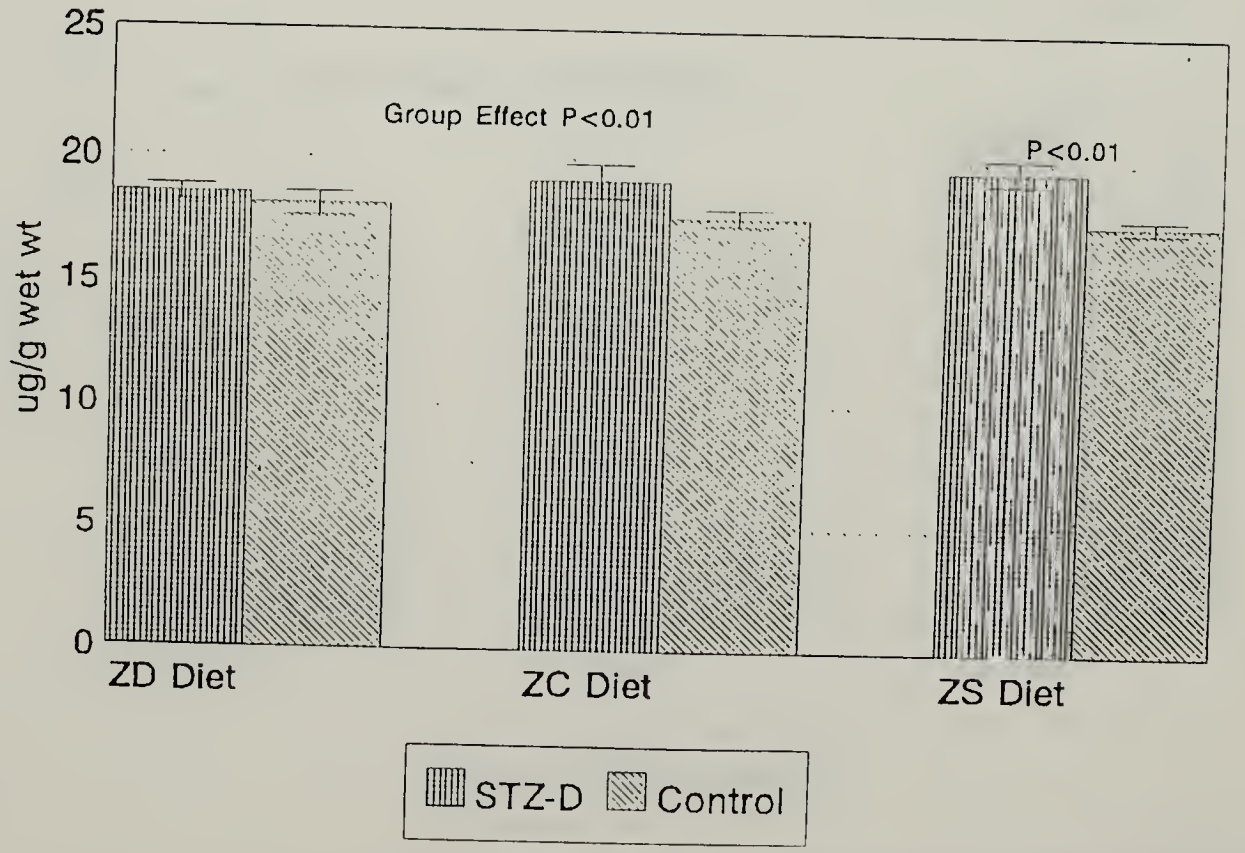

Figure 4.4 Comparison of Kidney Zn Concentrations between STZ-Diabetic and Control Rats Fed Three Levels of Zn

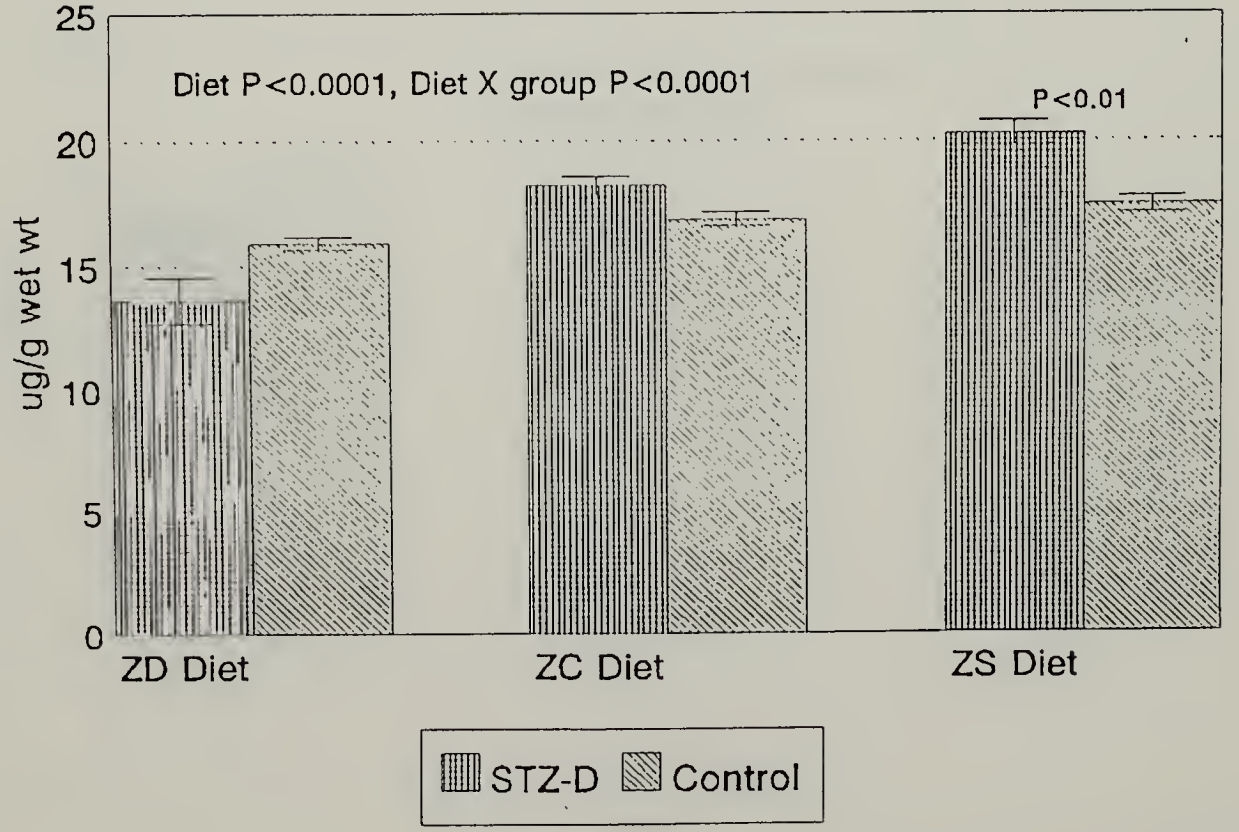

Figure 4.5 Comparison of Duodenal Zn Concentrations between STZ-Diabetic and Control Rats Fed Three Levels of Zn

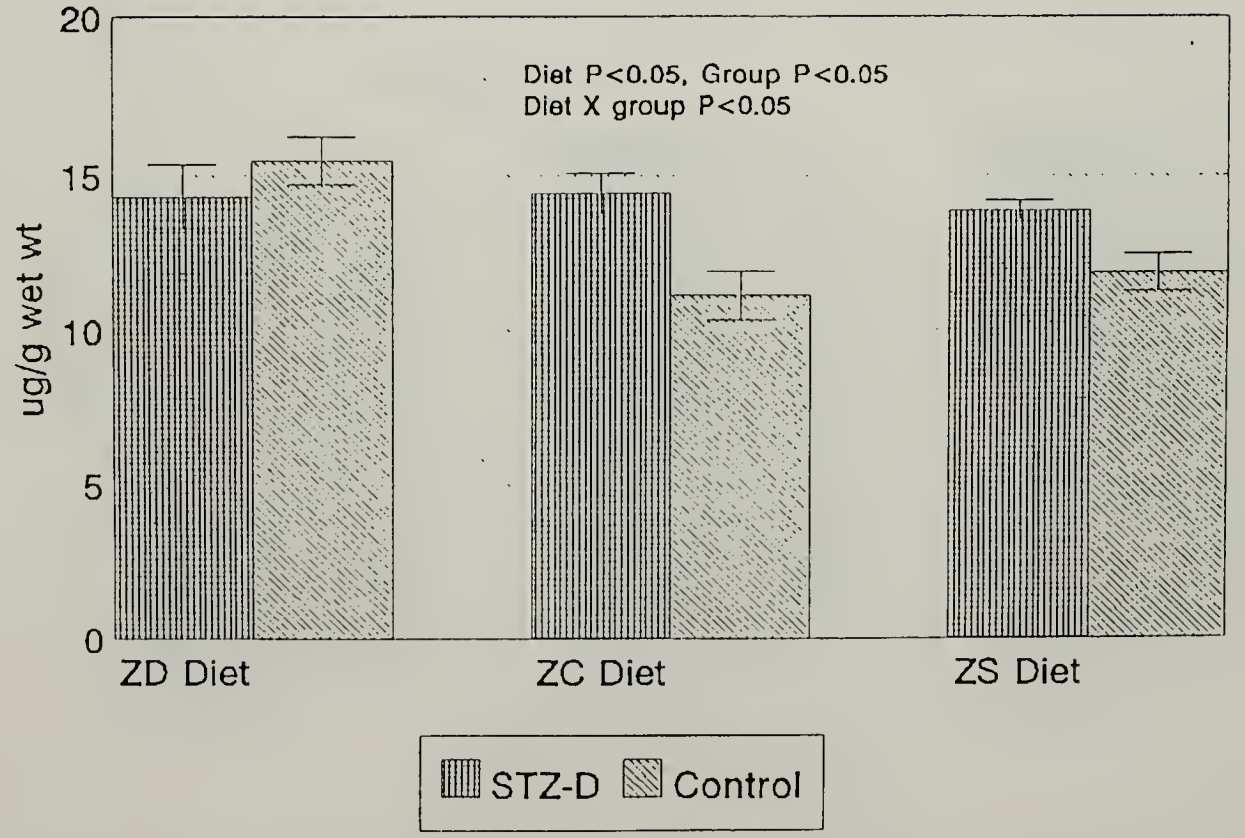

Figure 4.6 Comparison of Pancreas Zn Concentrations between STZ-Diabetic and Control Rats Fed Three Levels of Zn

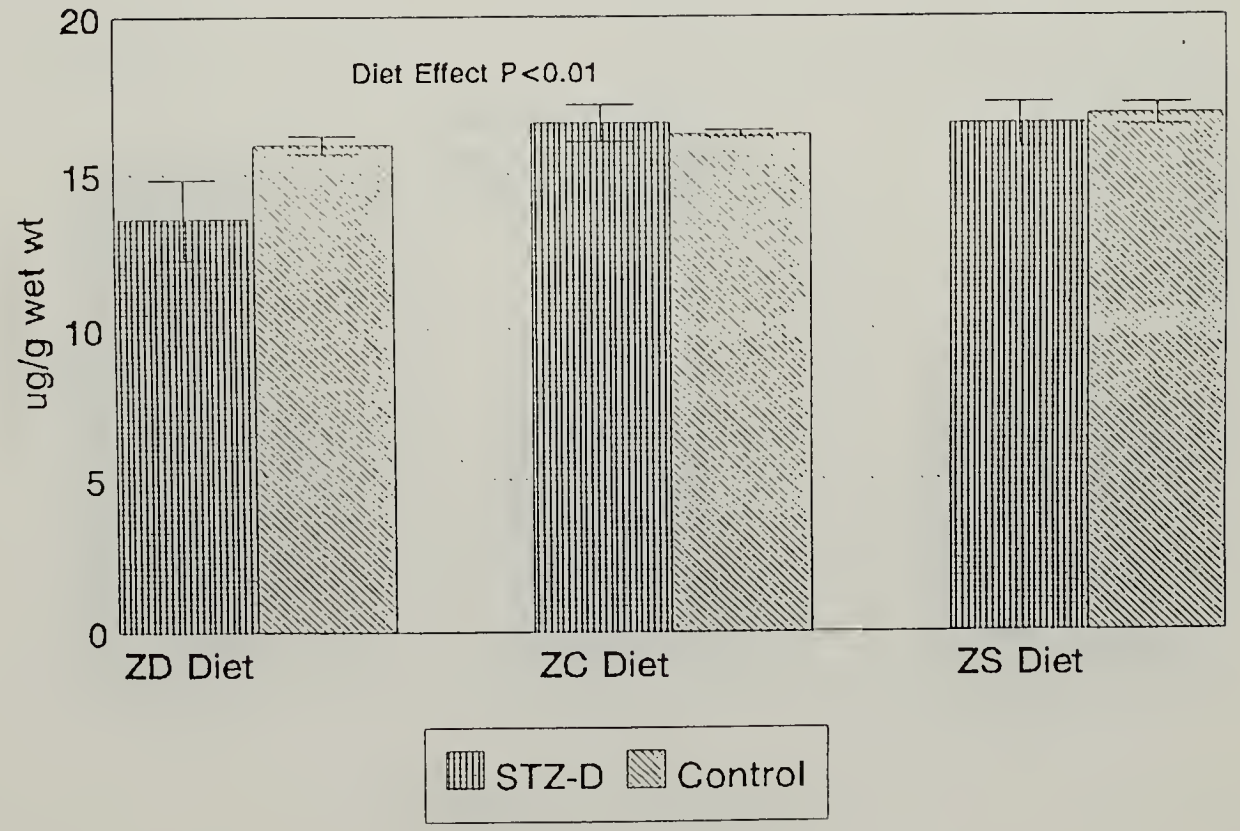

Figure 4.7 Comparison of Spleen Zn Concentrations between STZ-Diabetic and Control Rats Fed Three Levels of Zn

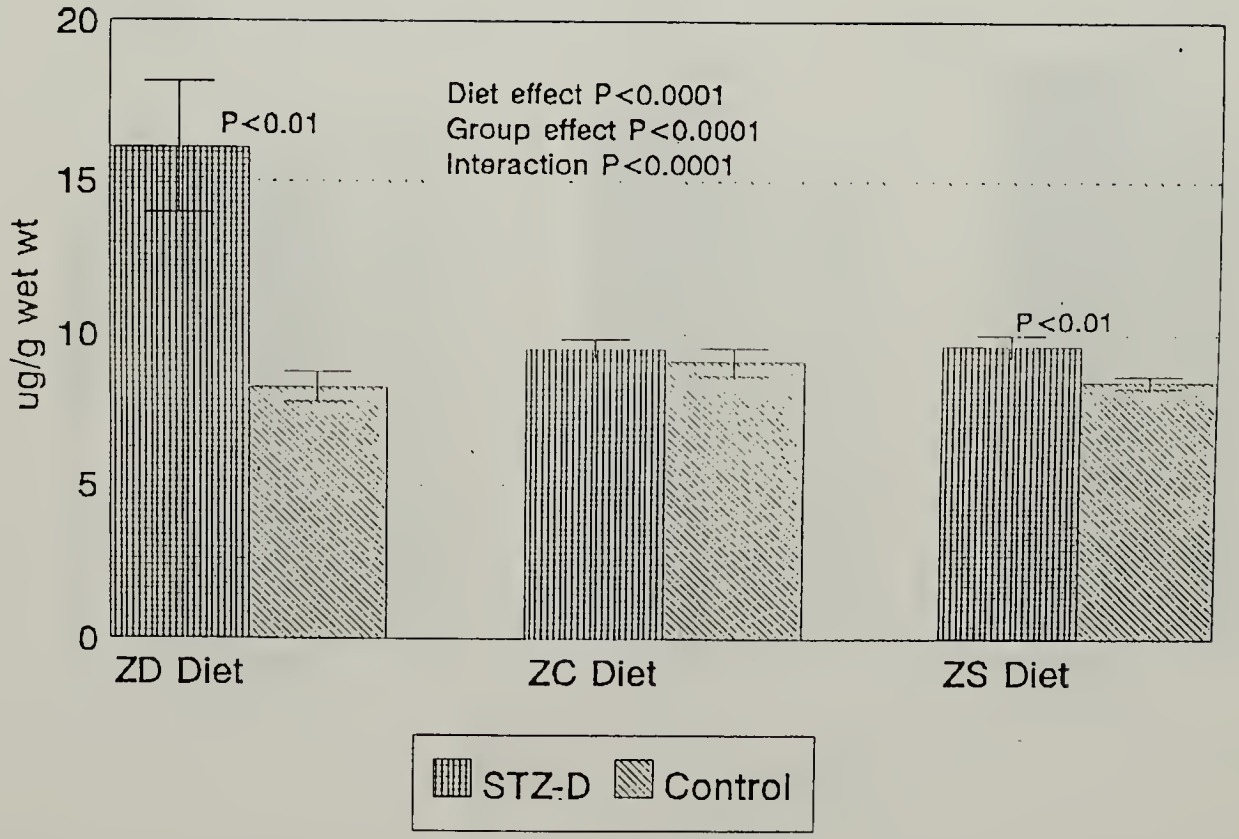

Figure 4.8 Comparison of Muscle Zinc Concentrations between STZ-Diabetic and Control Rats Fed Three Levels of Zn

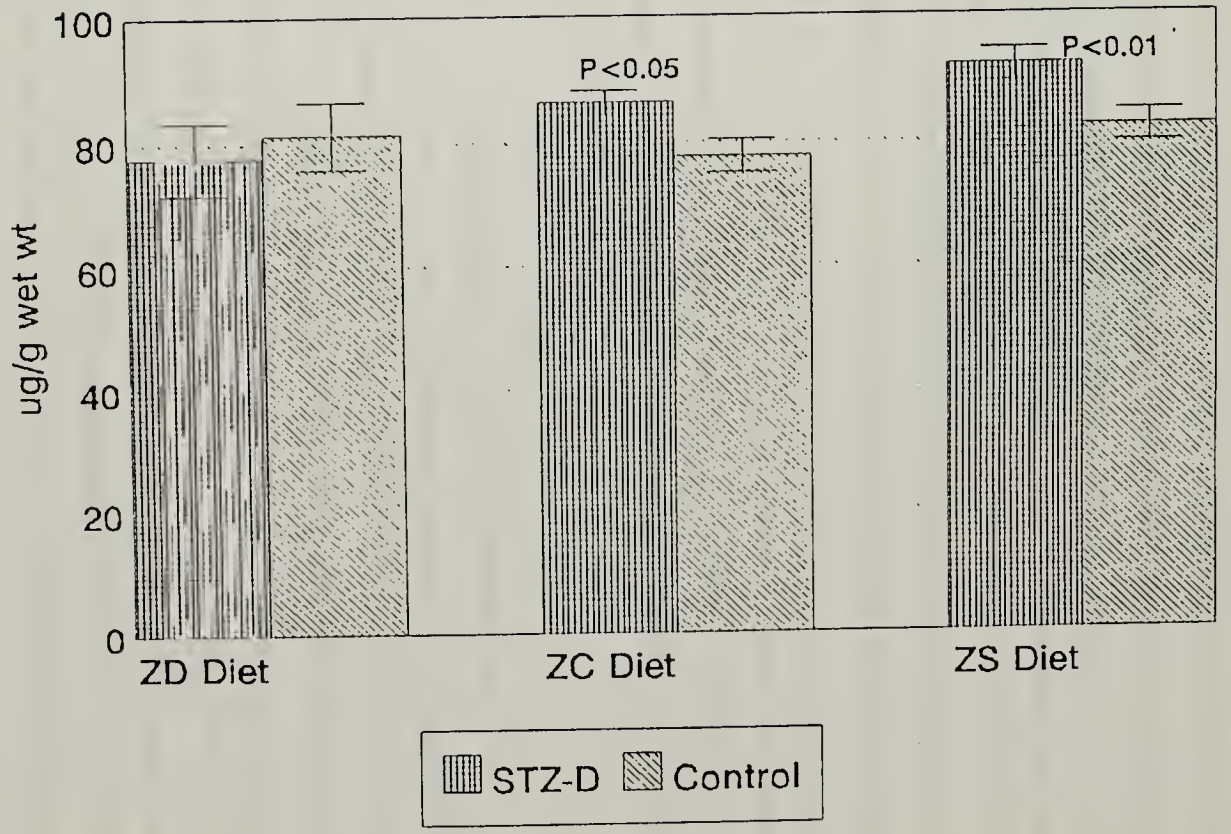

Figure 4.9 Comparison of Femur Zinc Concentrations between STZ-Diabetic and Control Rats Fed Three Levels of Zn

4.3.4.2 Whole Blood Zn Concentrations in STZ-Diabetic Rats

STZ-diabetic rats had higher, but not significantly ($P>0.05$), whole-blood Zn concentrations than their corresponding controls at all the three dietary Zn levels (Table 4.4 and Figure 4.2). Animals of both diabetic and control groups on the ZD diet had a 12% increase in whole-blood Zn concentration when compared to those on the NC and ZS diets. It seems that dietary Zn deficiency decreased plasma Zn concentration (Figure 4.1) but it did not decreased total circulating Zn level. It also seems that more Zn was sequestrated by blood cells within the blood when dietary Zn levels were low. Analysis of variance revealed a significant effect of dietary Zn levels on whole-blood Zn concentrations ($P<0.05$). Diet, but not diabetes, had significant effect on whole-blood Zn levels ($P<0.05$). There were no significant interactions between diet and diabetes, indicating this effect of dietary Zn level on whole blood Zn levels was independent of the diabetic condition of the animals. The differences in effects noted between whole blood Zn and plasma Zn concentrations must be attributed to Zn found in the various cellular components of blood.

4.3.4.3 Liver Zn Concentrations of STZ-Diabetic Rats

At each dietary Zn level, STZ-diabetic rats had an significantly ($P<0.01$) higher liver Zn concentration than that of control rats (Table 4.3 and Figure 4.3). The data are similar to those reported by Failla and Kiser (1981, 1983). Both diabetic and control rats had showed significant decrease in liver Zn concentrations as dietary Zn increased. Both dietary Zn levels and diabetes significantly altered liver Zn levels ($P<0.05$) as confirmed by GLM analysis. There was no significant interaction between the dietary Zn levels and diabetes. The increased liver Zn concentrations in Zn deficiency may have important implications that liver Zn may be used to support certain more vital life processes over others.

4.3.4.4 Kidney Zn Concentrations of STZ-Diabetic Rats

As indicated in Table 4.3. and Figure 4.4, kidney Zn concentrations in control rats slightly decreased as dietary Zn was increased, but the changes were relatively small. STZ-diabetic rats had an opposite trend showing a steady increase in kidney Zn concentrations as their dietary Zn level increased, possibly a consequence of increased Zn absorption at increased dietary Zn intake, and the increased workload to excrete unwanted Zn. The differences in kidney Zn concentration between the diabetic and control rats on the ZD and NC diets were not significant ($P>0.05$), but became significant ($P<0.01$) when they were on the ZS diet. Diabetes, but no diet, affected kidney Zn concentration significantly as assessed by GLM analysis. There was no significant interaction between diet and diabetes.

4.3.4.5 Duodenum Zn Concentrations of STZ-Diabetic Rats

Table 4.3 and Figure 4.5 indicate that both diabetic and control rats showed increased duodenum Zn concentrations as dietary Zn levels increased ($P<0.0001$). STZ-diabetic rats fed on the ZD diet had lower duodenum Zn concentration than controls, but this difference was not significant ($P>0.05$). However, duodenum Zn concentrations of STZ-diabetic rats were significantly higher than those of their controls when they fed either NC ($P<0.05$) or ZS ($P<0.01$) diets. Analysis of variance suggested that diet was a significant factor affecting duodenum Zn level, but the effect of diabetes was not significant ($P>0.05$). The significant interaction between the dietary Zn levels and diabetes suggests that the effect of diet on duodenum Zn concentration was dependent on the condition of the animals.

