DIVERGENT EFFECTS OF ZINC, PROTEIN AND ENERGY DEFICIENCIES ON SKELETAL MUSCLE MASS, MUSCLE FIBER DIAMETER AND SERUM INSULIN-LIKE GROWTH FACTOR-1 CONCENTRATION IN GROWING RATS

By Alexia Prescod

A thesis submitted to the Department of Foods and Nutrition
in partial fulfillment of the requirements for the degree of
Master of Science

Department of Foods and Nutrition
University of Manitoba
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Canada

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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BY

ALEXIA PRESCOD

A Thesis/Practicum submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

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ABSTRACT

DIVERGENT EFFECTS OF ZINC, PROTEIN AND ENERGY DEFICIENCY ON MUSCLE MASS, MUSCLE FIBER DIAMETER AND SERUM INSULIN-LIKE GROWTH FACTOR-1 CONCENTRATION IN GROWING RATS

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Zinc (Zn), protein and energy deficiencies arrest the growth and development of young mammals. The mechanisms behind this arrested growth are unclear. What is clear is that children who suffer from protein-energy malnutrition (PEM) are unable to fully recover their skeletal muscle mass and muscle fiber diameter following nutritional rehabilitation. Recovery from PEM is also associated with tenuous Zn status. Zn supplementation during recovery from PEM enhances the recovery of skeletal muscle mass. The mechanisms behind this improvement are also unclear.

Insulin-like growth factor-1 (IGF-1) is an anabolic peptide associated with skeletal muscle hypertrophy and regeneration. The concentration of IGF-1 in circulation and in tissues is strongly influenced by nutritional state, including Zn status. It is speculated that tenuous Zn status during recovery from PEM results in decreased IGF-1 concentrations, which leads to incomplete recovery of skeletal muscle mass. Since it would be difficult to explore this speculation in a single study, the hypothesis of this study is that full recovery of muscle fiber diameter following Zn, protein and energy deficiencies is dependent upon the full recovery of serum IGF-1 concentration and Zn status. The objective of the present study was twofold. Firstly, to explore the effect of Zn, protein and energy deficiencies on skeletal muscle mass and fiber diameter in relation to serum IGF-1 concentration in growing rats. Secondly, to investigate the effects of a 30 ppm Zn diet on the recovery of muscle mass, muscle fiber diameter and serum IGF-1 concentration.

To fulfill these objectives, eight 3-week-old rats were terminated upon arrival (baseline group, W) and the remaining 80 rats were fed one of the following diets for 3 weeks (deficiency phase): Zn deficient (Z, <1 ppm Zn), protein deficient (P, 2% protein), Zn and protein deficient (ZP, <1 ppm Zn + 2% protein), energy restricted (E, pair-fed control) or control (C, 30 ppm Zn & 20% protein). After the deficiency phase, eight rats from each group were fed a 30 ppm Zn diet for 3-weeks (recovery phase). Growth was assessed using anthropometry (e.g. body weight and length). Nutritional status was assessed using serum albumin and zinc concentrations, liver lipid concentration and inguinal fat weights. Skeletal muscle growth and development were assessed via gastrocnemius biopsy, histochemical staining and image analysis of muscle fiber diameter.

After the 3-week deficiency phase, the deficient rats had significantly smaller body, gastrocnemius muscle and fat pad masses, muscle fiber diameters and serum IGF-1 concentrations compared to the C group. Different types of malnutrition were produced by the

deficient diets. Assessment of serum albumin and liver lipid concentrations and inguinal fat pad mass revealed that the ZP and P groups were protein deficient and that the Z and E groups were energy deficient. The Z, ZP and P groups had serum and femur Zn concentrations that were significantly less than the W and C groups, indicating that these rats were Zn deficient. After the recovery phase, the serum albumin, liver lipid and serum IGF-1 concentrations of the deficient groups were similar to the C group. The P and E groups had a complete recovery of inguinal fat pad mass and an incomplete recovery of gastrocnemius mass and muscle fiber diameter. Recovery from Zn and combined Zn + protein deficiency was associated with full recovery of muscle fiber diameter, but incomplete recovery of inguinal fat and gastrocnemius mass.

In general, changes in serum IGF-1 concentration did not parallel changes in gastrocnemius mass or muscle fiber size. Full recovery of serum IGF-1 and Zn status was not associated with full recovery of muscle fiber diameter in the P and E groups. The recovery phase results indicated that previous nutritional status was the primary factor determining the degree of gastrocnemius mass and muscle fiber diameter recovery.

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I. Literature Review

Introduction

Normal growth and development is a complex interplay of genetics, trophic growth factors and nutrition. Growth and development can be derailed by micro and macro nutrient deficiencies. Rehabilitation from nutritional deficiency is accompanied by accelerated body mass accretion that is not simply the reinitialization of normal growth. Skeletal muscle mass and muscle fiber diameter do not fully recover to expected age and sex parameters despite rapid tissue accretion during nutritional rehabilitation. This literature review will explore relationships between skeletal muscle histology, zinc status, and insulin-like growth factor-1 during malnutrition and after nutritional rehabilitation.

Growth

What is the most rigorous law of our being? Growth. No smallest atom of our moral, mental, or physical structure can stand still a year. It grows—it must grow; nothing can prevent it.
Mark Twain (1835–1910), U.S. author. "Consistency," paper, read in Hartford, Connecticut, in 1884 (published 1923; repr. in *Complete Essays*, ed. by Charles Neider, 1963).

Growth is a physiological state in which an organism undergoes intensive tissue development and accretion. Growth is not an inevitable force of the universe. Growth is ultimately defined by the genetic code (Mitchell, 1997). In turn, the genetic code regulates the production and actions of endogenous anabolic hormones and peptides. The nutritional adequacy of the organism's diet is one of the most potent exogenous factors influencing the magnitude of growth (Mitchell, 1997). Without adequate nutrition powerful anabolic compounds become proteinaceous debris. Intensive periods of growth can occur at one or more points in the life cycle of an organism. These periods are usually associated with significant developmental changes and maturation. For humans and most mammals the intrauterine, infancy and pubescent periods are times of intensive growth and development (Forbes, 1987). These periods are also times of high nutritional requirements. The diet must be adequate in order to maintain past growth and

development as well as initiate and sustain new growth and development. The progression or impediment of growth can be assessed by monitoring the rate of tissue accretion (anthropmeterics), the state of tissue development (histology) and by the concentration of anabolic peptides in serum and tissues (biochemistry). It is during this time of growth, development and high nutritional requirements that malnutrition can have the greatest impact.

Insulin-like Growth Factor-1 (IGF-1)

IGF-1 is a 70 amino acid single chain member of the insulin family with a molecular weight of 7649 daltons (Thissen et al., 1994; Rechler and Nissley, 1991; Gammeltoft, 1989). 1989). Using hypophysectomized rats, Salmon and Daughaday (1957) first identified the physiological powers of IGF-1. These animals were unable to produce growth hormone (GH) and their bone and cartilage did not undergo normal growth and development. This defect was identified by the altered incorporation of radioactive sulfate [^{35}S] into cartilage. When the rats were exposed to exogenous GH this defect was corrected, but a preparation of cartilage segments did not show increased [^{35}S] incorporation with *in vitro* exposure to GH. When the *in vitro* preparation was exposed to normal rat serum, [^{35}S] incorporation increased indicating cartilage growth. Hypophysectomized rat serum had no effect. This and other early research lead to the hypothesis that the growth promoting effects of GH were mediated by other factors, namely, somatomedins. As further research elucidated the nature of the somatomedins, different types of somatomedines were identified- somatomedin A and C and multiplication stimulation activity (Rechiler and Nissley, 1991). Eventually it became clear that the somatomedins were the same as serum factors that had insulin-like activity (Doughaday et al., 1987). The somatomedins and the insulin-like serum factors became known as insulin-like growth factors.