4.3.4.6 Pancreas Zn Concentrations of STZ-Diabetic Rats

As shown in Table 4.3. and Figure 4.6 that STZ-diabetic rats did not have decreased pancreatic Zn concentrations when their dietary Zn levels were normal or increased. Actually STZ-diabetic rats had a 20% increase in their pancreatic Zn concentration when compared with their control rats on the diet with normal Zn level

($P<0.05$). STZ-diabetic rats on the ZD diet had a decrease, but not significantly, in their pancreatic Zn concentration. Both STZ-diabetic and controls fed the ZS diets did not differ significantly in their pancreatic Zn concentrations. GLM analysis showed that dietary Zn level and diabetes were both significant factors affecting pancreatic Zn concentrations ($P<0.05$). A significant interaction between the dietary Zn level and diabetes suggests pancreas Zn concentration responded to dietary Zn levels differently between the diabetic and control rats.

4.3.4.7 Spleen Zn Concentrations of STZ-Diabetic Rats

Both NC and ZS diets had little effect on spleen Zn concentration in either the STZ-diabetic or control groups (Table 4.3 and Figure 4.7). However, when fed the ZD diet, STZ-diabetic rats had a 20% decrease in spleen Zn concentrations, while the controls had only a slight change (<2%). The lowest spleen Zn concentration was in the STZ-diabetic rats fed the ZD diet. GLM analysis indicated that only dietary Zn level affected spleen Zn concentration, diabetes had no significant effect on spleen Zn concentration. No significant interaction existed between diet and diabetes.

4.3.4.8 Muscle Zn Concentrations of STZ-Diabetic Rats

STZ-diabetic rats had the greatest muscle Zn concentration on the ZD diet than either NC or ZS diet (Table 4.3 and Figure 4.8). STZ-diabetic rats had a comparable muscle Zn levels when they were fed the ZD and ZS diets, but these levels were substantially lower than that when they fed the ZD diet. The control rats, on the other hand, had relatively stable muscle Zn concentration when fed either of the three diets. The STZ-diabetic rats had consistently higher muscle Zn concentrations at all the three dietary Zn levels, and the differences were very significant when they were on the diets containing either marginal Zn or supplemented Zn. Both dietary Zn levels and diabetes were significant factors affecting muscle Zn concentrations ($P<0.0001$), The interaction between dietary Zn levels and diabetes was also highly significant ($P<0.0001$), indicating the differential effect of diabetes when animals fed the three dietary Zn levels.

4.3.4.9 Femur Zn Concentrations of STZ-Diabetic Rats

STZ-diabetic rats had significantly higher femur Zn concentrations when fed the NC or the ZS diet ($P<0.05$ and $P<0.01$, respectively, Table 4.3 and Figure 4.9) than noted for their controls. Animals of both groups fed the ZS diet showed further increased femur Zn concentrations than when on the NC diet. However, when they were on the diet that was marginal for Zn, the control rats had an increased femur Zn concentrations than they were on the NC and ZS diets, while the STZ-diabetic rats had decreased femur Zn concentrations than they were on the NC and ZS diets. GLM analysis showed no significant effect from either diet or diabetes, there was also no significant interaction between dietary Zn levels and diabetes.

In summary, data in Table 4.3. and Figure 4.1 through 4.9 indicate that there was no evidence of decreased tissue or organ Zn levels in STZ-diabetic rats when fed on the normal Zn diets. STZ-diabetic rats even had significantly higher liver, duodenal, and femur Zn concentrations than their controls when fed the normal diet. Feeding the ZS diet further increased all but plasma Zn concentrations in STZ-diabetic rats, however, ZS had no significant effect in these tissues and organs in the control rats. Intake of the ZD diet resulted in decreased Zn concentrations in plasma, duodenum, pancreas, spleen, and femur, but an increased Zn concentrations in liver and muscle in STZ-diabetic rats. These changes in organ and tissues Zn concentrations suggest a redistribution of Zn among body compartments in STZ-diabetic rats. Furthermore, these changes do not reflect Zn deficiency in STZ-diabetic rats on the NC and ZS diets. But under condition of Zn deficiency, diabetics tend to become Zn depleted in some of their tissues and organs. Alternatively, they may suggest that the influence of STZ-diabetes on specific tissue or organ may be related to both the general homeostasis of Zn and the particular function of Zn in the tissue. The normal influence that the levels of Zn intake would have on tissue Zn concentrations appear to be modulated by an over-riding effect of diabetes.

4.3.4.10 Whole Body Zn Contents of STZ-Diabetic Rats

The calculated total body Zn contents (See section 4.2.4 for calculations) of STZ-diabetic rats were consistently less when compared with that of their controls (Figure 4.10 and Table 1 in Appendix). However, due to the small body weight in STZ-diabetic rats, total body Zn contents did not mean a Zn deficiency in STZ-diabetic rats. GLM analysis indicates that whole body Zn contents was affected by both dietary Zn levels and diabetes at the levels of $P<0.01$.

4.3.4.11 Whole Body Mean Zn Concentrations in STZ-Diabetic Rats

Calculation of total body mean Zn concentrations of STZ-diabetic rats (Figure 4.11) revealed that STZ-diabetic rats had consistently higher Zn concentration than their controls ($P<0.0001$). GLM analysis indicates that the effect of dietary Zn on body mean Zn concentration is significant at the level of $P<0.01$, and the effect of diabetes is significant at the level of $P<0.0001$, indicating that the significant differences in total body mean concentrations is mainly the effect of diabetes. The results in Figure 4.11 further suggest that not only is there no evidence that Zn depletion occurs in STZ-diabetic rats, but that there also may be more Zn in the body of STZ-diabetic rats.

4.3.4.12 Body Zn Distribution among Selected Organs and Tissues

Figure 4.12 and Table 2 in Appendix summarizes results of Zn distribution among organs and tissues of diabetic and control animals at the three dietary Zn levels. As noticed from Figure 4.12, the amount of Zn in bone constitutes more than half of the total body Zn store, except for STZ-diabetic rats on the ZD diet. Muscle Zn constitutes about one third of total body Zn. The third largest Zn reservoir is liver, which constitutes about 8-10% of the total body Zn. Next are GI tract, kidney and spleen Zn. Zn in circulation (plasma and blood clot) constitutes about 1% of total body Zn.

On the NC diet, the organs and tissues of STZ-diabetic rats were significant altered are kidney and liver. STZ-diabetic rats obviously accumulated Zn in their livers and kidneys. The percentages of body Zn distributed in these two organs in STZ-diabetic rats

were significantly higher than that of their controls ($P<0.01$). STZ-diabetic rats also had decreased percentage of Zn in plasma, blood, GI tract, spleen, muscles, and bone, and an increased Zn content in pancreas, but, these alterations were not significantly different from that of their controls ($P>0.05$).

On the ZD diet, STZ-diabetic rats had a further increased the accumulation of Zn in liver, which was not significantly higher than their controls ($P>0.05$). Kidney Zn was still significantly higher in STZ-diabetic rats than in control rats ($P<0.05$). STZ-diabetic rats decreased significantly Zn content in their plasma ($P<0.05$), GI tract ($P<0.05$), pancreas ($P<0.05$), spleen ($P<0.01$), and bone ($P<0.01$). These changes may be a results of increased Zn content in muscle, which was nearly doubled in the STZ-diabetic rats than those fed the NC and ZS diets. Both diabetic and control rats had a decreased Zn content in the circulation, indicating when Zn deficiency existed, total Zn in plasma was affected. There was no significant changes in Zn distribution in whole blood in both groups. The percentage of pancreatic Zn was increased in both groups, however, the STZ-diabetic rats had a lesser magnitude compared with their controls.

In animals fed the Zn supplemented diet, distribution of Zn among organs and tissues of the STZ-diabetic rats was more comparable to that of their controls. The only significant changes left were the significantly increased Zn in kidney ($P<0.0001$) and liver ($P<0.05$), and significantly decreased Zn in plasma in the STZ-diabetic rats in comparison with that of their controls ($P<0.01$). The distribution of total body Zn in other body compartments were not significantly different between the STZ-diabetic and control groups.

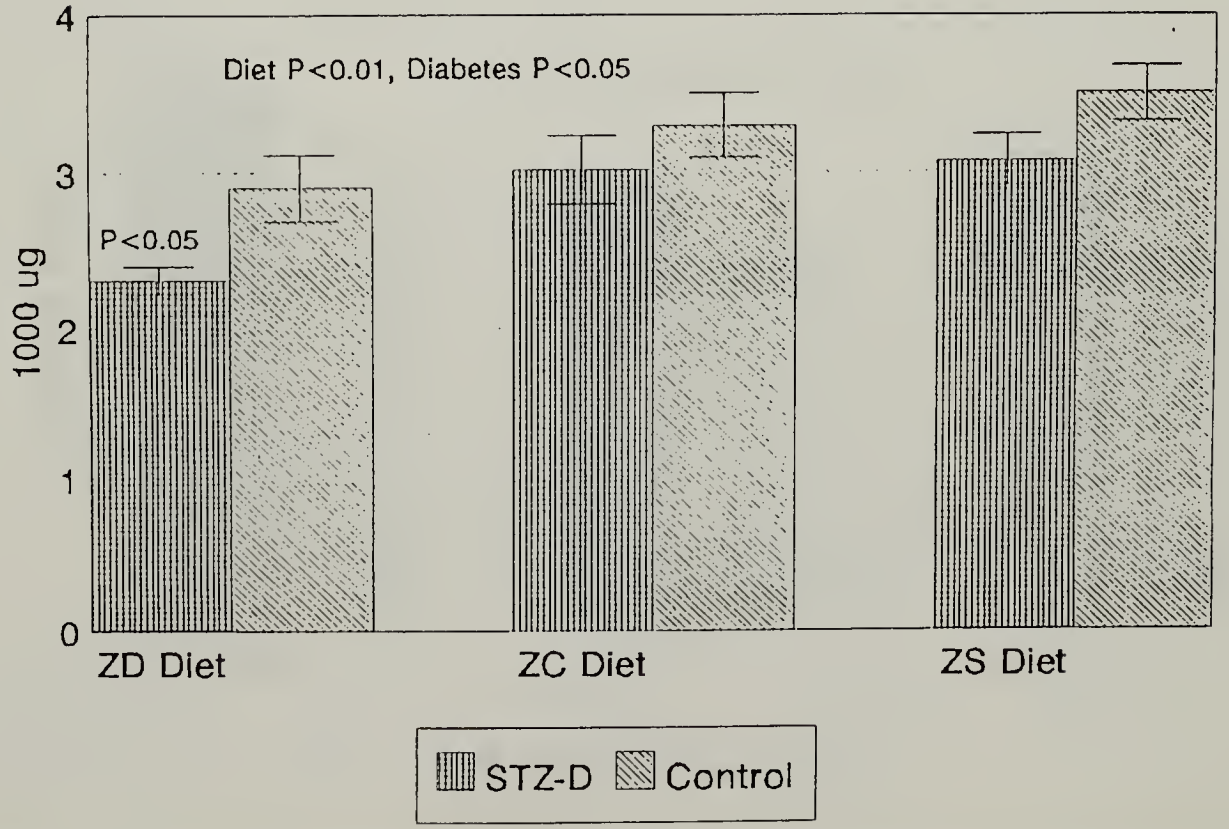

Figure 4.10 Comparison of Whole Body Zinc Contents between STZ-Diabetic and Control Rats Fed Three Levels of Zn

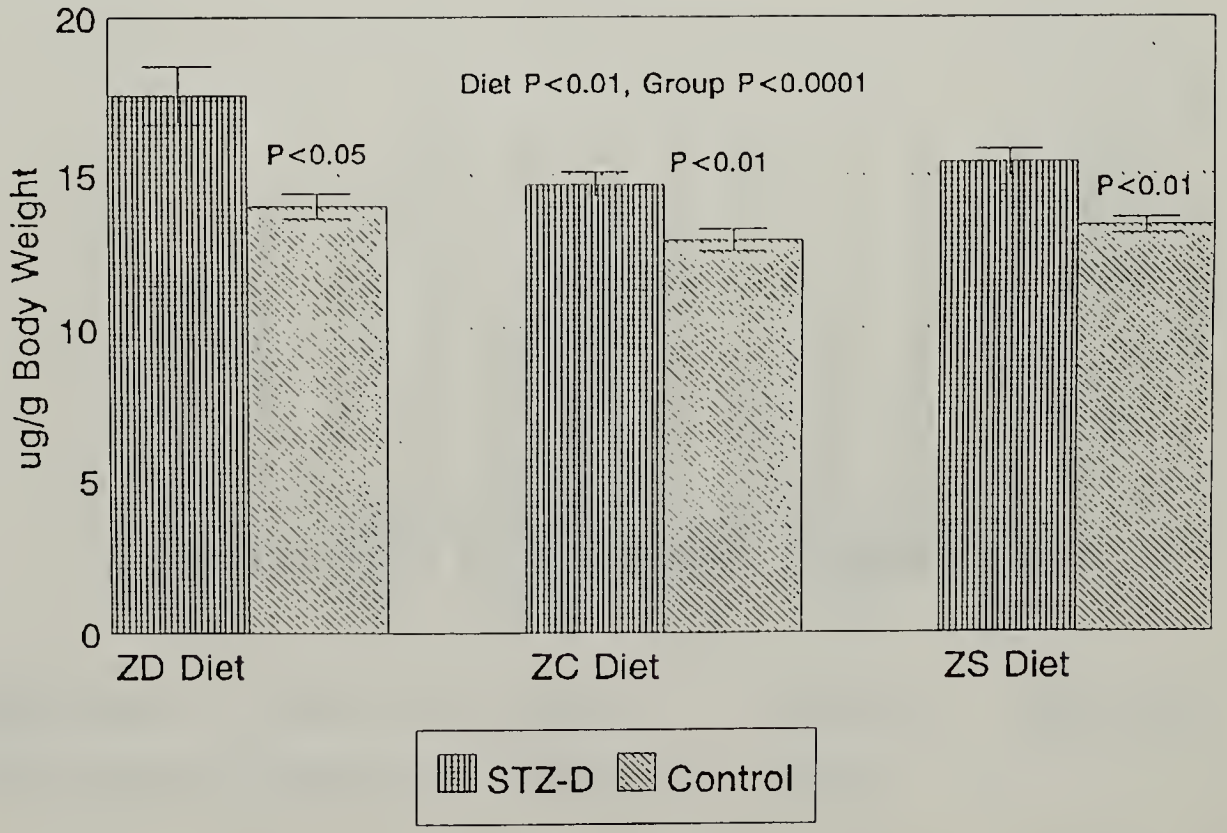

Figure 4.11 Comparison of Whole Body Mean Zn Concentrations between STZ-Diabetic and Control Rats Fed Three Levels of Zn

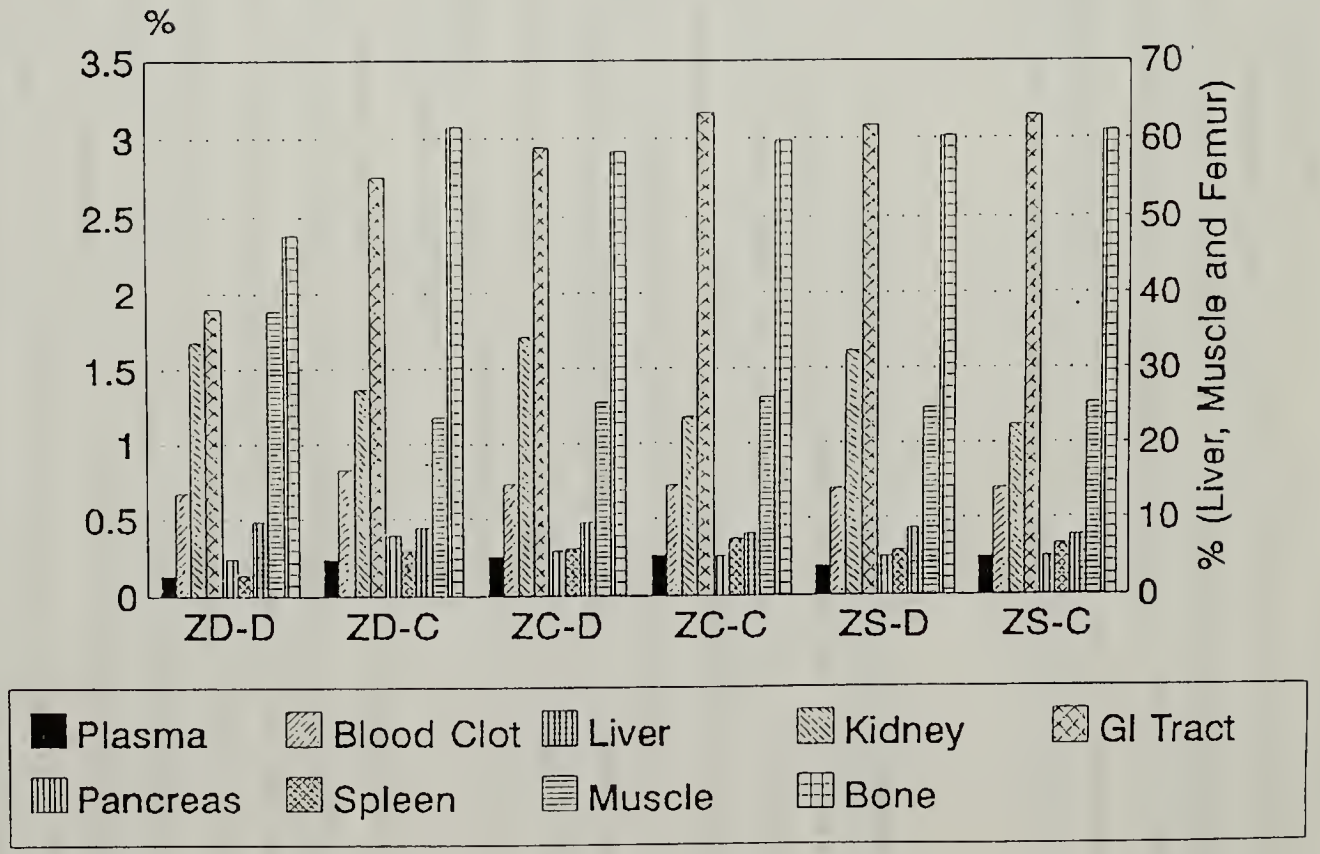

Figure 4.12 Comparison of Zn Distribution between STZ-Diabetic and Control Rats Fed Three Levels of Zn

4.3.5 The Effect of Diabetes and Dietary Zn Levels on Organ and Tissue Cu Concentrations

The metabolism of Zn, Cu and Fe is mutually linked. There is evidence that they may share common storage molecules and they do appear to compete with one another for absorption (Craft and Failla 1983; Davis 1980; Flanagan et al. 1980; 1983; Hill 1988). Because changes in Zn concentration in the diet may influence the absorption of Cu and Fe. It was of interest to determine if their status was altered due to changes in dietary Zn levels.

4.3.5.1 Cu Concentrations of Organs and Tissues of STZ-Diabetic Rats

Cu concentrations of organs and tissues of diabetic and control rats are presented in Table 4.4. When fed the NC diet, STZ-diabetic rats had higher Cu concentrations in nearly all organs and tissues analyzed, except muscle; these differences were significant in liver ($P<0.05$), duodenum ($P<0.05$), kidney ($P<0.01$) and pancreas ($P<0.01$) between the STZ-diabetic rats and their corresponding controls. A similar trend held true when they were on the ZS diet. When fed the ZD diet, STZ-diabetic rats still had the higher Cu concentrations in all organs and tissues analyzed, except kidney, which was lower in STZ-diabetic rats than in control rats.

There were no significant differences in plasma Cu concentrations between the diabetic and control rats at any of the three dietary Zn levels. In STZ-diabetic rats, highest plasma Cu concentration occurred when they were on the NC diet, while control rats on the NC diet displayed the lowest plasma Cu concentration. The ZD diet did not significantly affect plasma Cu in STZ-diabetic rats, as it did the control rats. STZ-diabetic rats on the ZS diet showed a decrease of about 30% in plasma Cu concentrations than that noted for those on the NC and ZD diets. The control rats fed both ZD and ZS diets showed a slight increase in plasma Cu concentration when compared to those fed the NC diet. All these changes in plasma Cu concentrations in both diabetic and control rats were not significant as assessed by analysis of variance. Both STZ-diabetic and control rats had

Table 4.4 Organ and Tissue Cu Concentrations of Diabetic and Control Rats

Dietary Groups	Zn Deficient	Normal	Zn Supplement
Plasma (mg/L)			
Diabetic	0.784+0.094 (4)	0.815+0.099 (8)	0.564+0.069 (10)
Control	0.621+0.076 (10)	0.549+0.040 (10)	0.678+0.132 (13)
Whole Blood (mg/L)a@$			
Diabetic	0.883+0.065 (6)	0.792+0.049 (13)	0.780+0.020 (10)*
Control	0.782+0.042 (11)	0.740+0.031 (10)	0.643+0.048 (14)*
Liver (ug/g wet wt)b@@			
Diabetic	4.927+0.393 (5)	7.550+1.186 (13)*	7.080+1.218 (12)*
Control	4.096+0.090 (11)	3.998+0.114 (11)*	3.633+0.171 (13)*
Kidney (ug/g wet wt)c@@$#			
Diabetic	5.000+0.321 (5)	13.414+2.353 (13)**	15.526+2.634 (10)**
Control	5.712+0.314 (11)	5.108+0.217 (11)**	6.004+1.448 (13)**
Duodenum (ug/g wet wt)@			
Diabetic	1.699+0.258 (5)	1.859+0.115 (12)*	1.910+0.090 (12)
Control	1.690+0.046 (11)	1.550+0.066 (11)*	1.623+0.123 (12)
Pancreas (ug/g wet wt)b@@			
Diabetic	1.103+0.061 (5)*	1.129+0.146 (16)**	1.061+0.063 (10)***
Control	0.902+0.049 (10)*	0.649+0.046 (9)**	0.639+0.053 (11)***
Spleen (ug/g wet wt)a@$$			
Diabetic	2.012+0.388 (5)	1.342+0.080 (11)	1.459+0.101 (13)
Control	1.477+0.139 (11)	1.261+0.049 (11)	1.232+0.107 (11)
Muscle (ug/g wet wt)a@#			
Diabetic	1.564+0.318 (5)	1.152+0.059 (13)	1.074+0.056 (13)
Control	1.054+0.045 (11)	1.177+0.068 (11)	1.083+0.098 (12)
Femur (ug/g wet wt)@			
Diabetic	2.654+0.0662 (5)	2.565+0.135 (13)	2.576+0.053 (11)*
Control	2.448+0.099 (11)	2.392+0.070 (10)	2.335+0.077 (12)*

*, **, ***: P<0.05, P<0.01, and P<0.0001 between diabetic and control groups from T-test. The following are from GLM: a..b, c.: model significance at P<0.05, 0.01 and 0.0001 level; $, $$, $$$: Diet effect at P<0.05, 0.01 and 0.0001 level; #, ##, ###: Significant interaction between dietary Zn level and diabetes at P<0.05, 0.01 and 0.0001 levels. @, @@, @@@: Groups effect at P<0.05, 0.01 and 0.0001 levels. (number): number of rats.

decreased whole blood Cu concentration as their dietary Zn levels increased. However, STZ-diabetic rats had higher whole blood Cu concentrations than controls at all the three dietary Zn range. The difference became significant in the ZS diet groups ($P<0.05$). Analysis of variance indicated that both diabetes and dietary Zn levels were the significant factors affecting whole blood Cu concentration. There was no significant interaction between diet and diabetes.

STZ-diabetic rats had a nearly two fold increase in liver Cu concentrations when compared with the controls when fed both the ZC and ZS diets ($P<0.05$). However, when fed the ZD diet, liver Cu concentrations of both groups were comparable. STZ-diabetic rats on the ZD diet decreased their liver Cu concentration about 40-50% when compared with STZ-diabetic groups fed either the ZC or ZS diets. But this decrease in liver Cu concentration was not seen in the control rats when they fed the ZD diet. Diabetes was the only significant factor affecting liver Cu concentration as assessed by analysis of variance. There was no significant interaction between the two variables.

Kidney Cu concentrations followed the same pattern as liver Cu concentration. STZ-diabetic rats fed on the ZC and ZS diets had a kidney Cu concentration which was more than double that of the control rats ($P<0.01$). The STZ-diabetic rats had a lower, but not significantly so, kidney Cu concentration when fed the ZD diet compared with that of their controls ($P>0.05$). Again, STZ-diabetic rats given the ZD diet showed a more than 50% decrease in their kidney Cu levels when compared to STZ-diabetic rats fed either the ZC or ZS diets. This change was not present in the control rats. Analysis of variance indicated that both diabetes and diet significantly affected kidney Cu concentration. The effect of diet was also dependent on the condition of the animals as there was a significant interaction between the two variables ($P<0.05$).

STZ-diabetic rats had 15% and 17% increase in their duodenum Cu concentration compared with their controls on the ZS and ZC diet respectively ($P<0.05$). STZ-diabetic rats also had higher (but not significantly so) duodenum Cu concentration when fed the ZD diets than that of their controls. Diabetes had significant effect on duodenum Cu

concentration as assessed by analysis of variance, there was no significant interaction between the diabetes and dietary Zn levels.

Pancreatic Cu concentrations were consistently higher in the STZ-diabetic rats than in their controls ($P<0.05$ for ZD, $P<0.01$ for NC, and $P<0.0001$ for ZS). There was little change in pancreatic Cu concentrations in STZ-diabetic rats when their dietary Zn increased or decreased. However, in control rats, there was about 30% increase in their pancreatic Cu concentration when fed ZD diet in comparison with that when they fed the NC and ZS diets. Analysis of variance indicated that diabetes was the only factor affecting pancreatic Cu concentration ($P<0.001$), the effect of dietary Zn was not significant, there was no significant interaction between the two variables.

When fed the ZC and ZS diets, STZ-diabetic rats had about 5-15% higher spleen Cu concentration than their controls. Both diabetic and control rats increased their spleen Cu concentration when fed ZD diet compared with those given either ZC or ZS diets, suggesting an antagonistic effect between Zn and Cu in this organ. The magnitude of this increase was much greater in STZ-diabetic rats than in control rats. Analysis of variance indicated that both diet and diabetes were significant factors affecting spleen Cu concentration. No significant interactions existed between the two variables.

STZ-diabetic rats had comparable muscle Cu concentrations when fed either the ZC or ZS diets as that of their controls, however, muscle Cu concentration increased about 30% in STZ-diabetic rats fed the ZD diet than they were on ZC and ZS diets, another antagonistic effect between the two metals occurred here. There was little change in muscle Cu concentrations in control rats on the ZD diet. Diabetes was a significant factor affecting muscle Cu concentration, there was also a significant interaction between diet and diabetes.

STZ-diabetic rats had a consistently higher femur Cu concentrations when compared with their controls, but the difference became significant when the ZS diet was fed ($P<0.05$). There was little variation in their femur Cu concentration over the dietary Zn range for both the STZ-diabetic rats and their controls. This was also confirmed by analysis of variance. Dietary Zn levels were not a significant factor affecting femur Cu

concentration, but diabetes significantly affected femur Cu concentration ($P<0.05$). There was no significant interaction between diet and diabetes.

4.3.5.2 Total Cu Contents of STZ-Diabetic and Control Rats

Total body Cu contents of STZ-diabetic and control rats are presented in Figure 4.13. The absolute amount of Cu was highest in STZ-diabetic rats on the normal Zn diet, which was significantly higher than that in controls ($P<0.05$). The total body Cu contents of STZ-diabetic rats and control rats were not different significantly when fed diets either deficient for or supplemented with Zn.

4.3.5.3 Whole Body Mean Cu Concentration of STZ-Diabetic and Control Rats

The whole body mean Cu concentration, as presented in Figure 4.14, showed that STZ-diabetic rats had consistently higher Cu concentrations than their controls at each levels of dietary Zn. In both STZ-diabetic and control groups, the whole body mean Cu concentrations were not affected by dietary Zn levels, and remained relatively stable.

4.3.5.4 Body Cu Distribution among Selected Organs and Tissues

Cu distribution among organs and tissues of STZ-diabetic rats and their controls at the three levels of dietary Zn is presented in Figure 4.15 and Table 4 in Appendix. The distribution of Cu in body organs and tissues is obviously different from that for Zn. Muscle is the major tissue that contains almost half of total body Cu. Liver is also another major organ that contains 20-30% of total body Cu. Followed by bone which contained about 20-25% of total body Cu. Kidney Cu constitutes about 5% to 6% of total body Cu in control rats, but this figure was more than doubled in STZ-diabetic rats. GI tract contained less than 5% of total body Cu. Cu in plasma and blood comprised 3-5% of total body Cu. Cu in pancreas and spleen contain only about 0.2% and 0.4% of total body Cu each.

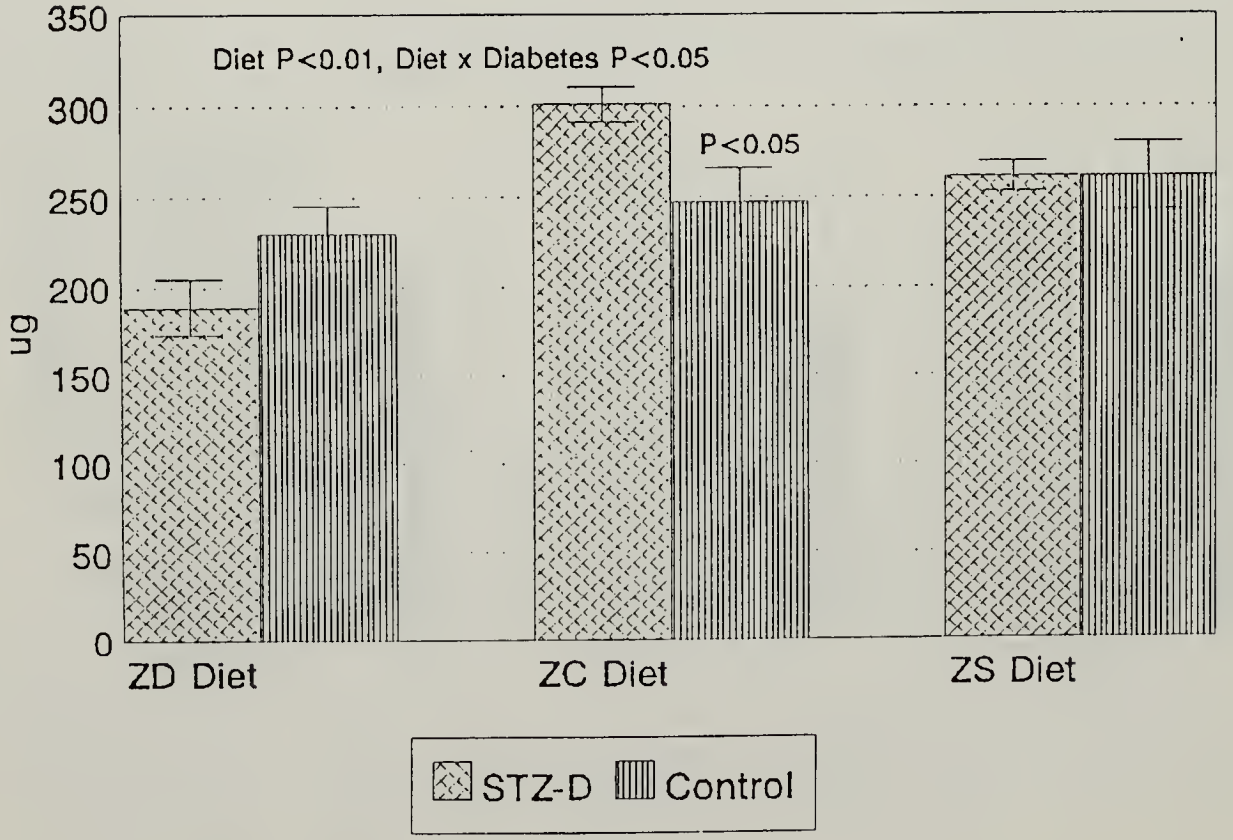

Figure 4.13 Comparison of Whole Body Cu Contents between STZ-Diabetic and Control Rats Fed Three Levels of Zn

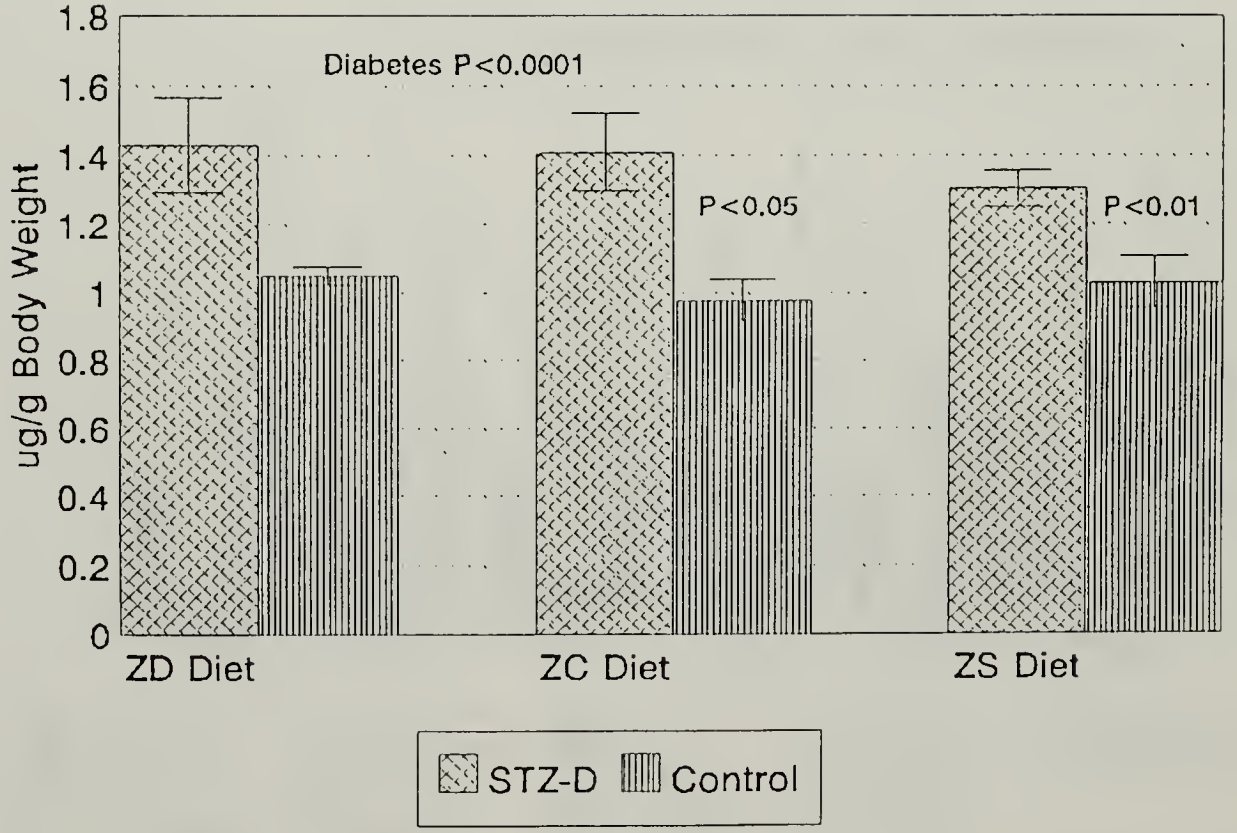

Figure 4.14 Comparison of Whole Body Mean Cu Concentrations between STZ-Diabetic and Control Rats Fed Three Levels of Zn

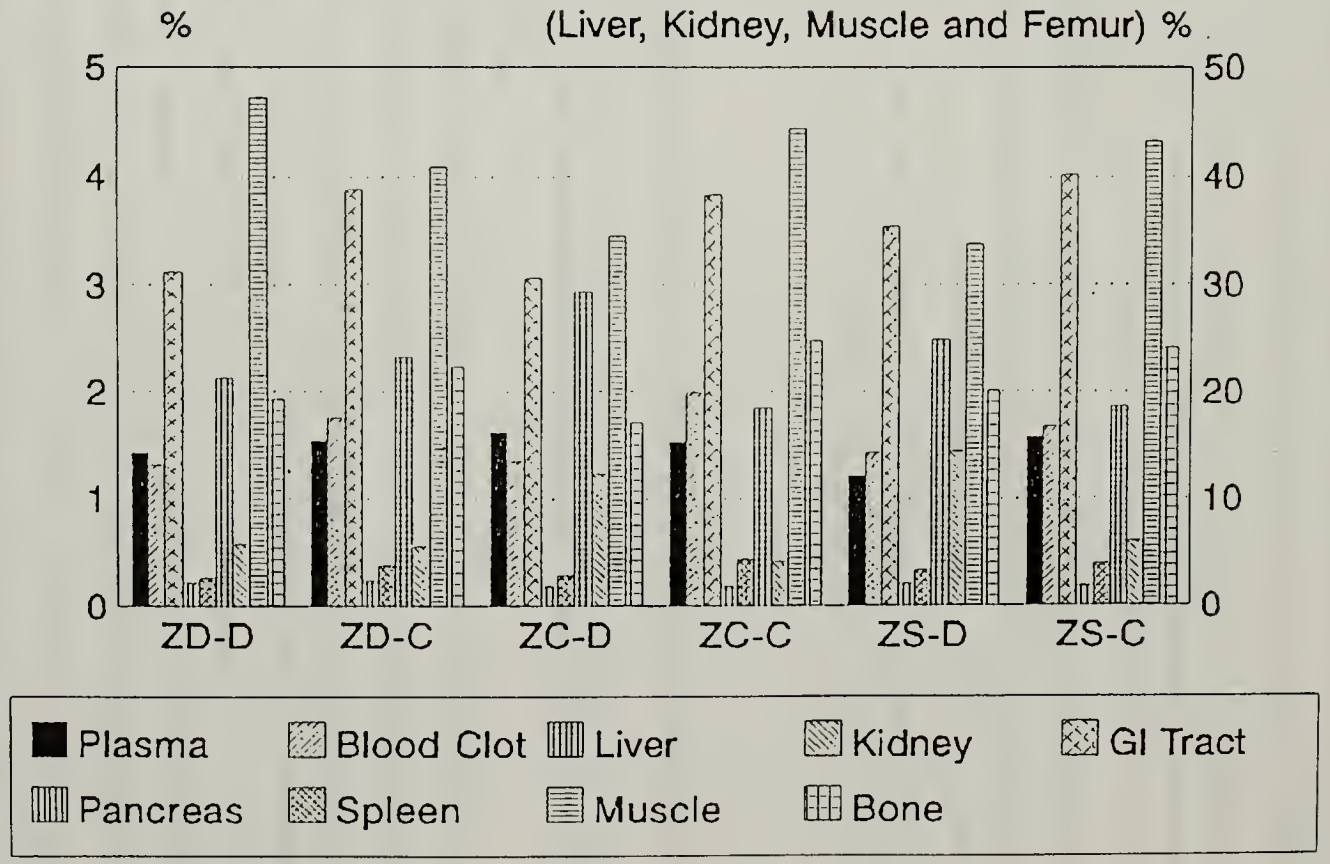

Figure 4.15 Comparison of Cu Distribution between STZ-Diabetic and Control Rats Fed Three Levels of Zn

On normal dietary Zn, Cu distribution in STZ-diabetic was also characterized by accumulation of Cu in liver and kidney in the STZ-diabetic rats compared with that of their controls. STZ-diabetic rats also had a significantly decreased Cu in spleen ($P<0.0001$). Muscle and GI Cu were significantly decreased in STZ-diabetics than in their controls ($P<0.01$). Percentage of Cu in plasma, blood, pancreas did not have significant difference between diabetic and control rats ($P>0.05$). On the ZD diet, Cu distribution among these organs were not significantly different from that of their corresponding controls. On the other hand, diabetic animals on ZS diet had a significantly increased Cu in kidney ($P<0.05$) and liver ($P<0.01$), significantly decreased Cu in muscle ($P<0.01$), GI tract ($P<0.05$) and bone and marrow ($P<0.05$) compared with their controls. There were no significant differences in Cu distribution in plasma, blood, pancreas and spleen ($P>0.05$).

4.3.6 The Effect of Diabetes and Dietary Zn Levels on Tissue and Organ Fe Concentrations

4.3.6.1 Fe Concentrations of Organs and Tissues of STZ-Diabetic and Control Rats

Table 4.5 presents mean values of organ and tissue Fe concentrations of diabetic and control animals, together with their statistical analysis. STZ-diabetic rats fed the normal Zn diet had comparable Fe concentrations in all tissues except femur, which was significantly higher when compared with their controls. Significantly increased Fe concentrations in pancreas, muscle and femur were also observed in STZ-diabetic rats fed the ZS diet compared with their controls. A significantly decreased duodenum Fe concentration was also seen in STZ-diabetic rats on ZS diet in comparison with that of their controls. When fed the ZD diet, STZ-diabetic rats had significantly increased femur and liver Fe concentrations when compared with their controls. The detailed comparisons are summarized below.

STZ-diabetic rats had a slightly but not significantly higher plasma Fe concentration when fed either ZC or ZS diet than when the ZD diet. The plasma Fe

Table 4.5 Organ and Tissue Fe Concentrations of Diabetic and Control Rats

Dietary Groups	Zn Deficient	Normal	Zn Supplement
Plasma (mg/L)a$			
Diabetic	0.946+0.198 (4)	1.727+0.149 (8)	1.649+0.146 (10)
Control	0.831+0.143 (10)	1.686+0.128 (10)	1.498+0.131 (12)
Whole Blood (mg/L)			
Diabetic	464.313+17.454 (5)	435.221+17.554 (13)	432.833+8.380 (10)
Control	434.574+14.166 (11)	416.983+7.356 (10)	415.662+7.548 (14)
Liver (ug/g wet wt)b$$@@			
Diabetic	137.667+15.801 (5)*	96.830+10.837 (13)	83.997+5.180 (12)
Control	92.929+6.727 (11)*	80.597+4.785 (11)	70.897+4.959 (12)
Kidney (ug/g wet wt)$			
Diabetic	71.020+7.046 (5)	63.025+4.917 (13)	58.548+2.692 (11)
Control	68.788+5.037 (11)	65.324+3.185 (11)	57.419+3.796 (12)
Duodenum (ug/g wet wt)a@@			
Diabetic	20.224+2.416 (5)	25.271+3.104 (13)	20.137+1.216 (12)**
Control	24.997+2.846 (11)	31.200+1.872 (11)	30.904+2.828 (12)**
Pancreas (ug/g wet wt)a@@			
Diabetic	22.360+1.973 (6)	30.925+6.353 (11)	25.881+1.928 (9)***
Control	20.923+3.249 (7)	21.300+1.534 (4)	15.562+1.656 (7)***
Spleen (ug/g wet wt)c$$$			
Diabetic	304.578+35.844 (5)	206.710+11.404 (13)	198.493+9.038 (14)
Control	279.330+27.327 (11)	189.489+6.984 (11)	190.626+10.111 (11)
Muscle (ug/g wet wt)c@@@$$$			
Diabetic	22.952+5.660 (5)	15.253+0.858 (13)	16.592+0.689(12)***
Control	13.020+1.106 (11)	15.421+0.588 (11)	12.685+0.702(12)***
Femur (ug/g wet wt)c@@$$##			
Diabetic	107.241+16.513 (5)*	55.294+4.368 (13)*	52.563+4.1677 (13)*
Control	54.089+8.847 (12)*	41.688+1.738 (13)*	40.221+1.764 (12)*

*, **, ***: P<0.05, P<0.01, and P<0.0001 between diabetic and control groups from T-test. The following are from GLM: a.,b. c.: model significance at P<0.05, 0.01 and 0.0001 level: $, $$, $$$: Diet effect at P<0.05, 0.01 and 0.0001 level; #, ##, ###: Significant interaction between dietary Zn level and diabetes at P<0.05, 0.01 and 0.0001 levels. @, @@, @@@: Groups effect at P<0.05, 0.01 and 0.0001 levels. (number): number of rats.

concentrations of STZ-diabetic rats were also higher than that of their controls when given either the ZC or ZS diets. Feeding the ZD diet caused their plasma Fe concentration to decrease nearly 45% to 50% respectively in both diabetic and control rats. Analysis of variance indicated that diet, but not diabetes significantly affected plasma Fe concentration. There was no significant interaction between diet and diabetes on plasma Fe levels.

Whole blood contained the highest amount of Fe amongst the organs and tissues analyzed. STZ-diabetic rats had a slightly higher (about 4-5% more) whole blood Fe than their controls at all the three dietary Zn levels, the differences were not significant ($P>0.05$). Both STZ-diabetic rats and their controls fed the ZD diet had a slight increase in whole blood Fe than when fed either ZC or ZS diets. Analysis of variance indicated that neither diet, nor diabetes had significant effect on whole blood Fe concentration.

Liver Fe concentrations of STZ-diabetic rats increased as their dietary Zn levels decreased. An increase in liver Fe concentration in control rats on the ZD diet was also observed, but the magnitude was much smaller than that of the STZ-diabetic rats. STZ-diabetic rats had a 30% more liver Fe concentration compared to controls fed the ZD diet ($P<0.05$), and 20% more liver Fe than when given the ZC and ZS diets, but the differences were not significant. However, analysis of variance indicated that both diabetes and diet had very significant effect on liver Fe concentration ($P<0.01$). There was no significant interaction between the two variables.

Kidney Fe concentration of both STZ-diabetic and control rats increased slightly with decreasing dietary Zn. However, there were no significant differences between the two groups at any of the three dietary Zn levels. Analysis of variance indicates that dietary Zn level significantly affects kidney Fe levels.

Duodenum Fe concentration was slightly lower in STZ-diabetic rats compared with control rats fed the ZC diet. The difference was about 25%, however, this difference was not significant ($P>0.05$). Both ZD and ZS diets caused about 25% decrease in duodenum Fe concentration in STZ-diabetic rats. However, feeding ZS did not affect duodenum Fe concentration in control rats as much as it did in the STZ-diabetic rats. Thus

the difference between the two groups fed the ZS was significant ($P<0.05$). While both groups decreased their duodenum Fe concentrations when fed the ZD diet, there was no significant difference between the two groups. As assessed by analysis of variance, only diabetes, but not diet, had significant effect on duodenum Fe concentration.

The highest pancreatic Fe concentration observed in STZ-diabetic rats was when they were on ZC diet; STZ-diabetic rats on both the ZD and ZS diets had decreased pancreatic Fe concentrations compared with those on the ZC diet. The highest pancreatic Fe concentration in control rats was observed when they were fed the ZC diet. Pancreatic Fe concentrations in STZ-diabetic rats were higher than those of control rats at all the three dietary Zn levels, and the difference became significant when they were on the ZS diet ($P<0.0001$). Analysis of variance indicated that only diabetes had significant effect on pancreatic Fe concentration.

STZ-diabetic rats had a slightly higher spleen Fe concentration at all three dietary Zn levels when compared with their corresponding controls, but the differences were not significant. Both STZ-diabetic rats and control rats increased their spleen Fe concentrations when they were fed the ZD diet. Analysis of variance indicated that dietary Zn levels had a significant effect on spleen Fe concentration, while diabetes did not affect spleen Fe, nor was there any significant interaction between the two variables.

Muscle Fe concentration was not different between the two groups on the ZC diet. However, feeding both the ZD and ZS caused the muscle Fe concentration of STZ-diabetic rats to increase, while that of control rats to decrease. The highest spleen Fe concentration was in STZ-diabetic rats fed the ZD diet, but it was not significantly different from that of control rats. STZ-diabetic rats had a very significantly higher spleen Fe concentration than that of their controls when fed the ZS diet ($P<0.0001$). Analysis of variance indicates that both dietary Zn levels and diabetes had significant effect on muscle Fe concentrations.

STZ-diabetic rats had consistently significantly higher femur Fe concentration than their corresponding controls at all the dietary Zn levels ($P<0.05$). Decreasing dietary Zn levels caused femur Fe concentrations of both groups to increase. Analysis of variance

indicated that both diet and diabetes had significant effect on femur Fe concentrations, there was no significant interactions between the two variables.