IGF-1 (nee somatomedin C) can be differentiated from insulin-like growth factor-2 and its other family members (insulin, proinsulin and relaxin) by its unique genetic, amino acid, binding protein and receptor profile. The rat and human IGF-1 genes are made up of 6 exons and 4 introns. The rat gene is able to produce 3 lengths of mRNA while the human gene produces 2 sizes of mRNA (Daughaday and Rotwein, 1989 ; Straus, 1994). There is a strong inter-species

conservation of amino acid sequences. Rat, mouse and human IGF-1 differ at less than 5 amino acid positions (Rechler and Nissley, 1991). The majority of serum and tissue IGF-1 is bound to one of six high affinity-binding proteins (Straus, 1994). The binding proteins are thought to act as transport vehicles and modulators of IGF-1's availability, half-life and physiological actions (Clemmons and Underwood, 1991; Straus, 1994; Froesch et al., 1985). As with IGF-1, nutritional status and GH influence the concentrations of the different binding proteins (Clemmons and Underwood, 1991). IGF-1 also has its own high affinity receptor known as the IGF-1 receptor (IGF-1R). The IGF-1R resembles the insulin receptor with its twin alpha and beta sub-units (Rechler and Nissley, 1985 & 1991; Gammeltoft, 1989). The post receptor sequence of events is not entirely clear. Reviews by Rechler and Nissley (1991& 1985) and Gammeltoft (1989 and references there in) point to phosphorylation of the beta sub-units and to tyrosine kinase activity following IGF-1-receptor union.

Origin and Roles in Growth

Work by D'Ecrole et al. (1984) and other researchers have helped to determine that the liver is the primary producer of the IGF-1 peptides found in circulation. IGF-1 and its mRNA have been found in many different extra-hepatic mammalian tissues including heart, lung, kidney, brain, fat and skeletal muscle (Daughaday and Rotwein, 1989; D'Ecrole et al., 1984; Orlowski and Chernusek, 1988, Turner et al., 1988; Hua et al., 1993 and Edwall et al., 1989). Due to the localized production of IGF-1 mRNA and IGF-1 peptide in many tissues, IGF-1 is though to act in autocrine and paracrine fashions (Daughaday and Rotwein, 1989; Clemmons and Underwood, 1991; Rechler and Nissley, 1991). As noted by Daughaday and Rotwein (1989) some tissues with high IGF-1 gene activity and peptide concentrations have anatomic barriers that would impede the transportation of IGF-1 from circulation to tissue (i.e. testis and brain). The discordance between serum and tissue IGF-1 concentration found by D'Ecrole et al. (1984) and Orlowski and Chernausek (1988) lent support to the theory of localized production and action of IGF-1. These researchers used GH deficient rats that were treated with exogenous GH. After GH treatments, they found tissue concentrations of IGF-1that did not correlate with serum concentrations. The increased IGF-1 production in the liver and kidney was greater and swifter

than that seen in serum. This implied that these tissues were able to alter their IGF-1 concentration independently of serum concentrations.

The physiological actions of IGF-1 have been investigated through many different avenues of research. Observational studies by Merimee et al. (1981) and Eigenmann et al. (1984) reiterated the role of IGF-1 in overall growth and development. These studies looked for links between body size, genetics and IGF-1. Merimee et al. (1981) studied the differences in serum IGF-1 concentration in African Pygmies, GH deficient subjects and normal controls. Previous studies by the authors had found that the Pygmies had normal GH secretion, yet the Pygmies had a body size and metabolic characteristics similar to a GH deficient individual. The Pygmies had serum IGF-1 concentrations that were similar to that of the GH deficient group. Since the Pygmies had normal serum GH concentrations along with low serum IGF-1 concentrations and a smaller body size, the authors speculated that the Pygmies had flawed IGF-1 production. Eigenmann et al. (1984) took advantage of the body size variations of Poodles in order to examine the genetic and endocrinological basis for this variation. The 3 sizes of Poodle (standard, miniature and toy) have evolved through years of selective breeding. The 3 types of Poodle had no signs of GH deficiency, secreted similar amounts of GH, but had different mean plasma IGF-1concentrations. The standard Poodle had a mean plasma IGF-1 concentration that was 6 times greater than the toy Poodle. The miniature Poodle with the intermediate body size had intermediate mean plasma IGF-1 concentration. The authors found a significant correlation ($r= 0.88$; $P<0.001$) between body weight and plasma IGF-1 concentration. The researchers concluded that this study provided indirect evidence of the growth-promoting role of IGF-1 *in vivo*.