4.3.6.2 Total Body Fe Contents of STZ-Diabetic and Control Rats

The total body Fe contents of STZ-diabetic and control rats at each dietary Zn level are presented in Figure 4.16. STZ-diabetes decreased total body contents in STZ-diabetic rats when compared with control rats, but the differences were not significant ($P>0.05$). GLM analysis indicates that STZ-diabetes was not a significant factor affecting total body Fe contents, but the effect of dietary Zn levels was very significant ($P<0.01$).

4.3.6.3 Whole Body Mean Fe Concentration of STZ-Diabetic and Control Rats

Figure 4.17 illustrates whole body mean Fe concentration of STZ-diabetic rats in comparison with that of their controls. Similar to Zn and Cu, whole body mean Fe concentrations of STZ-diabetic rats on the diet marginal for Zn was the highest among the groups. The whole body mean Fe concentration in STZ-diabetic rats was significantly higher than that of their controls when both were on the diet marginal for Zn. The significant difference in whole body mean Fe concentration was presented in both STZ-diabetic and control rats fed the diet supplemented with Zn ($P<0.01$). When they were on diet containing normal amount of Zn, their whole body mean Fe concentration was not different significantly.

4.3.6.4 The Effect of Diabetes and Dietary Zn Levels on Body Fe Distribution among Selected Organs and Tissues

Fe distribution among organs and tissues of diabetic and control rats on the three dietary Zn levels is summarized in Figure 4.18 and Table 6 in Appendix. Different from that of Zn and Cu distribution, blood is the major tissue that contains about more than 40% of the total body Fe. Followed by bone and marrow (20%), muscle (16-18%), liver

(11-13%), GI tract (5-8%), kidney (2-5%), spleen (2%), pancreas (0.2%). Plasma contains negligible amount of Fe (0.05%).

On the normal dietary Zn intake, STZ-diabetic rats had increased Fe accumulation in kidney ($P<0.01$), and a significantly decreased Fe concentrations in muscle ($P<0.05$) and GI tract ($P<0.01$) when compared with that of the control rats. Fe distribution in other remaining organs and tissues was not significantly different between the diabetic and control groups.

When the ZD diet was fed, significant change in Fe distribution occurred in blood. In the diabetics there was a 30% decrease in total blood Fe distribution (compared with a 5% decrease in control rats). STZ-diabetic rats also had significantly decreased Fe in GI tract ($P<0.05$) and spleen ($P<0.01$). There were no other significant differences among other organs and tissues between the STZ-diabetics and the controls. STZ-diabetic rats fed the ZD diet had a 50% increase in percentage of Fe in bone and marrow ($P<0.01$) compared to those fed the ZC and ZD diets. This increase did not occur in controls. The percentage of muscle Fe decreased by a third in control rats, but not in the diabetics.

Zn supplementation caused increased Fe accumulation in kidney ($P<0.01$), liver ($P<0.05$), and pancreas ($P<0.05$) in STZ-diabetic rats compared with their controls. The differences of Fe distribution in other organs and tissues was not significant between the STZ-diabetic and control groups.

Because levels of dietary Zn above requirement had no effect on Fe distribution, it is reasonable to conclude that higher levels of dietary Zn had no effect on Fe metabolism. When STZ-diabetic rats were fed the ZD diet, the percentage of blood Fe decreased significantly ($P<0.05$), suggesting a possible risk of Fe depletion.

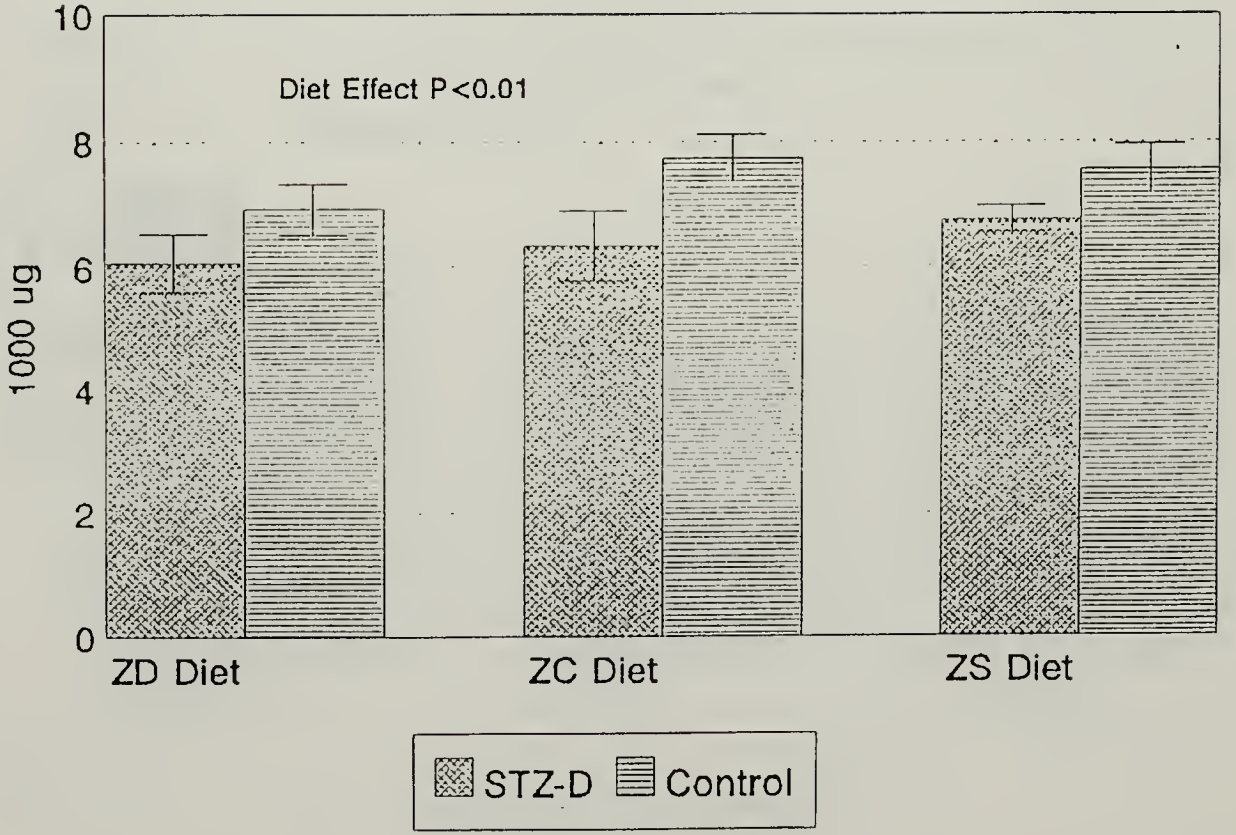

Figure 4.16 Comparison of Whole Body Iron Contents between STZ-Diabetic and Control Rats Fed Three Levels of Zn

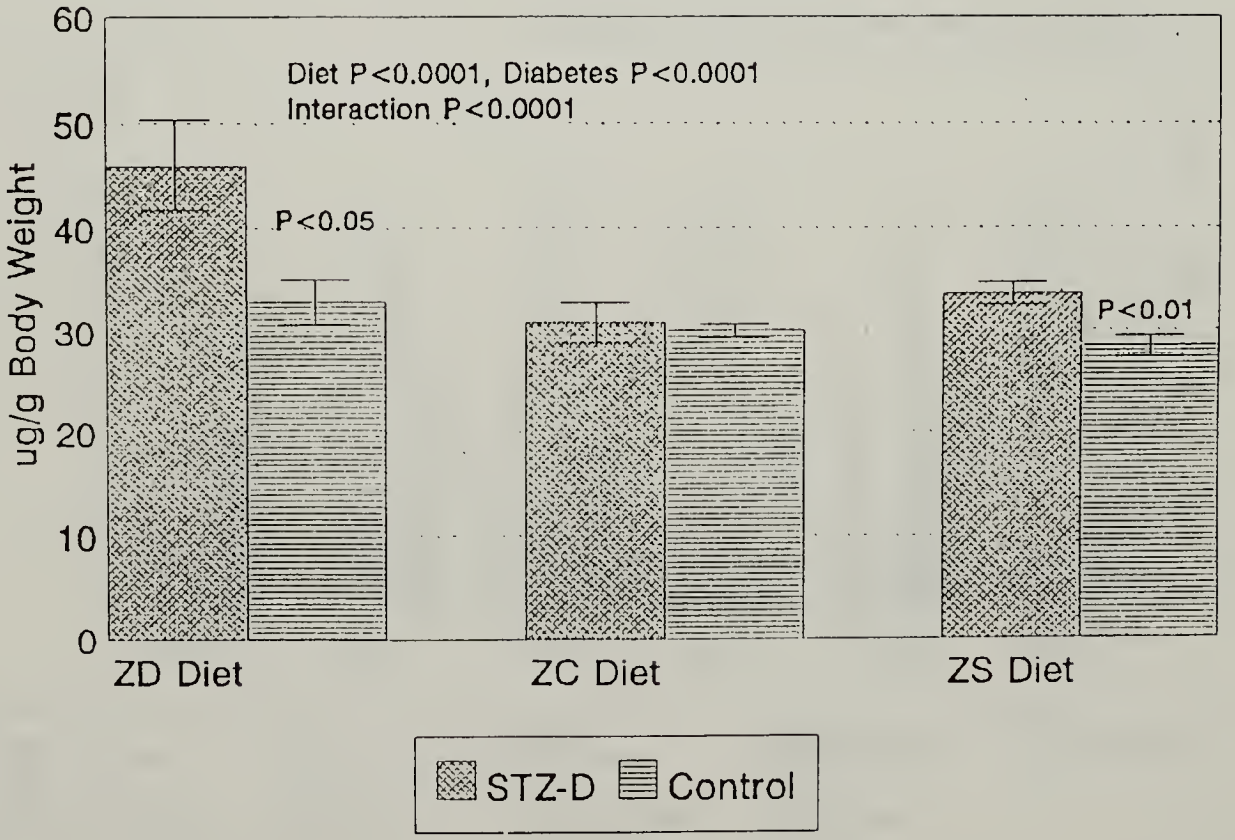

Figure 4.17 Comparison of Whole Body Mean Iron Concentrations between STZ-Diabetic and Control Rats Fed Three Levels of Zn

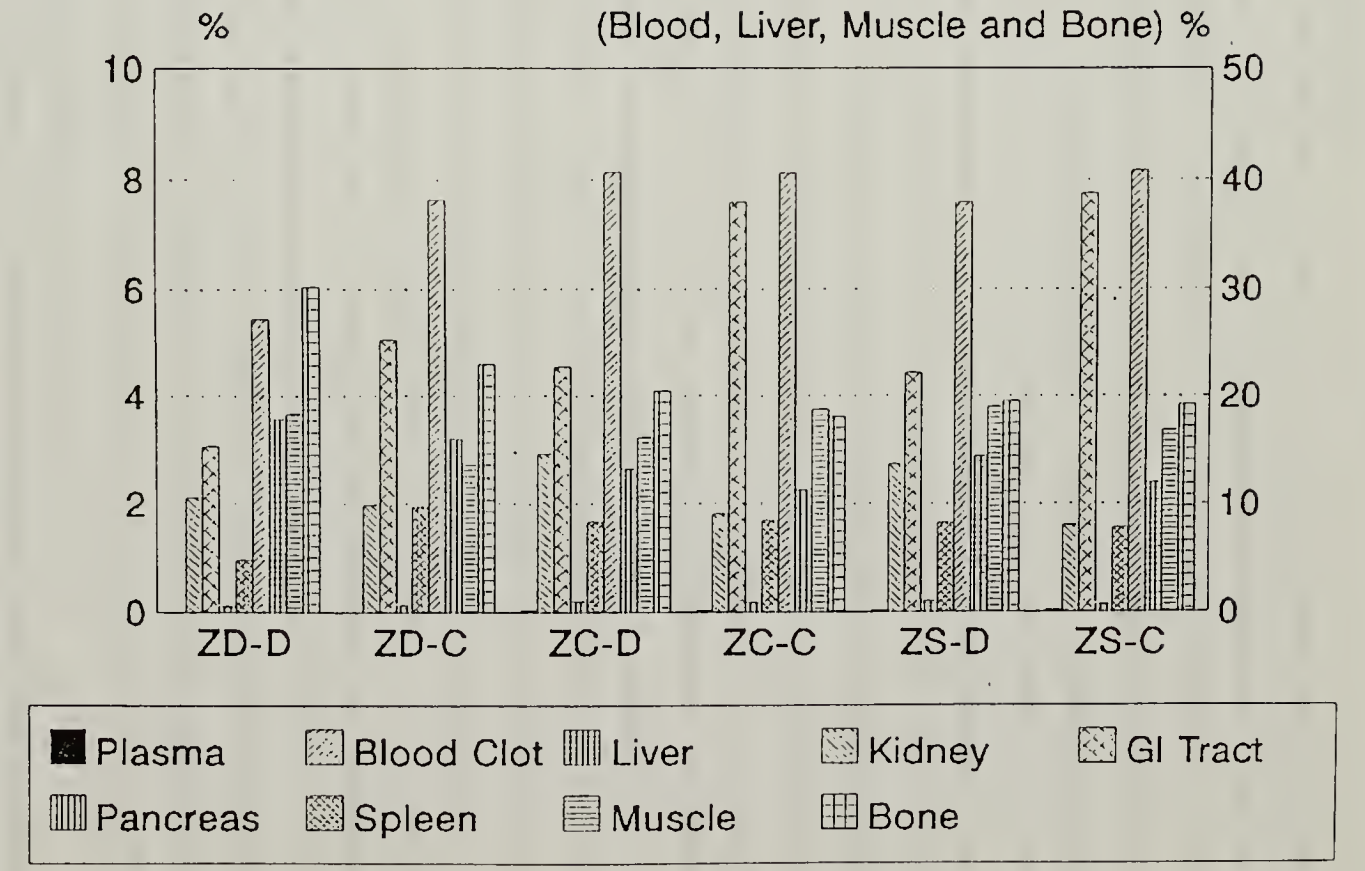

Figure 4.18 Comparison of Iron Distribution between STZ-Diabetic and Control Rats Fed Three Levels of Zn

4.4 Discussion

4.4.1 Increased Whole Body Mean Zn Concentration Excludes Zn Deficiency in STZ-Diabetic Rats

The finding that STZ-diabetic rats contained significantly higher Zn concentration per gram of body weight (Figure 4.11) excludes any possibility that Zn deficiency existed in STZ-diabetic rats. This finding is consistent in that all STZ-diabetic rats contained more Zn per gram body weight independent of their dietary Zn levels. A possible Zn toxicity in diabetes occurring is thus hypothesized for the first time. The increased whole body Zn concentration may be the ultimate result of increased Zn absorption, body catabolism, tissue breakdown and slower growth in body weight occurred in diabetes. The other findings that increased ^{65}Zn turnover rates and increased fecal excretion of ^{65}Zn, and higher Zn concentrations in all the tissues and organs analyzed when fed diets with normal and increased Zn contents all are evidence that there is more Zn in the diabetic body.

The finding that there is an increased Zn concentration in the STZ-diabetic rats leads naturally to reevaluation of many studies on Zn metabolism in both diabetic humans and animals. Previous studies in this laboratory (Fu 1991, Cunningham et al. 1994) showed that human IDDMs did not have decreased Zn levels in plasma, leukocytes, erythrocyte; and that Zn supplementation in a dose of 50 mg per day for 30 days to IDDMs did not show any beneficial effect as compared with their controls. On the other hand, Zn supplementation did aggravate their glycemic control as their glycosylated hemoglobin became worse as the study continued. There are other studies that showed that either an increased plasma Zn levels in diabetic subject (Heise et a. 1988; Canfield et al. 1984); or there is no significant difference in plasma Zn levels between IDDMs and controls (Kiilerich and Christiansen 1985; Hagglof et al. 1983; Chooi et al. 1976; Pidduck et al. 1971, Prout et al. 1969 and Rosner and Gorfien 1968). There is no evidence of zinc deficiency in diabetes as indicated in all these studies.

The decreased ^{65}Zn retention and absorption in human diabetic subjects as reported by Kiilerich et al. (1990) should be also the evidence that there was no Zn deficiency existed in these subjects.

4.4.2 Altered Zn Distributions and Concentrations at the Tissue Level

There was a peculiar change in body compartmental Zn distribution in STZ-diabetic rats, which reflect the natural result of this increased whole body mean zinc concentration (Figure 4.12). This altered tissue distribution was characterized by sequestration of more body Zn by kidney and liver when their dietary zinc levels were both adequate or increased. During zinc deficiency, muscle catabolism released large amount of Zn, thus Zn distributed into other tissues such as GI tract, spleen, pancreas, and femur decreased, but sequestration of Zn by their livers and kidneys still existed. The percentages of body Zn distributed to plasma were decreased in STZ-diabetic rats at all levels of dietary Zn (Figure 4.12 and Table 2. in Appendix). A decreased circulating Zn relative to other body compartments may be hypothesized.

When STZ-diabetic rats fed the diets either adequate for Zn or supplemented with Zn, their tissue or organ Zn levels were all elevated when compared with controls. On the other hand, despite the increased mean Zn concentration, STZ-diabetic rats when fed the diet marginal for Zn still had decreased Zn concentrations in some organs and tissues, such as plasma, femur, pancreas, GI tract and spleen (Table 4.3). These results may suggest that diabetic animals may have a peculiar change in Zn metabolism during Zn deficiency.

Plasma Zn concentration did not reflect dietary Zn intake in both STZ-diabetic and control rats. The plasma Zn concentrations were elevated in STZ-diabetic rats on normal Zn diet, but was decreased when STZ-diabetic animals fed diets either marginal for Zn or supplemented with Zn. Both Zn depletion and Zn supplementation did not significantly alter plasma Zn concentrations in the control rats, indicating plasma Zn is fairly tightly controlled in healthy rats (Table 4.4). However, this homeostatic regulation of plasma Zn seemed to be impaired in the STZ-diabetic rats as a decreased plasma Zn in STZ-diabetic rats when they fed either Zn deficient diet or Zn-supplemented diet.

Whole blood Zn concentration in STZ-diabetic rats fed all three levels of dietary Zn were increased but not significantly. Whole Blood Zn concentrations seemed to be not influenced by dietary Zn intake, neither by diabetic condition (Table 4.3). This lack of change in whole blood Zn levels in response to changes in dietary Zn intake may be due to

the relative short period of time covered in the present study. It is known that rat erythrocytes have a turnover rate of 30-40 days (Thompson 1991) and is not easily depleted within up to 9 weeks in human study (Baer and King 1984). The percentage of Zn in whole blood was also not different significantly between STZ-diabetic and control rats, indicating whole blood Zn levels were not affected by both diabetes and dietary Zn levels.

The present study found that the major change in Zn metabolism in diabetic animals was the increased liver and kidney Zn concentrations (Table 4.3). Since whole body Zn mean concentration was elevated in STZ-diabetic rats, the function of this Zn sequestration by liver and kidney may be beneficial in two aspects: one is to make free Zn in circulation less toxic to the body, another is to excrete quickly through kidney. This alteration in Zn metabolism in diabetes has been compared to stress stimulation (Hallmans and Lithner 1980). The sequestration of Zn by the liver and kidney was explained to be simply an adaptative response.

The increased mean Zn levels and increased kidney concentration in STZ-diabetic rats may lead to the conclusion that hyperzincuria may function to excrete more unwanted Zn from the Zn-over-saturated diabetic body. The statement that hyperzincuria in diabetes depletes body store of Zn seemed to be unwarranted.

Zn is absorbed through entire small intestine and duodenum is the major site of Zn absorption (Cousins 1985) and regulating site of Zn metabolism (Wastney et al. 1986). Analysis of duodenum Zn concentration reflects both Zn absorption and endogenous secretion. Intestinal Zn concentration was significantly higher in STZ-diabetic rats on both normal and Zn-supplemented diets, and decreased in STZ-diabetic rats on Zn-depleted diet. Changes in Zn concentrations in duodenum in both STZ-diabetic and control rats in response to dietary Zn intakes may indicate that both absorption and endogenous secretion of Zn are normally functioning in regulating body Zn metabolism.

Pancreas Zn concentrations in STZ-diabetic rats did not differ significantly between STZ-diabetic and control rats (Table 4.3). The possibility that insulin metabolism and function due to Zn deficiency did not exist in the present study.

Muscle Zn concentration in STZ-diabetic rats was elevated significantly in animals fed the diet marginal for Zn (Table 4.3). Since control rats did not have similar increase in muscle Zn concentration, it seems the elevation of muscle Zn is the unique response in STZ-diabetic rats in facing Zn deficiency. It is possibly an indication of tissue catabolism. Muscle Zn concentration did not reflect body store of Zn and Zn intake as observed in this study (Table 4.4 and Figure 4.8). This point of view is also shared by Cunnane (1988b), Giugliano and Millward (1984), who found that dietary Zn did not have any effect on muscle Zn level. Studies on humans also support that the mass of Zn in muscle is tightly regulated (Wastney et al. 1986; Brown et al. 1985).

A greater change in femur Zn concentrations was observed in STZ-diabetic rats. Higher femur concentrations of Zn occurred when fed the diets contained normal or increased amount of Zn, while it decreased when fed the diet marginal for Zn (Table 4.3 and Figure 4.9). However, femur Zn concentration in control rats did not change as much as it did in STZ-diabetic rats. The result from the present study seems supports the view of Levine et al. (1983) that bone Zn is a useful measure of Zn status in diabetes. however, it is not a particularly accessible tissue for in vivo study.

The results of present study agree generally with other studies in which tissue Zn concentration has been expressed as per gram of dry weight (Failla and Kiser 1981; Donaldson et al. 1987). This may exclude the influence of a possible dehydration of diabetic tissues on the tissue Zn analysis in the STZ-diabetic rats in the present study.

4.4.3 Alteration of Cu Metabolism of STZ-Diabetic Rats

Similar to Zn, total body Cu content was smaller in STZ-diabetic rats (Figure 4.13), but their whole body Cu concentration was elevated too (Figure 4.14). Alteration of tissue Cu concentrations was also characterized by increased concentrations of Cu in diabetic livers and kidneys when animals fed diets containing normal or increased levels of Zn. Zn supplementation in STZ-diabetic rats further aggravated the higher kidney Cu concentration (Table 4.5); On the other hand, when fed the diet marginal for Zn, the

accumulation of Cu in the diabetic kidney and liver was totally reversed. The similar but much lesser changes in Cu metabolism in STZ-diabetic suggests the similar influence of diabetes on both metals. The observation that feeding the diet marginal for Zn moved tissue Cu concentration and body Cu distribution toward that of control rats indicates an antagonizing effect between the two metals and Zn supplementation does affect Cu metabolism in STZ-diabetic rats.

4.4.4 Changes in Fe Metabolism of STZ-Diabetic Rats

The present study revealed that, similar to Zn and Cu, Fe metabolism in STZ-diabetic rats was also altered but not significantly as noted previously for Zn and Cu. The findings that the whole body mean Fe concentrations were also increased significantly in STZ-diabetic rats at all three dietary zinc levels suggest an alteration in Fe metabolism, which was similar with that of Zn and Cu (Figure 4.17). An antagonizing effect also exists between Zn and Fe as STZ-diabetic rats fed the diets either marginal for Zn or doubling the normal amount of dietary Zn caused whole body mean Fe concentration to increase significantly.

There was no significant effect of diabetes on Fe status when dietary Zn intake was normal. The most significant change was the increase in femur Fe concentration in STZ-diabetic rats persistently and significantly at all three dietary Zn levels.

There were no significant alteration in liver and kidney Fe concentrations in STZ-diabetic rats at all three dietary Zn levels, except liver Fe concentration was significantly increased in STZ-diabetic rats fed Zn deficient diet (Table 4.5).

Zn supplementation affected muscle Fe metabolism as seen that muscle Fe concentration continued to decrease in both STZ-diabetic rats and controls.

Zn supplementation may have an antagonistic effect on Fe metabolism in normal rats as seen in their decreased pancreatic, muscle and femur Fe. However, the effect of Zn supplementation on tissue levels of Fe in STZ-diabetic rats seems to be less obvious compared with those for Cu. On the other hand, Zn depletion selectively increase Fe concentration in femur, muscle, spleen and liver in both STZ-diabetic and control rats. The

significance of these effects is not clear and may be tied to the mutual interactions known for Zn, Cu and Fe (Davis 1980; Flanagan et al. 1980; 1983; Hill 1988).

In summary, Diabetes leads to alterations in Zn, Cu and Fe metabolism. A significant increase in whole body mean Zn, Cu and Fe concentrations was observed. Hyperzincuria in diabetes is not a depleting culprit, but an active and protective mechanism to eliminate extra unwanted Zn resulting from tissue catabolism. In diabetes mellitus, most tissues undergo catabolism, liver and kidney may function in both sequestration of circulating free Zn to decrease the harmful effect of free Zn or excrete extra body Zn via urine respectively. However, since STZ-diabetic rats when fed the diet marginal for Zn, which severely retarded their body growth, still had an increased whole body mean Zn concentration, this may suggest a disorder in utilization of Zn, Cu and Fe in diabetes.

CHAPTER 5
KINETIC STUDY ON ZINC METABOLISM IN INSULIN-DEPENDENT DIABETES MELLITUS

5.1 Introduction

This study was to measure the kinetic changes in ^{65}Zn distribution in STZ-diabetic and control rats at both the whole body and at the tissue levels for 12 days post ^{65}Zn loading. It is assumed that, after ^{65}Zn loading, its distribution among different tissues would have a characteristic distribution profile. The dynamic change and kinetics of this distribution can be followed and monitored. The differences in the distribution of ^{65}Zn within each tissue at each time point can be compared between the STZ-diabetic and control rats. The persistent changes or differences between the two groups should be due to the effect of diabetes mellitus and may reflect the changes of Zn metabolism in diabetes mellitus. The study would also provide more detailed data on changes, with time, in Zn metabolism in diabetes mellitus.

5.2 Methods and Study Design

5.2.1 Animals

Animals, induction of diabetes, animal diets and maintenance of animals were the same as described in Chapter 4.2.1. and 4.2.2. Animals on the ZC and ZS diets had a two week period of adaptation before injection of STZ; while a six week of adaptation were used for animals on ZD diet due to their slower growth in body weight.

5.2.2 Procedure of ^{65}Zn Loading, Tissue Sampling and ^{65}Zn Counting

^{65}Zn solution was prepared by dilution with sterilized saline solution to a final concentration of 20 μCi ^{65}Zn containing ~3.0 μg Zn per ml. Two hundred microliter isotope solutions were injected intraperitoneally into diabetic and control rats. Immediately after introduction of the ^{65}Zn solution , the animals were put into the counting chamber under the NaI-detector for whole body total radioactivity detection. Animals were then

put back into the metabolic cages for various periods of time. Then each rat was counted for radioactivity for 10 to 15 minutes daily thereafter. Animals were allowed to eat and drink freely throughout the entire study. At each period, urine and fecal samples were collected for later radioactivity counting.

Animals were sacrificed on the scheduled day after the last whole body counting under anesthesia by injection 0.5 ml of 0.3% sodium pentobarbital solution. The following tissues were taken for both ^{65}Zn counting: liver, kidney, pancreas, spleen, a section of duodenum, leg muscle (muscle vastus lateris, flexor digitorum profundus, and muscles covering the femur), and femur, together with urine and feces. These tissues were weighed immediately and then put in the counting vessels to count the radioactivity by a scintillation counter (Gamma 4000 Counting System, Gamma Counting Spectrometer, Beckman Instrument Inc.) for 10 minutes. One ml of whole blood sample was used for whole blood radioactivity counting. Plasma was separated by centrifugation at 1380 g for 15 minutes, and 1 ml was used for counting.

Data were expressed as percentage of count per tissue out of original dosing introduced. Total count for each tissue or organ was calculated based on the organ weight multiplied by counts/g of that tissue. For liver, kidney, spleen, and testes, the actual organ weight was used; and for muscle, bone, pancreas and gastrointestinal tract, organ weight was calculated according to Owen, (1964). The specific radioactivity of ^{65}Zn in tissues was calculated and expressed as CPM ^{65}Zn/g tissue divided by total μg Zn/g tissue. Tissue Zn concentration was analyzed according to the procedures described in section 3.2.4.

5.2.3 Whole Body ^{65}Zn Turnover Rate

The rate of ^{65}Zn turnover (% dose/day) was measured by using the formula: $0.693/\kappa$, κ = ln (reading of point one/reading of point 2)/length of time between the two points of time.

5.2.4 Statistical Treatment of Data

The total body counts were not the original dose introduced at 0 time, but the first day's count. This was due to the instability of the first day's count. The highest whole body count for most animals was attained 24 hours later. The data were expressed as the percentages of each organ count out of total body count. Mean values were based on values of 1 to 4 animals at each time point as indicated in Table 5.1. For calculating the total organ or tissue counts, either the actual weight of the organ was used (liver, kidney, spleen) or calculated based on the body weight (See Chapter 4.2.4). The percentage of each organ or tissue was plotted against each time point. Except for whole body count, no attempt was made to analyze the difference among the percentages of each organ or tissue at each time point. Radioactive decay were adjusted based on counts of the standard counts. Background counts were recorded and subtracted from the total counts each day.

The differences in percentage of ^{65}Zn retained for whole body and turnover rates between STZ-diabetic rats and control rats was analyzed by a T-test procedure by using the SAS Statistical Software (SAS Institute, Cary, NC).

5.3 Results

5.3.1 Kinetics of Whole Body ^{65}Zn in STZ-Diabetic Rats Compared to Controls

5.3.1.1 Whole Body Counting of ^{65}Zn in STZ-Diabetic Rats

Figure 5.1 presents the kinetics of whole body ^{65}Zn counting. Data are plotted as the percentage of the first day's count. Theoretically, the highest count should be the count immediately after ^{65}Zn loading, however, due to the slow distribution and exchange of ^{65}Zn within the animal body compartments and consequently the counting efficiency was gradually increased up to the highest at the first day's counting for most animals. Thus, each animal was calculated against their first day's counting value. The data of counting values at zero day and four hours after ^{65}Zn loading are also available for most animals, and are included in the Figures. The same applies for all the calculations and Figures which follow.

All three groups of STZ-diabetic rats retained less ^{65}Zn than their three corresponding control groups (Figure 5.1). This became more distinct from day 6 on till the end of the study. Dietary Zn levels affected ^{65}Zn retention in control rats, as can be seen that the highest ^{65}Zn retention was in the control rats on the ZS diet, followed by control rats on NC diet, and the lowest was in control rats on ZD diet. While in the STZ-diabetic rats, the effect of dietary Zn levels was not clear-cut. The lowest retention was in the STZ-diabetic rats on ZD diet. The highest was in the STZ-diabetic group on NC diet. At the end of the 12 day's study, control rats on the ZD, NC, and ZS diets retained 62.4%, 65.4% and 67% of their ^{65}Zn respectively based on the first day's count, while for STZ-diabetic rats, the retained ^{65}Zn was 46.5%, 52.3% and 49.8% of their first day's value respectively. The retention rates were significantly lower in STZ-diabetic rats than that of their corresponding controls from day 6 ($P<0.05$).

5.3.1.2 Whole Body ^{65}Zn Turnover Rates in STZ-Diabetic Rats

As seen from Figure 5.2 diabetic rats had a significant increase in their turnover rate of ^{65}Zn. The normal rats had a turnover rate of 412 hour (17.2 days), while this

turnover rate decreased to 262 hours for STZ-diabetics. Both Zn depletion and Zn supplementation decreased the turnover rates slightly in control rats. However, STZ-diabetic rats on the normal Zn diet had the fastest turnover rate than when they were either on Zn depleted diet or Zn supplemented diet. The difference among the turnover rates within either STZ-diabetic groups or control groups is much smaller than that between the STZ-diabetic groups and control groups. It may thus be concluded that diabetes mellitus has its effect on ^{65}Zn turnover rate. On the other hand, dietary Zn levels seemed to have little effect on ^{65}Zn turnover.

5.3.1.3 The Effect of Diabetes on ^{65}Zn Elimination

Figure 5.3 and Figure 5.4 present cumulated fecal and urine ^{65}Zn eliminated from the body. The percentage of ^{65}Zn excreted in urine was significantly higher for all three STZ-diabetic groups than for their corresponding control groups. Dietary Zn levels was not related to the percentage of ^{65}Zn excreted in urine of the three STZ-diabetic groups, nor in the three control groups. The highest percentage of urine ^{65}Zn excretion in the STZ-diabetic groups may indicate a higher catabolic status.

On the ZS diet, the percentage of fecal excretion of ^{65}Zn was nearly similar in STZ-diabetic and its control group throughout the study (Figure 5.4). However, when they were on the ZC diet, significantly higher fecal ^{65}Zn excretion was noted from the STZ-diabetic rats than from their control group ($P<0.05$). The percentage of fecal ^{65}Zn excretion was greater from both STZ-diabetic rats and control rats on the ZS diet than they fed the ZC diet within the 7 days of the study. However, the percentage of fecal ^{65}Zn in STZ-diabetic rats fed the NC diet became significantly higher than both STZ-diabetic rats and their controls fed the ZS diet. At the end of the study, both STZ-diabetic group and control group on the Zn supplemented diet excreted about 22% of ^{65}Zn from feces. The STZ-diabetic rats on the normal Zn diet excreted the highest fecal ^{65}Zn (24%) among the four groups, while the control rats on the NC diet excreted the lowest fecal ^{65}Zn (16%). It is apparent that the STZ-diabetic rats increased not only their urinary Zn

excretion, but also endogenous Zn secretion. There was no compensation of hyperzincuria through decreasing their fecal Zn excretion.

For control rats, the major route of Zn excretion was via feces (95%) with urinary excretion of ^{65}Zn accounting for only 4-5% of total ^{65}Zn excreted from the body. However, urine ^{65}Zn excretion increased to more than 30% of total body ^{65}Zn loss for the STZ-diabetic rats with 70% of ^{65}Zn eliminated from feces. These data indicate that the urinary Zn excretion increased substantially for STZ-diabetic rats, but major route of Zn excretion is still through the feces for both normal and diabetic rats (Table 5.1).

Table 5.1 The Ratio of Urine to Fecal ^{65}Zn Elimination

	STZ-Diabetics	Control
ZD Diet	-----	-----
NC Diet	35.342+3.117 (50)***	3.976+0.458 (43)***
ZS Diet	31.243+2.492 (47)***	5.156+1.941 (45)***

*** Significant difference between STZ-diabetic and control rats at $P<0.0001$.

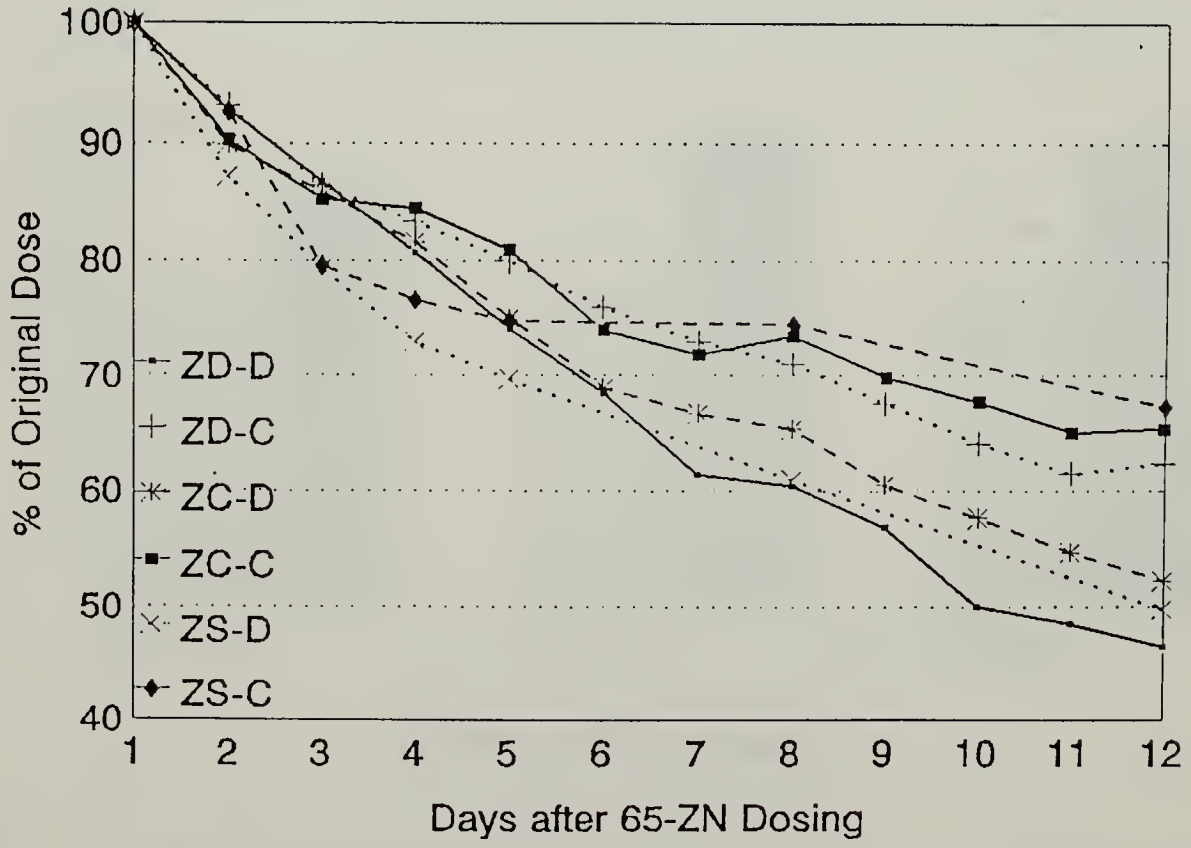

Figure 5.1 Whole Body 65-ZN Retention Curves of STZ-Diabetic and Control Rats Fed Three Levels of Zn

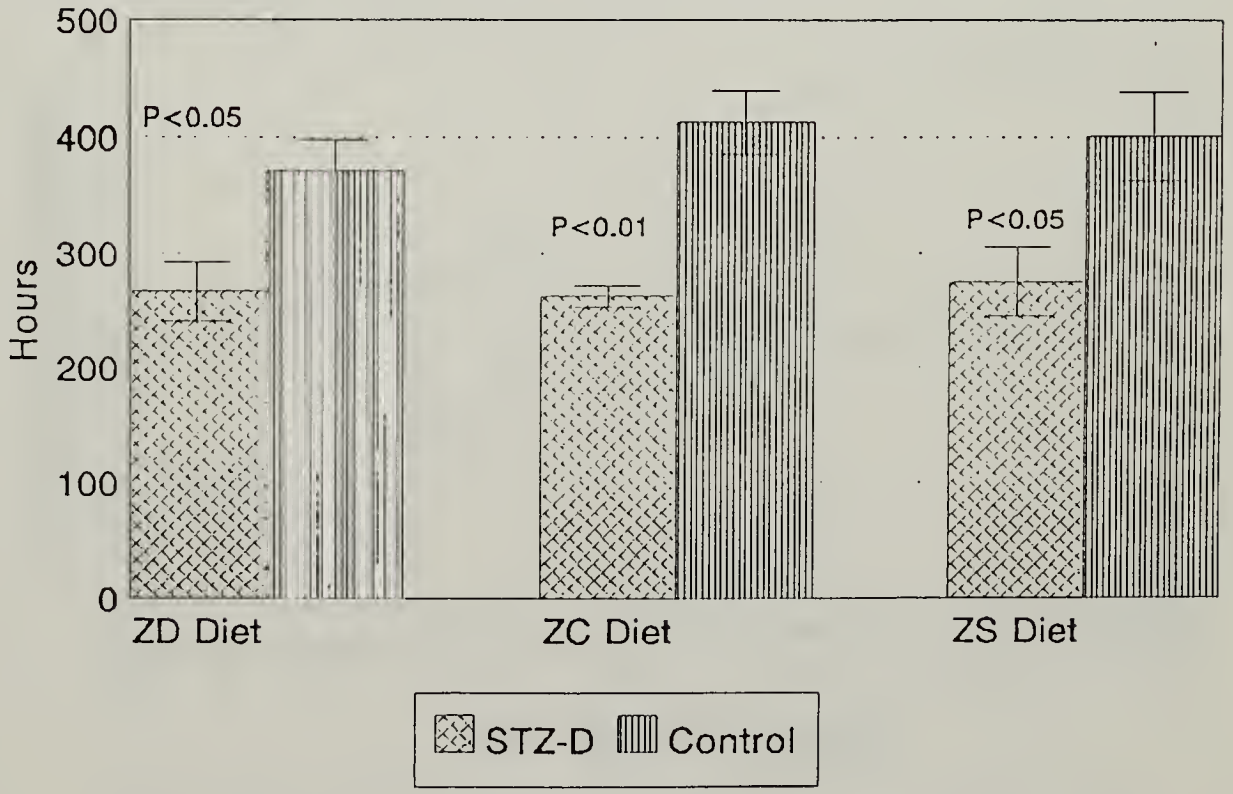

Figure 5.2 Comparison of 65-Zn Turnover Rates between STZ-Diabetic and Control Rats Fed Three Levels of Zn

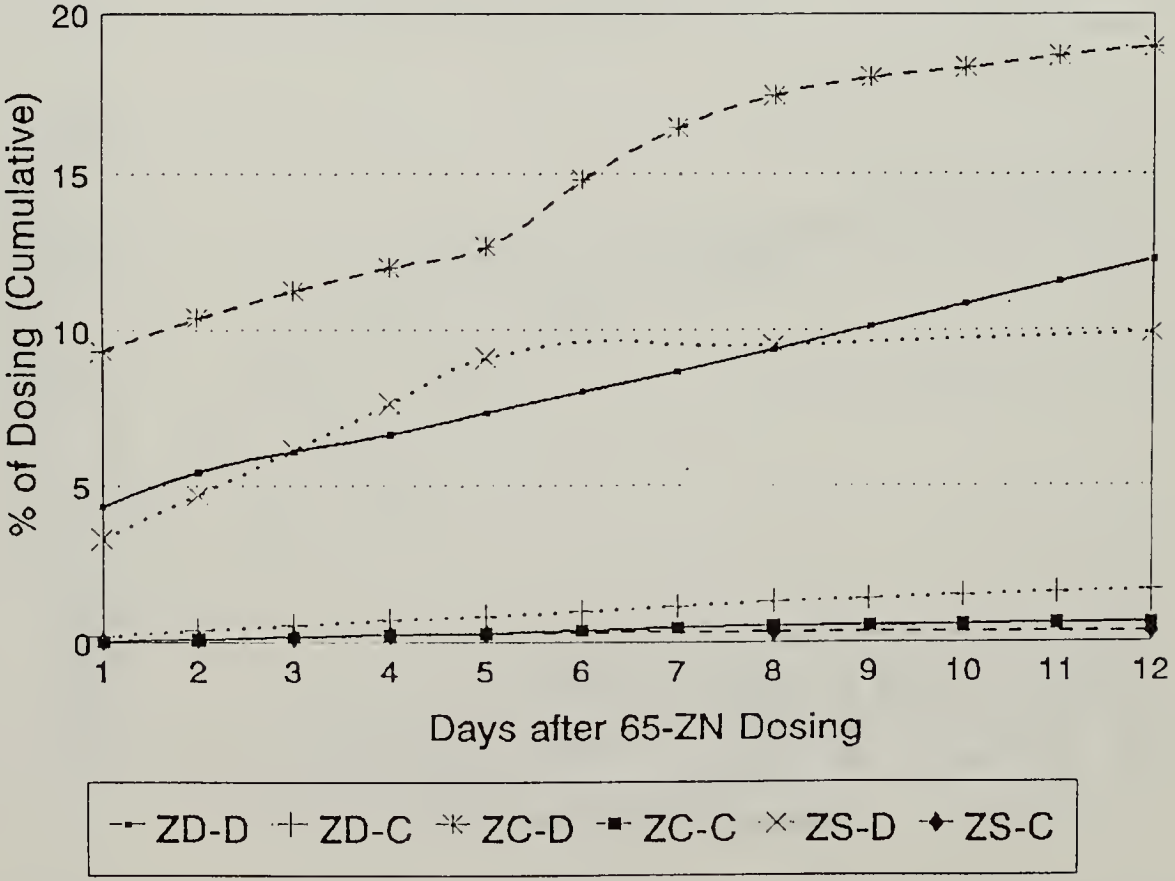

Figure 5.3 Comparison of Urine 65-ZN Excretion between STZ-Diabetic and Control Rats Fed Three Levels of Zn

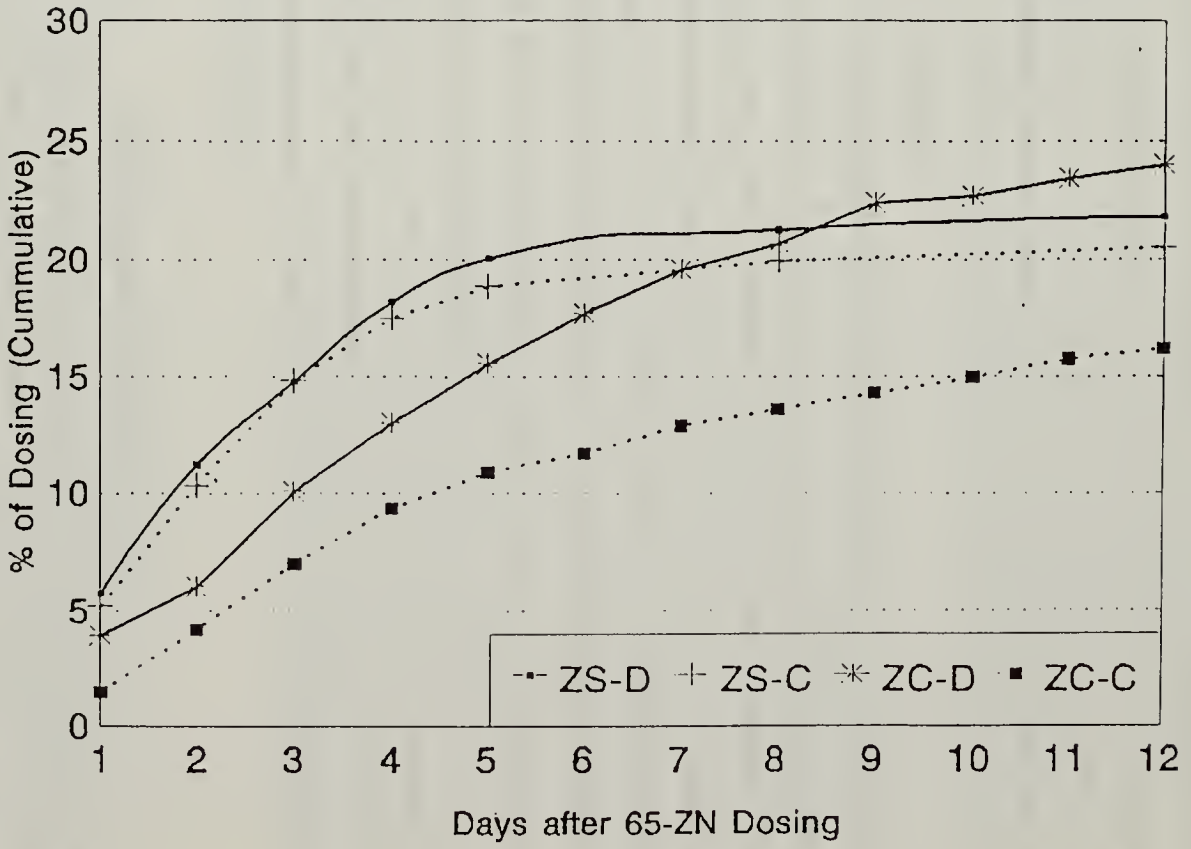

Figure 5.4 Comparison of Fecal 65-Zn Secretion in STZ-Diabetic and Control Rats Fed Two Levels of Zn

5.3.2 Partition of ^{65}Zn in Organs or Tissues

5.3.2.1 The Kinetics of Plasma ^{65}Zn

Figure 5.5 present kinetics of plasma ^{65}Zn of STZ-diabetic rats in comparison with that of their controls. The data is expressed as percentage of plasma ^{65}Zn count over first day's total body count. Plasma ^{65}Zn was not a major site of ^{65}Zn within the body in that it comprises less than 0.6% of total body ^{65}Zn. The percentage value of plasma ^{65}Zn of both STZ-diabetic groups and control groups underwent a dynamic change and maintained at about 0.15% of total body ^{65}Zn, suggesting a constant exchange of plasma Zn with other body compartments.