Through genetic and breeding manipulation Baker et al. (1993) and Lui et al. (1993) studied the embryonic and post-natal growth of mice with null mutations of the IGF-1 gene. In short, these animals were not able to produce biologically active IGF-1. The researchers observed that the IGF-1 mutated mice had increased post-natal mortality, poor weight gain, poor bone development, small reproductive organs and adult body weights that were 30% of that observed in control mice. These animals were also infertile. Hizuka et al. (1986) and Philipps et al. (1988) took the opposite approach. These research teams exposed weanling rats to

exogenous IGF-1 and observed their growth. Hizuka et al. (1986) infused weanling rats with synthetic IGF-1 via a minipump for 7 days. This resulted in significant increases in serum IGF-1 concentrations ($P<0.001$), body weights ($P<0.01$), body length ($P<0.05$) and significantly heavier kidney, liver and testis weights compared to control values. The IGF-1 treated rats also had significantly greater epiphyseal widths ($P<0.01$). The serum thyroxine concentration did not differ between the treated and control animals. The Philipps et al. (1988) study was 16 days long and biosynthetic IGF-1 was administered by twice daily injections. At the end of the study the body weights, tail lengths, heart, liver and testis weights were significantly greater ($P<0.05$) than control rats. These studies highlight the physiological importance of IGF-1 in growth and development.

Regulation by Growth Hormone and Nutrition

i. Growth Hormone

Growth Hormone (GH) and nutrition primarily control the production and anabolic actions of IGF-1. GH deficiency is associated with dwarfism (Howard et al., 1981; Furlanetto and Cara, 1986) and low IGF-1 concentrations (Merimee et al., 1981; D'Ecrole et al., 1984; Orlowski and Chernausek, 1988). GH deficient children treated with GH experienced a marked increase in linear growth (Howard et al., 1981). Unfortunately, changes in IGF-1 concentrations in response to GH treatment were not investigated. The administration of GH to GH deficient humans (Merimee et al., 1982), rats (D'Ecrole et al., 1984; Bates et al., 1993) or mice (Pell and Bates. 1992) resulted in increased serum and/or tissue IGF-1 concentrations. In sheep with normal GH secretion, exogenous GH was able to increase plasma IGF-1 concentrations (Hua et al., 1993). Isgaard et al. (1988) demonstrated a dose dependent relationship between exogenous GH and skeletal muscle IGF-1 mRNA expression. A minimal dose of 100 μg of GH was required to produce a significant increase in gastrocnemius IGF-1 mRNA in hypophysectomized rats. Turner et al. (1988) implanted GH secreting cells into non-growing rats with normal GH secretion capacity in order to induce hypertrophy. Thirty days after implantation, the body weights of the treated rats surpassed control values. There was an associated increase in plasma GH and

serum IGFs. Several tissues demonstrated GH induced hypertrophy including gastrocnemius, heart and liver tissues. By the end of the study the gastrocnemius, liver and heart weights were 1.5, 3 and 2.5 times, respectively, greater than age-matched controls. Along with the tissue hypertrophy, there was a concomitant increase in IGF-1 mRNA. This study demonstrated that GH induced IGF-1 gene activity and this was associated with tissue accretion.

The above mentioned studies and other works highlight the strong relationship between IGF-1 and GH. However, exogenous stimuli can induce localized IGF-1 production and anabolic actions without the presence of GH. Adaptive hypertrophy due to muscle overload or physical training was associated with increased IGF-1 mRNA and IGF-1 peptide concentration in GH deficient and GH suppressed rats in studies conducted by Zanconato et al. (1994) and Adams and Haddad (1996). The Adams and Haddad (1996) work included time course studies that demonstrated that muscle IGF-1 expression increased before the weight of the overloaded plantaris muscle increased. Recovery from ischemic injury to the extensor digitorum longus (EDL) muscle of GH deficient rats included increased muscle IGF-1 mRNA levels (Edwall et al., 1989). Therefore, exogenous stimuli of muscular hypertrophy and regeneration can induce local IGF-1 expression independently of GH.