If the percentage values of plasma ^{65}Zn between STZ-diabetic group and control group are compared at each dietary Zn level, all three STZ-diabetic groups had consistently lower values. However, dietary Zn levels seemed inversely related to their percentage values of plasma ^{65}Zn in either STZ-diabetic groups or control groups. At the first day, STZ-diabetic rats on the ZD diet had the lowest percentage of plasma ^{65}Zn counts, however, a peak emerged at day 2. From day 5 until day 12 there was neither increase nor decrease in percentage of plasma ^{65}Zn count in STZ-diabetic rats on ZD diet. The control rats on ZD diet had persistently higher percentage value of plasma ^{65}Zn among the six groups. For the rest of the data, STZ-diabetic rats on both ZS and NC diets at the first day had lower percentage of plasma ^{65}Zn count compared with that of their corresponding controls. Generally, on each diet, control rats had a higher value than that of STZ-diabetic rats.

5.3.2.2 The Kinetics of Whole Blood ^{65}Zn

Figure 5.6 presents data for whole blood ^{65}Zn from STZ-diabetic rats compared with that of their controls. Whole blood ^{65}Zn was also not a major source of ^{65}Zn as it constituted only less than 0.2% of total body ^{65}Zn. Notice that at each time point, all the three STZ-diabetic groups had a lower percentage of whole blood ^{65}Zn compared with that of their corresponding controls. This possibly indicates a slower entry of ^{65}Zn into

blood cells. The relationship between dietary Zn intake and whole blood ^{65}Zn was not clear-cut as seen from the Figure.

5.3.2.3 The Kinetics of Liver ^{65}Zn

The kinetics of liver ^{65}Zn of STZ-diabetic rats on three different dietary Zn levels in comparison with those of their controls are presented in Figure 5.7. Liver ^{65}Zn was one of the major source of body ^{65}Zn as it constituted about 15% of total body ^{65}Zn .Notice that STZ-diabetic on all three different levels of dietary Zn generally had higher percentage of liver ^{65}Zn count than their corresponding controls for the first 8 days of the study. At the end of the study, STZ-diabetic rats on ZD diet seemed to have exhausted its liver ^{65}Zn storage, while STZ-diabetic rats on the other two diets remained still higher than that of their controls. This difference was more obvious for the STZ-diabetic rats and their control rats on the ZD diet. The accumulation of ^{65}Zn by the liver of STZ-diabetic rats may inversely related to dietary Zn intake as seen from the Figure that animals on the ZD diet had generally the highest liver ^{65}Zn , the lowest liver ^{65}Zn was in the rats on ZS diet.

5.3.2.4 The Kinetics of Kidney ^{65}Zn

Kidney ^{65}Zn constituted about 2-5% of total body ^{65}Zn and STZ-diabetic rats had the higher amount of ^{65}Zn than their controls throughout the study (Figure 5.8). This may indicate an increased excretion of ^{65}Zn through the diabetic kidney, rather than reflected an accumulation of Zn.

5.3.2.5 The Kinetics of Gastrointestinal Tract ^{65}Zn

Figure 5.9 presents the kinetics of gastrointestinal tract (GI) ^{65}Zn of STZ-diabetic rats and their controls on the three dietary Zn diets. ^{65}Zn in gastrointestinal tract may represent a secretion of endogenous Zn into the feces. In this regard it may be concluded that STZ-diabetic rats on ZD diet have a decreased endogenous secretion of ^{65}Zn into the feces within the second day of the study. However, their GI ^{65}Zn increased up to the levels of STZ-diabetic rats on the NC and ZS diets. STZ-diabetic rats on other two diets had a

higher GI ^{65}Zn than their corresponding controls within the first 5 days of the study, which may indicate that STZ-diabetic rats still have an increased endogenous secretion of Zn under normal and increased Zn intake, but decreased endogenous secretion of Zn during Zn depletion.

5.3.2.6 The Kinetics of Pancreatic ^{65}Zn

The kinetics of pancreatic ^{65}Zn of STZ-diabetic rats on the three different Zn diets and their corresponding control groups are presented in Figure 5.10. Higher values are generally in the rats with higher dietary Zn intakes at the beginning of the study. The STZ-diabetic rats had generally higher value at the first day of the study. A peak occurred at day 5 when the pancreatic ^{65}Zn was much higher in both STZ-diabetic and their control rats on ZS diet and a control group on ZD diet. The trend reversed from the 8th day until the end of the study.

5.3.2.7 The Kinetics of Spleen ^{65}Zn

The kinetics of spleen ^{65}Zn of STZ-diabetic rats and their controls are presented in Figure 5.11 Notice that STZ-diabetic rats on the NC deficient diet had generally the lowest percentage of spleen ^{65}Zn. STZ-diabetic rats on a ZS diet had a surge of spleen ^{65}Zn at the 5th day of the study. The influence of either dietary Zn levels or diabetes on the kinetics of spleen ^{65}Zn was not clear.

5.3.2.8 The Kinetics of Muscle ^{65}Zn

The kinetics of muscle ^{65}Zn of STZ-diabetic rats and their controls on the three different dietary Zn are presented in Figure 5.12. Notice that at the beginning of the study, muscle ^{65}Zn was lower in all the STZ-diabetic rats, which may indicate a slower entry of Zn into the muscle in diabetes. However, from the 2nd day of the study until the end STZ-diabetic rats on ZD diet gradually increased their muscle ^{65}Zn; and the STZ-diabetic rats on the ZS diet had the highest muscle ^{65}Zn at the end of the study. It seems that diabetes does not necessarily deplete muscle Zn, and diabetes may increased muscle Zn

concentration when rats were fed at increased or decreased dietary Zn. The higher muscle ^{65}Zn of STZ-diabetic rats fed the ZD diet may reflect a greater catabolic status of these rats.

5.3.2.9 The Kinetics of Bone ^{65}Zn

Kinetics of bone ^{65}Zn of STZ-diabetic rats and their control rats on three dietary Zn levels are presented in Figure 5.13. Notice that diabetes at all levels of dietary Zn clearly resulted in decreased bone ^{65}Zn. The trend was persistent throughout the study, and the lowest value was in STZ-diabetic rats on the ZD diet. However, the highest reading was in control rats on ZD diet. The data may indicate that diabetes selectively suppress entry of ^{65}Zn into bone, and the lower bone ^{65}Zn in three STZ-diabetic groups may present as a sign of Zn deficiency due to a slower entry of Zn, particularly when STZ-diabetic rats were fed a marginal Zn deficient diet. This feature may be one of the most important feature of altered Zn metabolism in diabetes mellitus.

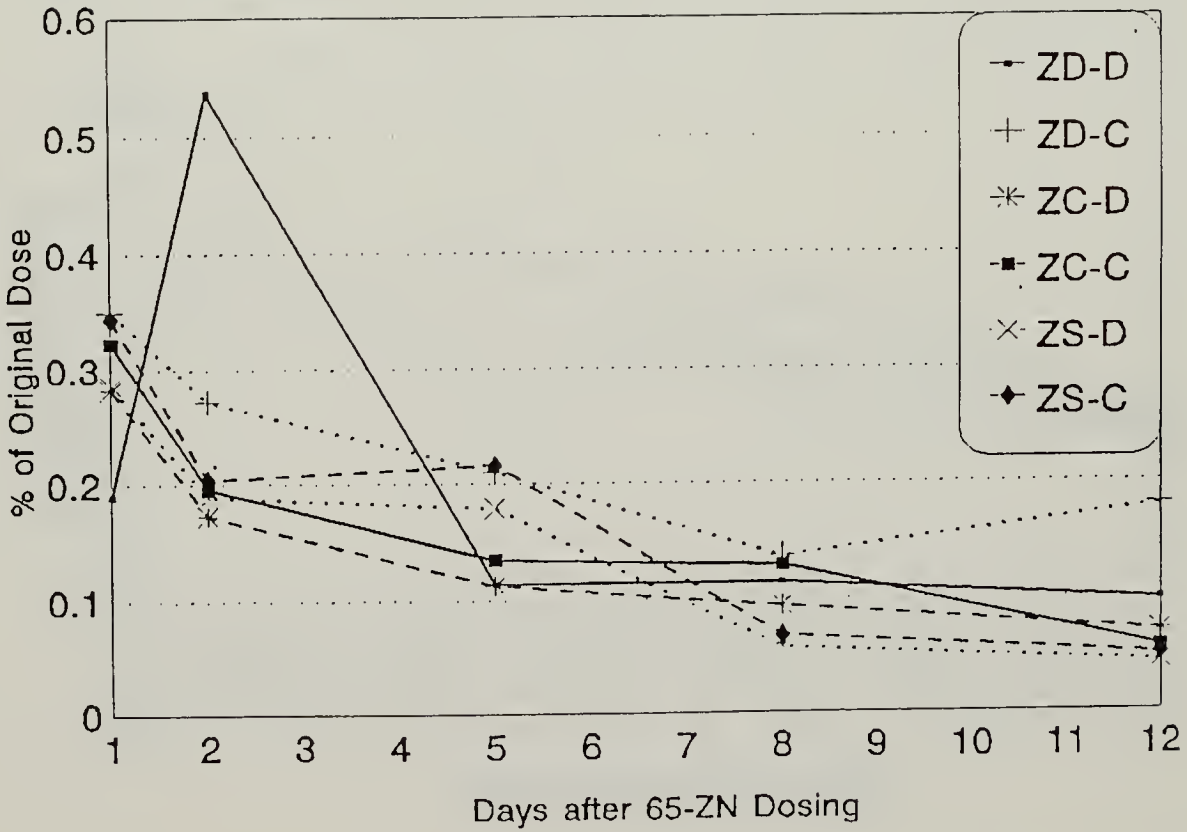

Figure 5.5 Kinetics of Plasma 65-ZN of STZ-Diabetic and Control Rats Fed Three Levels of Zn

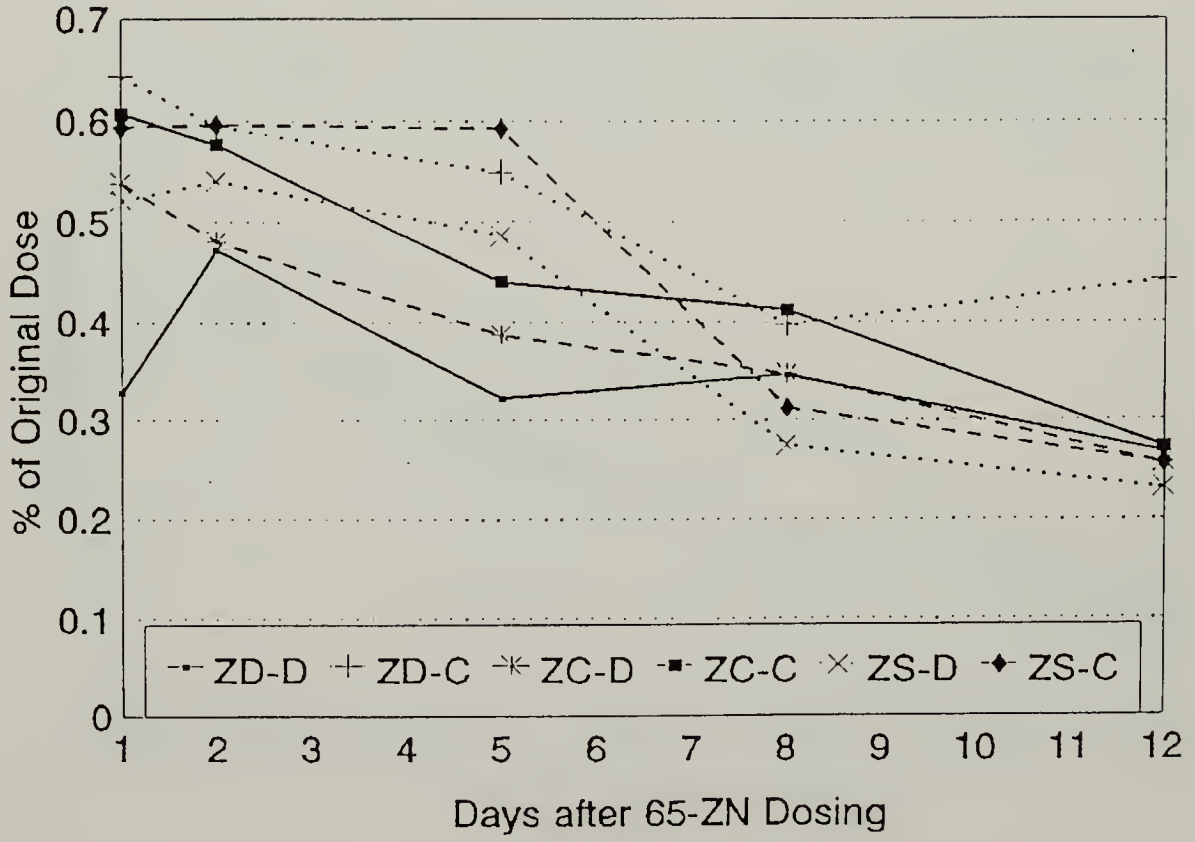

Figure 5.6 Kinetics of Whole Blood 65-ZN of STZ-Diabetic and Control Rats Fed Three Levels of Zn

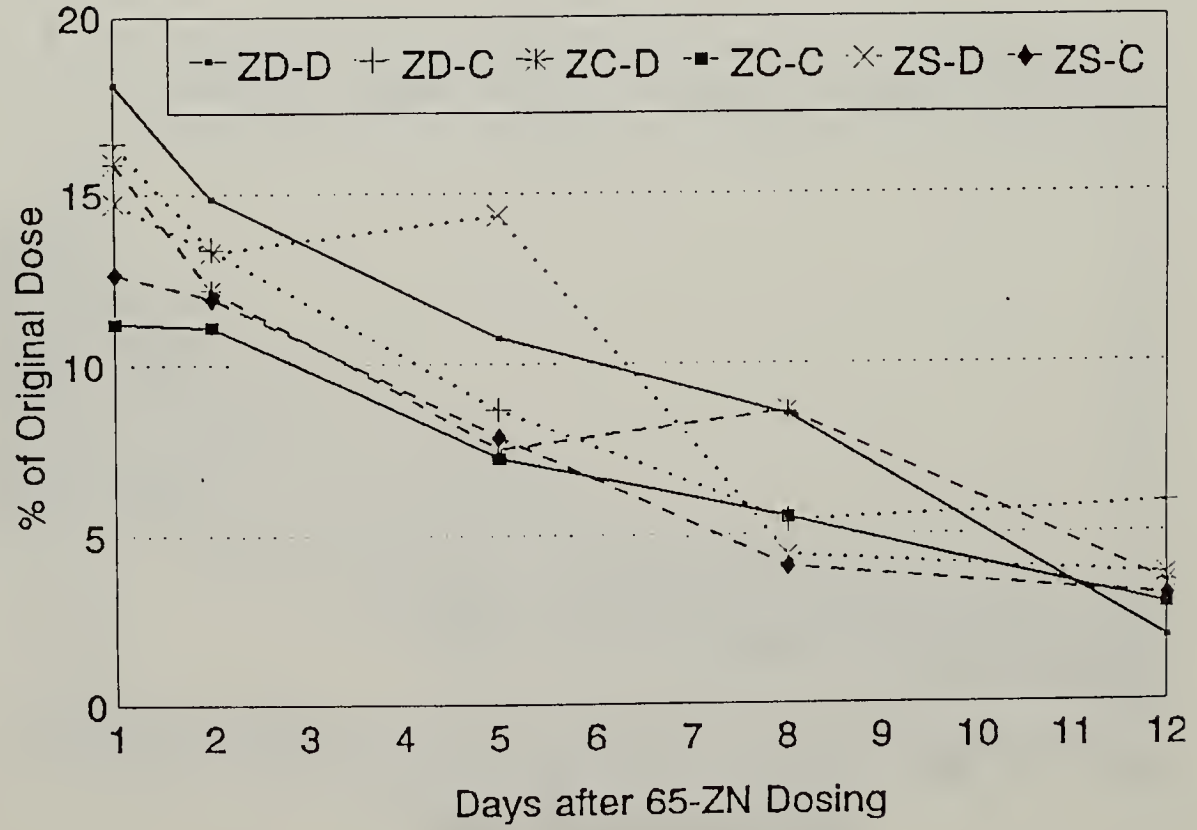

Figure 5.7 Kinetics of Liver 65-ZN of STZ-Diabetic and Control Rats Fed Three Levels of Zn

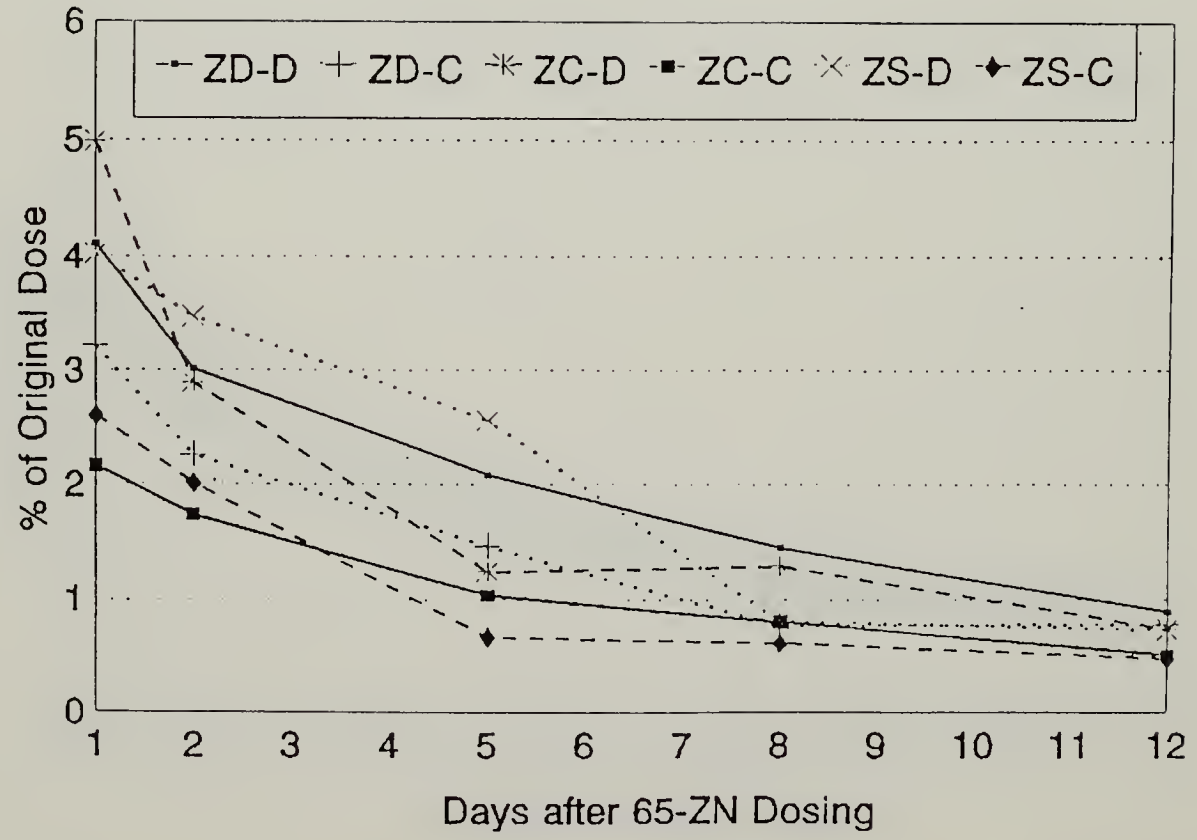

Figure 5.8 Kinetics of Kidney 65-ZN of STZ-Diabetic and Control Rats Fed Three Levels of Zn

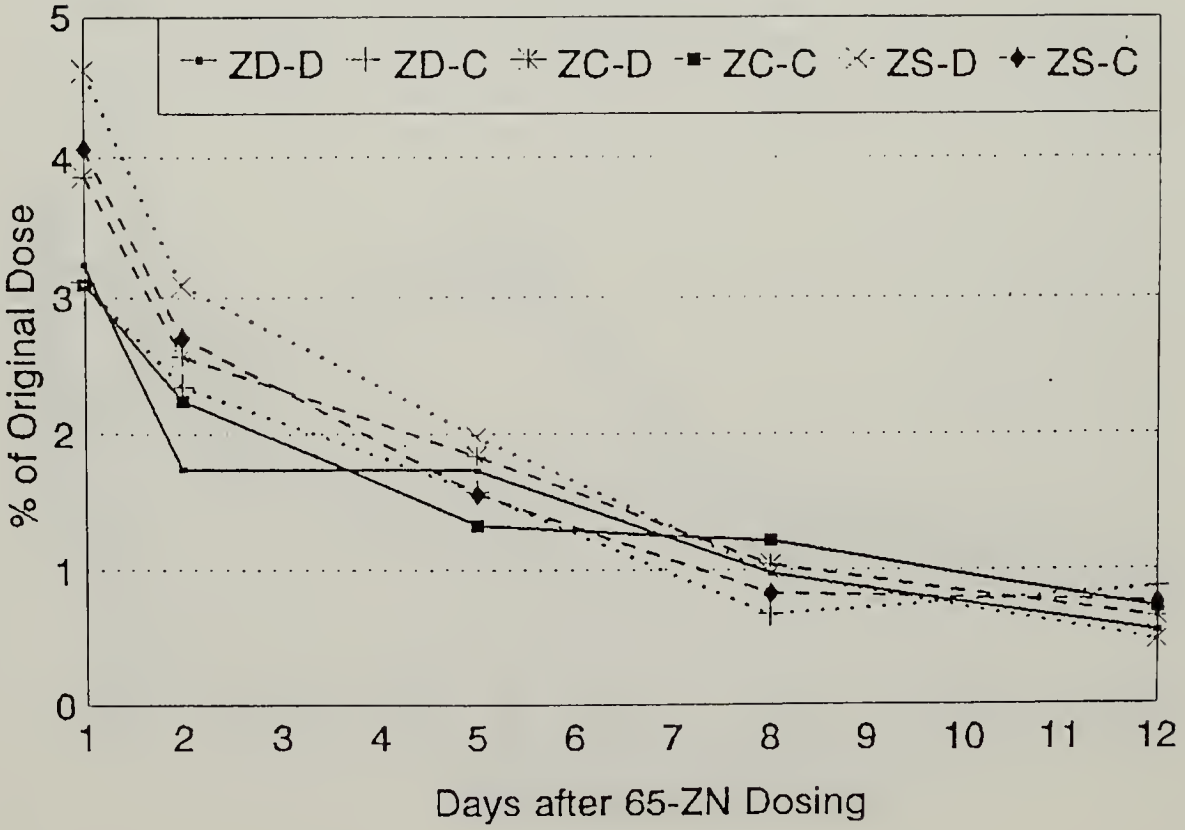

Figure 5.9 Kinetics of GI Tract 65-ZN of STZ-Diabetic and Control Rats Fed Three Levels of Zn

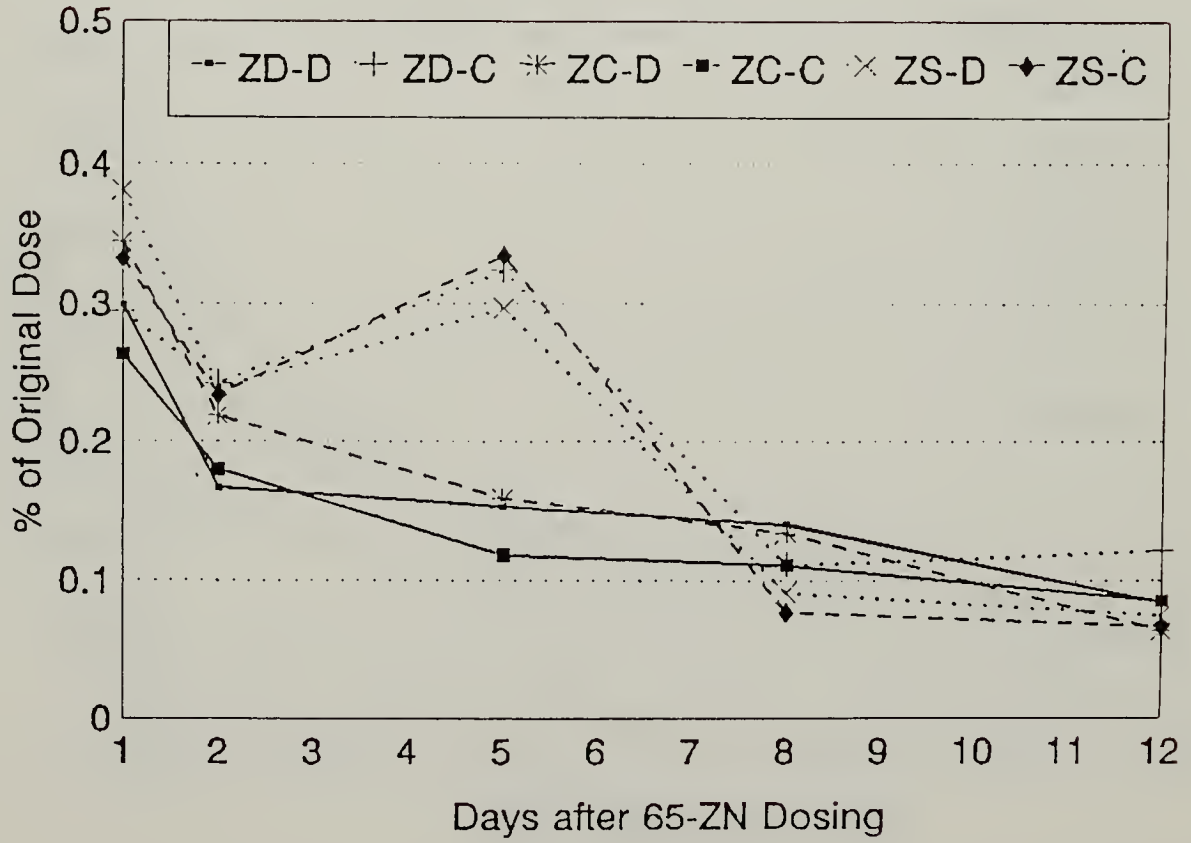

Figure 5.10 Kinetics of Pancreas 65-ZN of STZ-Diabetic and Control Rats Fed Three Levels of Zn

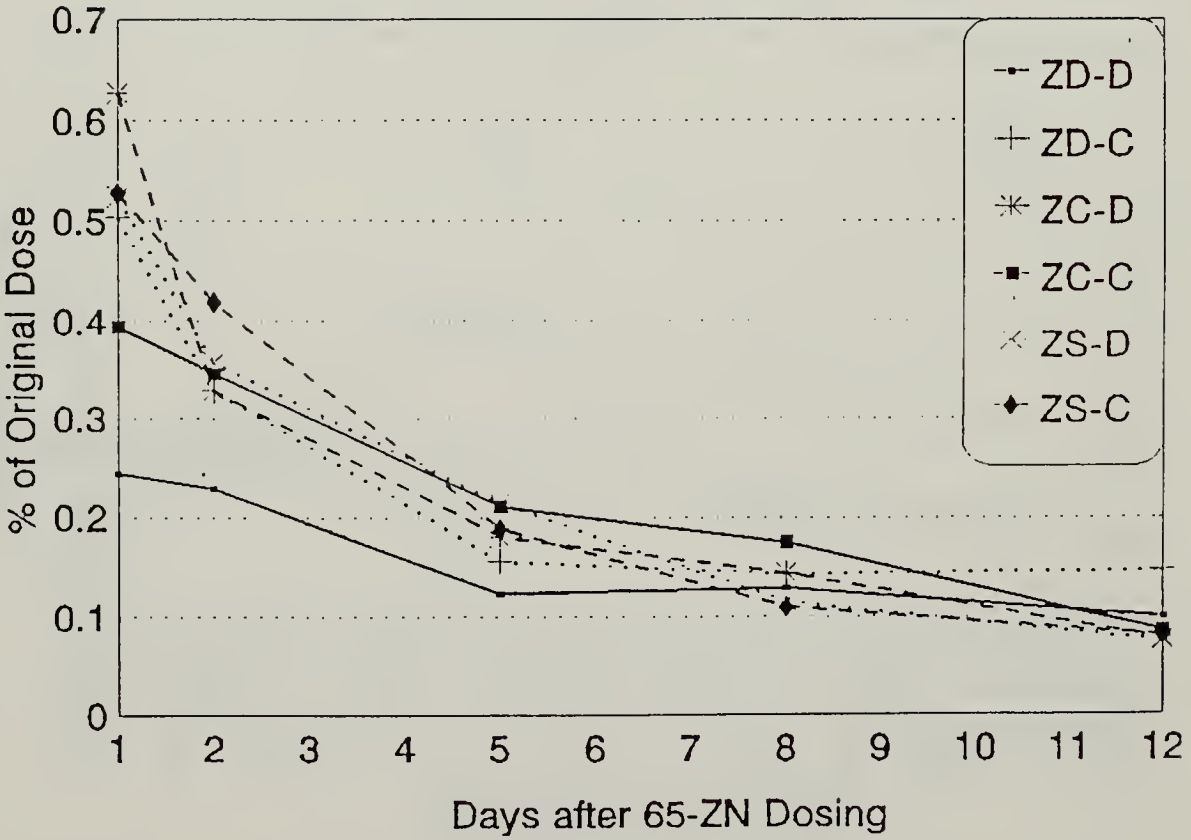

Figure 5.11 Kinetics of Spleen 65-ZN of STZ-Diabetic and Control Rats Fed Three Levels of Zn

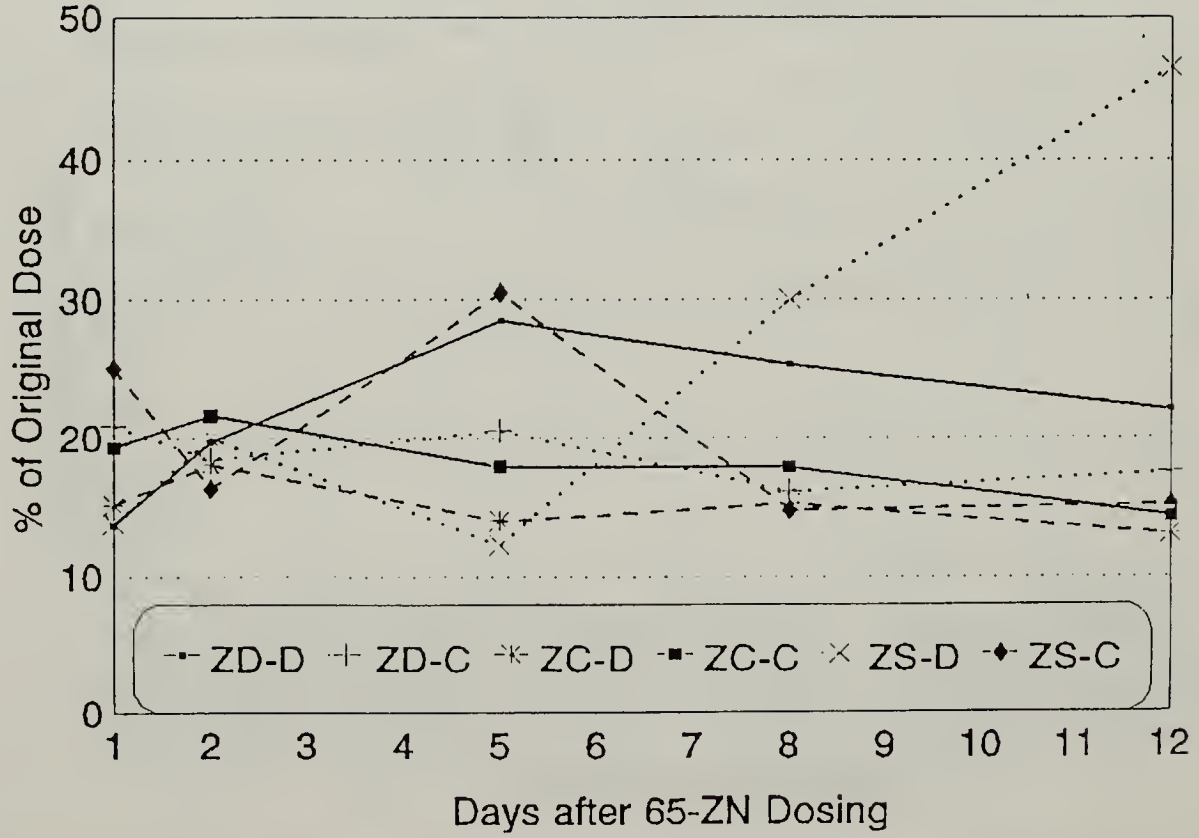

Figure 5.12 Kinetics of Muscle 65-ZN of STZ-Diabetic and Control Rats Fed Three Levels of Zn

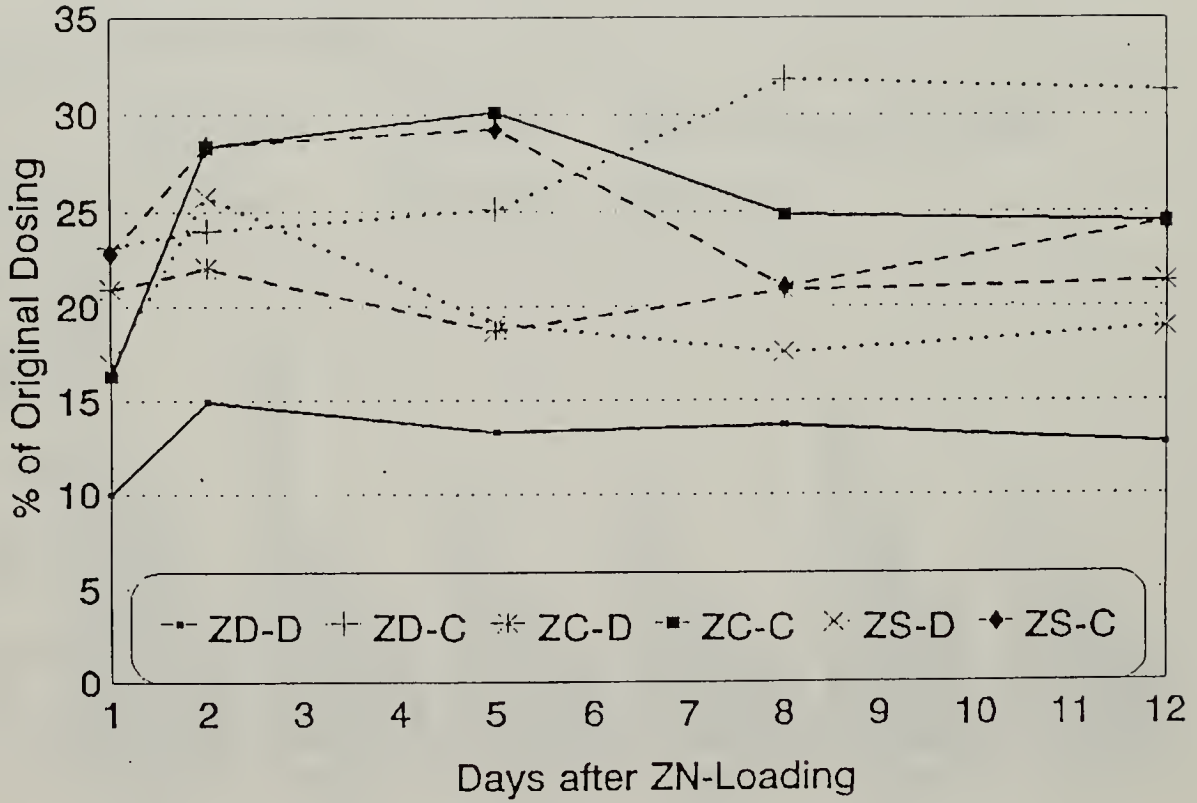

Figure 5.13 Kinetics of Bone 65-ZN of STZ-Diabetic and Control Rats Fed Three Levels of Zn

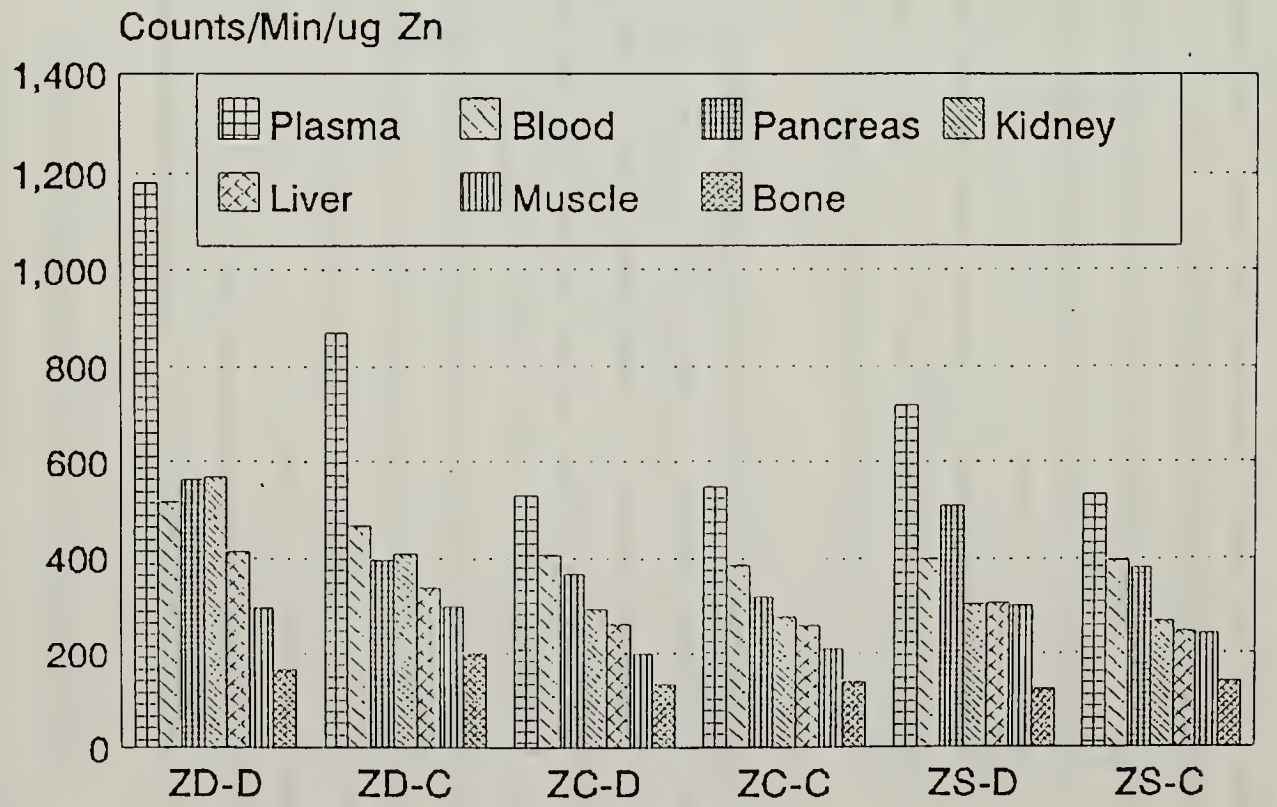

Figure 5.14 Comparison of Organ 65-Zn Specific Activity of STZ-Diabetic and Control Rats Fed Three Levels of Zn

5.4 DISCUSSION

5.4.1 Accelerated Turnover of Whole Body ^{65}Zn in STZ-Diabetic Rats

Whole body ^{65}Zn kinetics analysis revealed that the STZ-diabetic rats retained significantly less ^{65}Zn at all the three dietary Zn levels (Figure 5.1), indicating an accelerated excretion of body ^{65}Zn. These data are in agreement with the human IDDMs ^{65}Zn absorption reported by Kiilerich et al. (1990). The diabetic human retained less ^{65}Zn when compared with their controls. The decreased retention of whole body ^{65}Zn in these STZ-diabetic rats was mainly the result of faster whole body turnover of ^{65}Zn. The calculated turnover rates in the three STZ-diabetic groups were about a third faster than their controls. Dietary Zn intake seemed to have no significant effect on whole body ^{65}Zn turnover in both the STZ-diabetic and control groups.

5.4.2 Increased Ratios of Urine to Fecal ^{65}Zn Elimination in STZ-Diabetic Rats

The major route of ^{65}Zn elimination from body was the fecal secretion in both STZ-diabetic and control rats (Figure 5.4). The urinary excretion of ^{65}Zn accounted for only less than 5% of total body elimination of ^{65}Zn in control rats, while in the STZ-diabetic rats, the figures increased to about 35%. The total ^{65}Zn excreted in feces during the 12 days of study was not significantly different between the STZ-diabetic and control groups fed the normal zinc diet. However, STZ-diabetic rats had significantly increased their fecal ^{65}Zn when they were on the Zn supplemented diet. These results agree also with the conclusion by Johnson and Canfield (1985) that STZ-diabetic rats had an increased endogenous secretion of ^{65}Zn (The authors concluded that their STZ-diabetic rats had decreased endogenous secretion, but their results showed an increased fecal ^{65}Zn).

5.4.3 Changes in ^{65}Zn kinetics of Tissues in STZ-diabetic Rats

The most obvious changes in tissue ^{65}Zn kinetics of STZ-diabetic rats were their decreased ^{65}Zn retention in bone tissue (Figure 5.13). The decreased percentages of bone ^{65}Zn in STZ-diabetic rats were consistent at all dietary Zn levels. However, the bone of STZ-diabetic rats fed the ZD diet retained the least percentage of original ^{65}Zn dose,

followed by STZ-diabetic rats fed the Zn supplemented diet. The results suggest that dietary Zn did not affect bone ^{65}Zn kinetics in a linear manner.

The plots of liver and kidney ^{65}Zn kinetics revealed that at the first day after ^{65}Zn loading, the diabetic livers and kidneys contained the higher percentage of original ^{65}Zn dose. This may indicate that the absorbed ^{65}Zn was rapidly sequestrated by these two organs. The ^{65}Zn retained in these two organs in the STZ-diabetic rats was also eliminated faster than that of their control rats. This may suggest an increased ^{65}Zn turnover within these two diabetic organs.

Compared to control rats, muscles of STZ-diabetic rats retained greater percentages of total dosed ^{65}Zn on diets containing either marginal Zn or supplemented Zn, but not on normal Zn diet. This may indicate that the increased muscle Zn contents in STZ-diabetic rats came from both sources: from increased absorption and from tissue catabolism. The kinetics of other tissues were generally not distinguished.

5.4.4 Changes in Tissue ^{65}Zn Specificity in STZ-Diabetic Rats

The following organs of STZ-diabetic rats had increased tissue ^{65}Zn specificities: pancreas, kidney, liver, and plasma. The STZ-diabetic rats had consistently decreased bone ^{65}Zn specificity at all three dietary Zn levels. It seems the bone of the STZ-diabetic rats may be the special tissue with decreased utilization of total body Zn (See Figure 5.14).

CHAPTER 6
DISCUSSIONS AND CONCLUSIONS

6.1 The Zn Status of Stz-Diabetic Rats Has Been Altered but Is Not Deficient

The objective of this study was to find out whether Zn deficiency exists and what are the alterations of tissue Zn concentrations in diabetes mellitus. The significance of the present study is the findings that the WBMZCs in the STZ-diabetic rats were increased consistently and significantly. The major contributors of this increase in WBMZC in STZ-diabetic rats were liver and kidney when STZ-diabetic rats fed the Zn deficient diet; while the increased WBMZCs in STZ-diabetic rats fed the diets either adequate for Zn or supplemented with Zn were contributed by increased Zn concentrations of all organs and tissues.

The sources of this increased WBMZC in STZ-diabetes mellitus mainly came from the increased tissue breakdown resulting from greater body catabolism and decreased growth velocity, and was also contributed in part by their increased absorption due to greater dietary intake. However, this increased WBMZC can not be accounted for by decreasing endogenous secretion of Zn into feces since their fecal ^{65}Zn excretion was not decreased.

The findings of the present study lead to the reevaluation of Zn status of the diabetics, their hyperzincuria, and the nature of accumulation of Zn in liver and kidney.

6.2 Hyperzincuria in Diabetes Mellitus Is the Natural Consequence of Increased Total Body Zn Content

Hyperzincuria in diabetes has been regarded as the culprit depleting body Zn stores in diabetics. The results of the present study lead to the believe that hyperzincuria in diabetes is the consequence of Zn accumulation resulting from both the increased absorption and body catabolism. It can be envisioned that in diabetes most tissues, especially muscles, undergo catabolism and release Zn, at the same time liver is saturated with sequestrated Zn from circulation, while kidney is overloaded with unwanted extra Zn

needing for excretion. Hyperzincuria is the natural result of this readjusted Zn homeostasis in the diabetic body. It is thus not a culprit depleting body Zn stores, but an active and protective process, and an important component of Zn homeostasis in diabetes mellitus. Similarly, the increased urinary Cu and Fe excretion in diabetes mellitus (Lau and Failla 1984) can also be explained by the same mechanism.

6.3 The Adaptive Nature and Mechanisms of Increased Zn Levels in Diabetic Kidneys and Livers

Several alterations in diabetic rats have been identified. The most prominent of which is the accumulation or sequestration of Zn in the diabetic livers and kidneys. The absolute increase in Zn contents in these two organs are contributed by both increased concentration and organ volume or tissue hypertrophy.

The mechanism of the accumulation of Zn by diabetic livers and kidneys has been hypothesized to be previously related to hormone imbalance and stress reaction (Failla and Kiser, 1983; Hallmans, et al. 1984). However, The results of the present study for the first time challenge this view. The elevated WBMZC leads reasonably to the conclusion that these accumulations of Zn (and Cu) in the diabetic livers and kidneys may be the natural consequence of diabetic catabolism. The release of large amount of Zn from tissue catabolism lead to the increased amount of these metals in the circulation, which needs either to be stored in the liver for detoxification or excreted through the kidney.

Another finding of the present study is that decreased dietary Zn intake per se also leads to Zn concentration in the liver (but not kidney) in both normal and STZ-diabetic rats. It may also be possible that accumulation of Zn in the diabetic livers during Zn deficiency services a more vital function.

The present study found that the accumulation of Zn by the STZ-diabetic liver was not affected by the levels of Zn in the diet. Zn toxicity due to greater release of Zn from tissue catabolism is thus not existed. If Zn toxicity was present in the STZ-diabetic rats, Zn concentrations of livers of STZ-diabetic rats should fall when they fed the diet marginal for Zn. One possible explanation is that Zn accumulated in the liver may not be easily

eliminated by a reversible process. Whether this Zn accumulated in the diabetic liver is reusable or not needs further clarification. It may also be possible that it is a very slow process for the liver to eliminate stored Zn.

6.4 Zn Absorption in Diabetic Rats Is Not Altered

The percentage of ^{65}Zn absorption and retention were not significantly different between the STZ-diabetic rats and controls. However, STZ-diabetic rats absorbed more Zn due to their increase in food intake. The findings that STZ-diabetic rats can increase ^{65}Zn absorption when "post-fasted" indicate that the diabetics are capable of absorbing and retaining enough Zn to meed their body needs of Zn.

The findings of feeding pattern influenced ^{65}Zn absorption and retention in the present study may have significant implications in several aspects. First, the total amount of Zn absorbed is determined by the duration of post-fasting. Second, this is also a factor that may account for different results observed by different studies (Kiilerich et al. 1990; Craft and Failla 1983); Third, these findings are also important in future studies in which feeding pattern should be considered in study designs.