ii. Nutrition

Nutritional status is a powerful exogenous factor modulating IGF-1 expression and anabolic potential. Malnutrition in many mammals including humans, sheep and dogs is accompanied by GH resistance characterized by high GH secretion and low IGF-1 circulating (Vance et al., 1992; Straus, 1994). Merimee et al. (1982) exposed 7 human subjects with isolated GH deficiency to exogenous GH while in a fed and in a fasted state. During the fasting, the exogenous GH increased serum IGF-1 concentrations, but the increase was 10 times smaller and significantly less ($P<0.001$) than the rise seen in the fed state. Phillips and Young (1976) administered GH to rats with normal GH secretion during a 72 hours fast. The exogenous GH was unable to prevent the drop in serum IGF potency (assessed by porcine cartilage bioassay) that was associated with fasting. Bates et al. (1993) found that GH treatments in

hypophysectomized rats resulted in significant increases in feed intake ($P<0.001$), body weight ($P<0.01$), muscle ($P<0.01$) and serum ($P<0.001$) IGF-1 concentrations. When feed intake was limited, the GH was unable to induce increases in body mass and IGF-1 concentrations equivalent to those observed in the well-fed rats. Since these studies employed exogenous GH treatments during malnutrition, the poor response of IGF-1 could be due to GH resistance and the influence of nutrition on GH binding, receptors and signaling (Vance et al. 1992). Therefore fasting and/or energy restriction could influence IGF-1 expression indirectly through changes in GH physiology. The 5% protein deficient diet employed by VandeHaar et al. (1990) did not cause critical changes in GH binding, yet there were marked changes in IGF-1 mRNA expression.

Nutrition can also have a direct influence on IGF-1 expression. As mentioned before, Philipps et al. (1988) injected exogenous IGF-1 into rat pups. These IGF-1 injections resulted in increased somatic and organ growth. This study went one step further- the litter size for some pups was increased to induce environmental malnutrition. The environmental malnutrition was effective as the control animals in these large litters had an overall drop in body weight and reduced organ weights. The IGF-1 treated animals in the large litters did not demonstrate significant increases in growth like their normal litter-sized counterparts. Thissen et al. (1991a) reported that a 5% protein diet suppressed the anabolic effects of IGF-1 infusions in non-hypophysectomized/ intact young rats. The IGF-1 infusions were delivered via an osmotic minipump at a rate of 1μl/h for 8 days. The IGF-1 infused rats fed the 5% protein diet had body weights, tail lengths or epiphyseal widths that were similar to that of rats fed the 5% protein diet only. Intact rats that were not infused with IGF-1 and fed a 15% protein diet displayed significantly greater growth than the protein deficient IGF-1 infused rats ($P<0.01$). The IGF-1 infusions normalized the serum IGF-1 concentrations in the 5% protein rats, yet failed to promote growth. Thissen et al. (1991a) concluded that the protein deficient diet caused tissue specific resistance to the anabolic effects of exogenous IGF-1.

In summary, GH and nutrition are powerful regulators of IGF-1 expression and anabolic actions. The ability of GH to induce IGF-1 production and for IGF-1 to induce growth is dependent on nutritional status.

Zinc

Zinc deficiency and Growth

Zinc (Zn) is a ubiquitous non-transition metal that is essential for the function of over 200 mammalian enzymes (Cousins and Hempe, 1990; Wardlaw and Insel, 1990). Zn has been found to be essential for the growth, development and differentiation of microorganisms, and plant and animal cells (Wu and Wu, 1987; Cousins and Hempe, 1991). The biochemical mechanisms through which Zn plays its critical role in growth and the relationship between dietary Zn deficiency and its biochemical and physiological manifestations have not been fully elucidated (Wu and Wu, 1987). What is clear is that growth failure is the major symptom of Zn deficiency in developing mammals (Golden, 1989; Cousins and Hempe, 1990).

Growth retardation, delayed sexually development, rough/dry skin and geophagia were the hallmark characteristics of a syndrome documented in young Iranian and Egyptian males 40 years ago (Prasad, 1984). Treatment with Zn supplementation and an adequate diet resulted in increased growth and sexual development that was greater than the changes seen with iron supplement and adequate diet alone (Sandstead et al., 1967). This syndrome seen in the Iranian and Egyptian males was identified as the first documented cases of human Zn deficiency.