6.5 Endogenous Excretion of Zn Changes with Dietary Zn Intake in Stz-Diabetic Rats; and Endogenous Zn Excretion Does Not Decrease in Stz-Diabetic Rats

Fecal ^{65}Zn excretion was not increased in STZ-diabetic rats when fed the diet containing normal level of Zn, suggesting under normal conditions, endogenous secretion of Zn in diabetics is not decreased. Analysis of duodenal Zn concentration revealed that a significant increase in duodenal Zn concentration occurred in STZ-diabetics, which was also dependent on dietary intake of Zn. The observation may also support the view that endogenous secretion of Zn in diabetics is not decreased. This finding suggests that the increased WBMZC is not due to decreasing endogenous Zn secretion as suggested by Johnson and Canfield (1985). The findings that both endogenous Zn secretion and Zn absorption were not significantly altered in STZ-diabetic rats suggests that the major

source of increased WBMZC and Zn accumulation in liver and kidney comes from body tissue catabolism.

6.6 Muscle Zn Increased Significantly in Stz-Diabetic Rats During Zn Deficiency, Suggesting Greater Catabolism, and Release of Large Amount of Zn

Muscle Zn concentrations in STZ-diabetic rats were generally higher than that of their controls. The observation that muscle Zn concentration in STZ-diabetic rats when fed the diet marginal for Zn increased significantly, but this phenomenon was not observed in control rats, indicating that an altered Zn metabolism in muscle tissue in diabetics. The increased muscle Zn concentration in STZ-diabetic rats during Zn deficiency may indicate a greater catabolism of the tissue and it also becomes the major source of increased WBMZC and complements increases in Zn contents of liver and kidney, and urine loss. The findings from the kinetic study that the muscle from STZ-diabetic rats also contained greater amount of ^{65}Zn may indicate that a greater amount of absorbed Zn goes to muscles.

The findings from the present study indicate that muscle Zn concentration does not reflect food Zn intake and is also not a valid indicator of Zn status.

6.7 Plasma Zn Levels Do Not Reflect Dietary Zn Intakes and Do Not Reflect Whole Body Mean Zn Concentration

The present study observed that plasma Zn levels did not respond to dietary Zn intake in both STZ-diabetic rats and controls. Plasma levels of Zn and ^{65}Zn in STZ-diabetic rats were observed to be affected by feeding pattern as observed in the kinetic study. The results that plasma Zn levels did not reflect dietary Zn intake confirmed again that plasma Zn concentration is not a good indicator of Zn status.

6.8 Zn Utilization by Diabetic Bone May Be Compromised as Bone ^{65}Zn Turnover Is Lower in STZ-Diabetic Rats

The STZ-diabetic rats had consistently lower percentages of ^{65}Zn in bones. This finding agrees well with the results of decreased bone ^{65}Zn specificity in the diabetic bone in all three STZ-diabetic rat groups. The observations that severely retarded body growth, significantly higher WBMZC, and the lower ^{65}Zn specificity in diabetic bone exist simultaneously in STZ-diabetic rats when they fed a diet marginal for Zn may represent a disorder of Zn utilization in diabetes mellitus.

6.9 Future Studies

The above 8 conclusion drawn based on the animal study may be valid for STZ-diabetic rats only, whether these changes occur also in human diabetes have to be confirmed. It may be possible that all these alterations of zinc concentration and distribution, particularly that occurred in diabetic livers and kidneys, observed in the STZ-diabetic rats may be the results of toxic effect of streptozotocin. Thus all these observations may not represent what happens in human diabetes. Future studies may continue to explore the mechanisms of these changes in zinc, copper and iron metabolism as observed in the present study.

1. Determine whether these changes in STZ-diabetic rats reflect true changes in human diabetes. This can be done by analyzing livers and kidneys from human diabetic subjects. The possible toxic effect of STZ could be ruled out.
2. Determine whether changes in zinc concentration and distribution, particularly changes in diabetic livers, kidneys and muscle, reflect the natural results of catabolism; This could be done by fasting the animals to see whether similar changes occur.
3. Confirm whether increases in zinc concentration and alteration in diabetic livers and kidneys are reversible. This can be done in normal animals by insulin clamp techniques.
4. Confirm whether increases in trace element concentrations in diabetic livers and kidneys provide any beneficial effect to diabetic animals and humans.

5. Determine how STZ-diabetic animals increased their total body mean zinc, copper and iron concentrations.

6. Characterize changes in the diabetic bone. To explain why zinc concentration was lower even if STZ-diabetic animals had increased total body mean zinc, copper and iron concentration, and

7. To distinguish whether changes in Zn concentration and distribution, as well as increased WBMZC of STZ-diabetic rats are toxic effect of STZ or purely result of diabetes

APPENDIX

ADDITIONAL TABLES

Table 1 Zinc Contents of Organs and Tissues in STZ-Diabvetic Rats

Diet	ZD Diet	ZC Diet	ZS Diet
Plasma Zinc (ug)b$$@@			
STZ-Diabetics	3.1+0.3 (4)**	7.8+0.9 (9)	5.9+0.6 (9)**
Controls	7.0+0.9 (7)**	8.9+0.9 (6)	8.6+0.7 (9)**
Blood Clot Zinc (ug)a@@			
STZ-Diabetics	15.4+1.5 (4)**	21.7+1.5 (9)	21.3+0.9 (9)
Controls	23.9+1.5 (7)**	23.5+2.0 (6)	24.0+1.4 (9)
Liver Zinc (ug)			
STZ-Diabetics	223.5+16.3 (4)	286.0+15.0 (9)	265.5+9.4 (9)
Controls	260.4+25.4 (7)	262.9+13.6 (6)	269.0+ 9.0 (9)
Kidney Zinc (ug)c$$@@@##			
STZ-Diabetics	39.0+0.9 (4)	50.1+1.7 (9)***	49.0+1.4 (9)***
Controls	38.5+0.1 (7)	38.4+1.0 (6)***	38.6+0.6(9)***
GI Tract Zinc (ug)c$$$@@@			
STZ-Diabetics	43.8+4.7 (4)**	88.4+5.4 (9)*	95.2+5.6 (9)
Controls	79.6+5.3 (7)**	103.6+4.2 (6)*	109.2+4.0 (9)
Spleen Zinc (ug)c$$$@@@			
STZ-Diabetics	3.10+0.49 (4)**	9.4+0.9 (9)*	9.0+1.0 (9)*
Controls	8.38+0.98 (7)**	12.0+0.7 (6)*	11.4+0.4 (9)*
Pancreas Zinc (ug)a@@#			
STZ-Diabetics	5.6+0.5 (4)*	8.7+ 0.5 (9)	7.7+0.4 (9)
Controls	11.8+2.2 (7)*	8.6+0.7 (6)	8.7+0.5 (9)
Muscle Zinc (ug)a#			
STZ-Diabetics	878.6+95.3 (4)	772.2+57.4 (9)	761.5+39.1 (9)
Controls	667.3+34.0 (7)	866.0+64.7 (6)	889.0+47.6 (9)
Bone Zinc (ug)b$$@@			
STZ-Diabetics	1101.3+37.7 (4)**	1774.9+139.9 (9)	1865.1+110.8 (9)
Controls	1808.3+162.2 (7)**	1978.7+137.4 (6)	2146.4+130.0 (9)

*, **, ***: $P<0.05$, $P<0.01$, and $P<0.0001$ between diabetic and control groups from T-test. The following are from GLM: a.,b. c.: model significance at $P<0.05$, 0.01 and 0.0001 level; $, $$, $$$: Diet effect at $P<0.05$, 0.01 and 0.0001 level; #, ##, ###: Significant interaction between dietary Zn level and diabetes at $P<0.05$, 0.01 and 0.0001 levels. @, @@, @@@: Groups effect at $P<0.05$, 0.01 and 0.0001 levels. (number): number of rats.

Table 2 Zinc Distribution among Organs and Tisssues in STZ-Diabetic Rats

Diet	ZD Diet	ZN Diet	ZS Diet
Plasma Zinc (%)b$$@@			
STZ-Diabetics	0.14+0.02 (4)**	0.26+0.02 (9)	0.19+0.01 (9)**
Controls	0.24+0.02 (7)**	0.27+0.02 (6)	0.25+0.02 (9)**
Blood Clot Zinc(%)			
STZ-Diabetics	0.67+0.07 (4)	0.73+0.04 (9)	0.70+0.03 (9)
Controls	0.83+0.02 (7)	0.72+0.07 (6)	0.70+0.05 (9)
LiverZinc (%)a$@@			
STZ-Diabetics	9.68+0.69 (4)	9.62+0.43 (9)**	8.69+0.24 (9)*
Controls	8.85+0.34 (7)	8.02+0.28 (6)**	7.76+0.29 (9)*
Kidney Zinc (%)c@@@			
STZ-Diabetics	1.69+0.07 (4)*	1.72+0.13 (9)**	1.62+0.07 (9)***
Controls	1.37+0.11 (7)*	1.18+0.06 (6)**	1.12+0.05 (9)***
GI Tract Zinc (%)c$$@@@###			
STZ-Diabetics	1.91+0.24 (4)*	2.95+0.09 (9)	3.09+0.06 (9)
Controls	2.76+0.09 (7)*	3.17+0.12 (6)	3.15+0.12 (9)
Spleen Zinc (%)b$$@@			
STZ-Diabetics	0.14+0.02 (4)**	0.31+0.02 (9)	0.29+0.03 (9)
Controls	0.29+0.03 (7)**	0.37+0.02 (6)	0.33+0.02 (9)
Pancreas Zinc (%)b##			
STZ-Diabetics	0.24+0.02 (4)*	0.29+0.01 (9)	0.25+0.01 (9)
Controls	0.40+0.06 (7)*	0.26+0.02 (6)	0.25+0.02 (9)
Muscle Zinc (%)c$$$@@@###			
STZ-Diabetics	37.79+2.95 (4)**	25.58+0.52 (9)	24.77+0.47 (9)
Controls	23.61+1.70 (7)**	26.23+0.95 (6)	25.42+0.72 (9)
Bone Zinc (%)c$$$@@@###			
STZ-Diabetics	47.74+1.91 (4)**	58.54+0.63 (9)	60.41+0.69 (9)
Controls	61.66+1.54 (7)**	59.79+1.21 (6)	61.02+1.01 (9)

*, **, ***: P<0.05, P<0.01, and P<0.0001 between diabetic and control groups from T-test. The following are from GLM: a.,b. c.: model significance at P<0.05, 0.01 and 0.0001 level: $, $$, $$$: Diet effect at P<0.05, 0.01 and 0.0001 level; #, ##, ###: Significant interaction between dietary Zn level and diabetes at P<0.05, 0.01 and 0.0001 levels. @, @@, @@@: Groups effect at P<0.05, 0.01 and 0.0001 levels. (number): number of rats.

Table 3 Copper Contents of Selected Tissues of STZ-Diabetic Rats

Diet	Zinc Deficient	Normal	Zinc Supplement
Plasma Copper (ug)			
STZ-Diabetics	2.7+0.4 (4)	4.9+0.8 (6)	3.2+0.4 (10)
Controls	3.4+0.3 (10)	3.7+0.3 (7)	4.5+1.1 (11)
Blood Clot Copper (ug)@			
STZ-Diabetics	2.4+0.1 (4)***	4.1+0.4 (6)	3.7+0.2 (10)
Controls	4.0+0.2 (10)***	5.0+0.8 (7)	4.6+0.7 (11)
Liver Copper (ug)c$$@@###			
STZ-Diabetics	40.2+3.5 (4)*	88.7+10.8 (6)**	65.6+3.8 (10)**
Controls	53.8+3.7 (10)*	46.2+4.1 (7)**	48.1+2.9 (11)**
Kidney Copper (ug)b$@@			
STZ-Diabetics	10.6+1.3 (4)	36.5+8.7 (6)*	37.2+7.3 (10)*
Controls	12.3+0.4 (10)	10.1+0.6 (7)*	16.6+4.5 (11)*
GI Tract Copper (ug)a$$@			
STZ-Diabetics	5.6+1.1 (4)*	9.3+0.6 (6)	9.3+0.8 (10)
Controls	8.9+0.6 (10)*	9.4+0.8 (7)	10.3+0.8 (11)
Spleen Copper (ug)a$@@			
STZ-Diabetics	0.5+0.1 (4)**	0.9+0.1 (6)	0.9+ 0.1 (10)
Controls	0.8+0.1 (10)**	1.1+0.1 (7)	1.0+0.1 (11)
Pancreas Copper (ug)#			
STZ-Diabetics	0.4+0.03 (4)*	0.6+0.1 (6)	0.6+0.03 (10)
Controls	0.6+0.1 (10)*	0.5+0.04 (7)	0.5+0.03 (11)
Muscle Copper (ug)c@@@			
STZ-Diabetics	90.4+14.7 (4)	104.8+10.4 (6)	88.5+6.6 (10)*
Controls	94.8+7.1 (10)	110.8+10.8 (7)	115.5+10.4 (11)*
Bone Copper (ug)a$$@@			
STZ-Diabetics	35.7+0.8 (4)*	51.8+5.1 (6)	52.7+2.5 (10)
Controls	51.7+5.1 (10)*	60.6+3.7 (7)	60.7+3.5 (11)

*, **, ***: P<0.05, P<0.01, and P<0.0001 between diabetic and control groups from T-test. The following are from GLM: a.,b. c.: model significance at P<0.05, 0.01 and 0.0001 level; $, $$, $$$: Diet effect at P<0.05, 0.01 and 0.0001 level; #, ##, ###: Significant interaction between dietary Zn level and diabetes at P<0.05, 0.01 and 0.0001 levels. /@, /@@, /@@@: Groups effect at P<0.05, 0.01 and 0.0001 levels. (number): number of rats.

Table 4. Copper Distributions Among Tissues in STZ-Diabetic Rats

Diet	Zinc Deficient	Normal	Zinc Supplement
Plasma Copper (%)			
STZ-Diabetics	1.44+0.20 (4)	1.63+0.26 (6)	1.22+0.11 (10)
Controls	1.55+0.21 (10)	1.54+0.19 (7)	1.59+0.34 (11)
Blood Clot (%)a@@@			
STZ-Diabetics	1.32+0.11 (4)*	1.35+0.11 (6)*	1.43+0.06 (10)
Controls	1.76+0.04 (10)*	1.99+0.23 (7)*	1.68+0.20 (11)
Liver Copper (%)c@@@@##			
STZ-Diabetics	21.36+1.31 (4)	29.43+3.54 (6)*	24.95+0.92 (10)***
Controls	23.37+0.65 (10)	18.59+0.86 (7)*	18.67+0.50 (11)***
Kidney Copper (%)b@@			
STZ-Diabetics	5.75+0.80 (4)	12.40+3.38 (6)*	14.42+3.02 (10)*
Controls	5.60+0.49 (10)	4.15+0.13 (7)*	6.07+1.24 (11)*
GI Tract Copper (%)a@@			
STZ-Diabetics	3.13+0.73 (4)	3.06+0.10 (6)**	3.54+0.21 (10)
Controls	3.88+0.13 (10)	3.83+0.18 (7)**	4.02+0.20 (11)
Spleen Copper (%)a@@			
STZ-Diabetics	0.27+0.05 (4)	0.29+0.02 (6)**	0.34+0.04 (10)
Controls	0.38+0.03 (10)	0.44+0.02 (7)**	0.39+0.03 (11)
Pancreas Copper (%)b$			
STZ-Diabetics	0.22+0.01 (4)	0.19+0.01 (6)	0.21+0.01 (10)
Controls	0.24+0.01 (10)	0.19+0.01 (7)	0.18+0.01 (11)
Muscle Copper (%)b$@##			
STZ-Diabetics	47.187+3.930 (4)	34.51+2.92 (6)*	33.76+2.32 (10)**
Controls	40.875+1.088 (10)	44.43+1.59 (7)*	43.28+1.70 (11)**
Bone Copper (%)b@@			
STZ-Diabetics	19.31+1.57 (4)	17.14+1.56 (6)**	20.13+0.69 (10)*
Controls	22.34+1.43 (10)	24.86+1.17 (7)**	24.12+1.61 (11)*

*, **, ***: $P<0.05$, $P<0.01$, and $P<0.0001$ between diabetic and control groups from T-test. The following are from GLM: a.,b. c.: model significance at $P<0.05$, 0.01 and 0.0001 level: $, $$, $$$: Diet effect at $P<0.05$, 0.01 and 0.0001 level: #, ##, ###: Significant interaction between dietary Zn level and diabetes at $P<0.05$, 0.01 and 0.0001 levels. @, @@, @@@: Groups effect at $P<0.05$, 0.01 and 0.0001 levels. (number): number of rats.

Table 5 Iron Contents of Selected Organs or Tissues of STZ-Diabetic Rats

Diet	Zinc Deficient	Normal	Zinc Supplement
Plasma (ug)c$@@@			
STZ-Diabetics	3.26+0.69 (4)	8.97+1.06 (9)	8.13+0.74 (10)
Controls	4.47+0.75 (8)	11.43+0.95 (9)	10.84+1.30 (8)
Blood (ug)c$$@@@			
STZ-Diabetics	1777+69 (4)***	2649+181(9)*	1005+74 (11)
Controls	2792+141(8)***	3247+140 (9)*	1080+80 (7)
Liver (ug)			
STZ-Diabetics	1184+93.76 (4)	929+134 (9)	982+56 (10)
Controls	1180+94 (8)	949+79 (9)	990+78 (8)
Kidney (ug)b@@			
STZ-Diabetics	141.86+11.96 (4)	190.52+16.37 (9)*	186.26+ 10.00 (10)*
Controls	143.57+7.23 (8)	143.63+7.89 (9)*	141.25+11.93 (8)*
GI Tract (ug)c$$$@@			
STZ-Diabetics	196.39+26.15 (4)*	329.98+59.31 (9)**	321.99+30.06 (10)**
Controls	382.489+58.53 (8)*	689.10+71.98 (9)**	641.19+81.55 (8)**
Pnacreas (ug)a$$			
STZ-Disbetics	8.26+0.77 (4)*	13.74+1.45 (9)	14.80+1.60 (10)
Controls	11.13+0.83 (8)*	14.33+0.57 (9)	12.65+1.31 (8)
Spleen (ug)a@@#			
STZ-Diabetics	65.71+14.73 (4)**	111.15+8.83 (9)	115.73+13.20 (10)
Controls	147.35+20.12 (8)**	140.31+11.64 (9)	121.81+10.08 (8)
Muscle (ug)a#			
STZ-Diabetics	1253+338 (4)	1109+133 (9)*	1417+93 (10)
Controls	1018+104 (8)	1518+97 (9)*	1339+93 (8)
Bone Iron (ug)			
STZ-Daibetics	1439+260 (4)	988+113 (9)	967+70 (10)
Controls	1249+245 (8)	1034+63 (9)	1119+79 (8)

*, **, ***: P<0.05, P<0.01, and P<0.0001 between diabetic and control groups from T-test. The following are from GLM: a.,b. c.: model significance at P<0.05, 0.01 and 0.0001 level; $, $$, $$$: Diet effect at P<0.05, 0.01 and 0.0001 level; #, ##, ###: Significant interaction between dietary Zn level and diabetes at P<0.05, 0.01 and 0.0001 levels. @, @@, @@@: Groups effect at P<0.05, 0.01 and 0.0001 levels. (number): number of rats.

Table 6 Iron Distribution among Organs and Tissues of Diabetic Rats

Dietary groups	Zinc Deficient	Normal	Zinc Supplement
Plasma (%)$$			
Diabetic	0.014+0.003 (4)	0.042+0.006 (9)	0.042+0.009 (9)
Control	0.016+0.002 (8)	0.050+0.012 (7)	0.040+0.006 (8)
Blood (%)$$,#			
Diabetic	27.222+2.671(4)*	40.758+2.777 (9)	37.919+0.761 (9)
Control	38.209+2.379 (8)*	40.627+0.924 (7)	40.834+1.674 (8)
Liver (%)$$			
Diabetic	17.794+0.857 (4)	13.216+1.515 (9)	14.398+0.821 (9)*
Control	16.020+1.111 (8)	11.248+0.840 (7)	11.923+0.790 (8)*
Kidney (%)#			
Diabetic	2.127+0.082 (4)	2.927+0.238 (9)**	2.741+0.163 (9)***
Control	1.987+0.160 (8)	1.818+0.128 (7)**	1.622+0.100 (8)***
GI Tract (%)$$			
Diabetic	3.060+0.559 (4)*	4.554+0.556 (9)**	4.446+0.268 (9)**
Control	5.061+0.599 (8)*	7.599+0.474 (7)**	7.752+0.716 (8)**
Pancreas (%)$$, #			
Diabetic	0.124+0.011 (4)	0.204+0.009 (9)	0.213+0.023 (9)*
Control	0.151+0.012 (8)	0.183+0.012 (7)	0.149+0.013 (8)*
Spleen (%)#			
Diabetic	0.977+0.184 (4)**	1.679+0.069 (9)	1.652+0.186 (9)
Control	1.954+0.203 (8)**	1.710+0.179 (7)	1.568+0.144 (8)
Muscle (%)#			
Diabetic	18.285+3.911 (4)	16.129+0.644 (9)*	18.987+1.137 (9)
Control	13.600+1.112 (8)	18.742+0.820 (7)*	16.844+0.976 (8)
Bone system (%)$$			
Diabetic	30.399+4.719 (4)	20.491+1.118 (9)	19.601+1.307 (9)
Control	23.002+3.100 (8)	18.023+0.621 (7)	19.268+0.628 (8)

*, **, ***: P<0.05, P<0.01, and P<0.0001 between diabetic and control groups from T-test. The following are from GLM: a,b, c.: model significance at P 0.05, 0.01 and 0.0001 level; $, $$, $$$: Diet effect at P 0.05, 0.01 and 0.0001 level; #, ##, ###: Significant interaction between dietary Zn level and diabetes at P<0.05, 0.01 and 0.0001 levels. /@, /@/@, /@/@/@: Groups effect at P<0.05, 0.01 and 0.0001 levels. (number): number of rats.

Table 7. Whole body Mean Zinc, Copper and Iron Contents and Concentration of STZ-Diabetic Rats

Diet	Zinc Deficient	Normal	Zinc Supplement
Whole Body Zinc (ug)a$$@			
STZ-Diabetics	2313+87 (4)*	3019+214 (9)	3080+162 (9)
Controls	2905+211 (7)*	3303+207 (6)	3505+176 (9)
Whole Body Mean Zinc Concentation (ug/g wet wt)c$$@@@			
STZ-Diabetics	17.54+0.88 (4)*	14.69+0.36 (9)**	15.39+0.42 (9)**
Controls	13.99+0.40 (7)*	12.85+0.37 (6)**	13.34+0.27 (9)**
Whole Body Copper (ug)b$$#			
STZ-Diabetics	188.6+15.6 (4)	301.4+9.4 (6)*	261.8+8.7 (10)
Control	230.3+15.1 (10)	247.3+19.1 (7)*	261.8+19.1 (11)
Whole Body Mean Copper concentration (ug/g Wet Wt)c@@@			
STZ-Diabetics	1.431+0.135 (4)	1.410+0.112 (6)*	1.305+0.054 (10)**
Controls	1.051+0.025 (10)	0.978+0.061 (7)*	1.029+0.075 (11)**
Whole Body Iron (ug)$$			
STZ-Diabetics	6069+454 (4)	6328+564	6760+204 (11)
Controls	6928+407 (8)	7747+371	7564+388 (7)
Whole Body Mean Iron concentration (ug/g Wet Wt)c$$$@@@###			
STZ-Diabetics	46.12+4.23 (4)*	30.85+1.98 (9)	33.62+1.09 (11)**
Controls	32.94+2.17 (8)*	29.97+0.66 (9)	28.49+0.90 (7)**

*, **, ***: $P<0.05$, $P<0.01$, and $P<0.0001$ between diabetic and control groups from T-test. The following are from GLM: a.,b. c.: model significance at $P<0.05$, 0.01 and 0.0001 level; $, $$, $$$: Diet effect at $P<0.05$, 0.01 and 0.0001 level; #, ##, ###: Significant interaction between dietary Zn level and diabetes at $P<0.05$, 0.01 and 0.0001 levels. @, @@, @@@: Groups effect at $P<0.05$, 0.01 and 0.0001 levels. (number): number of rats.

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