Similarly, growth suppression is hallmark characteristic of weanling rats fed a semi-synthetic Zn deficient diet (Williams and Mills, 1970; Chesters and Quaterman, 1970). Along with retarded growth, the Zn deficient rats had decreased feed intakes (Chesters and Quaterman, 1970). Decreased dietary intake is another hallmark of Zn deficiency (Wardlaw and Insel, 1990). This decrease in voluntary feed intake necessitates the use of pair-fed groups in Zn deficiency studies in order to differentiate the effects of decreased feed intake from the effects of Zn deficiency per se. The pair-fed rats are fed a nutritionally complete diet, but the quantity of diet is limited to the amount consumed by the Zn deficient group.

Zinc deficiency and Insulin-like Growth Factor-1

Studies by various researchers have explored the potential mechanisms for the growth delay associated with Zn deficiency. Mild Zn deficiency has been associated with poor linear growth in children without endocrine deficiencies (Prentice, 1993; Nakamura et al., 1993). Nakamura et al. (1993) and Ghavami-Maibodi et al. (1983) found that healthy short children with mild Zn deficiency had delayed bone and sexual development as well as low serum IGF-1 concentrations. Oral Zn supplementation in these children resulted in significant increases in linear growth, bone development, and serum Zn and IGF-1 concentrations versus placebo treated counterparts (Ghavami-Maibodi et al., 1983; Nakamura et al., 1983).

Cossack (1986) explored the relationships between growth, dietary Zn and protein, and plasma IGF-1 concentrations in growing rats. Cossack found that the weight gains ($P<0.001$) and plasma IGF-1 concentrations ($P<0.005$) were significantly less in the low Zn rats (0.9 μg Zn /kg) regardless of dietary protein content. The author reported that increasing the Zn content of the low protein diet (75g protein/kg) resulted in increased weight gains and plasma IGF-1 concentrations. Cossack (1986) concluded that a nutritionally balanced diet plus adequate feed intake are required for maintenance of homeostatic concentrations of IGF-1. It is interesting to note that the rats in the Cossack (1986) study with the highest plasma IGF-1 concentrations also had the highest rate of weight gain.

Dorup et al. (1991) also examined the relationship between Zn deficiency and its associated growth retardation. Weanling rats were fed a Zn deficient diet for 14 days followed by a repletion period of 33 days. The Zn deficient rats exhibited decreased body mass accretion, serum Zn and IGF-1 concentrations. With Zn repletion, the Zn deficient rats experienced a rapid increase in growth as well as increased serum Zn and IGF-1 concentrations. The serum Zn and IGF-1 concentrations returned to normal after 3 and 17 days of Zn repletion, respectively. After the 33 days of repletion, the Zn deficient rats were unable to attain body weights that were similar to the control group. Unfortunately there was no analysis of the composition of the accelerated

weight gain during Zn repletion. The researchers speculated that the retarded growth seen in Zn deficiency involved decreased IGF-1 production. Work by McNall et al. (1995) lent support to the Dorup et al. (1991) speculation.

Zn deficient rats in the McNall et al. (1995) study exhibited the characteristic reduction in growth, serum and bone Zn concentrations, and serum IGF-1 concentrations associated with dietary Zn deficiency. Utilizing Northern blot analysis McNall et al. (1995) found that Zn deficient rats had altered production of liver IGF-1 mRNA, the primary source of serum IGF-1 (D'Ecrole et al., 1984). More specifically, McNall et al. (1995) found that the 7.5-kb IGF-1 mRNA transcript was markedly reduced in Zn deficiency while the other IGF-1 mRNA transcripts were more sensitive to decreased feed intake. This study also found that both GH receptor and GH binding protein mRNA concentrations were notably reduced in Zn deficiency. The researchers speculated that the growth retardation seen in Zn deficiency was associated with alterations in the GH signaling pathway.

These studies document the growth retardation associated with Zn deficiency as well as the parallel reduction in serum IGF-1 concentrations. The exact mechanisms of dietary Zn deficiency which lead to reduced IGF-1 and growth are not clear. The studies by Dorup et al. (1991) and McNall et al. (1995) point to defects in IGF-1 production and the GH signaling pathway. Both types of endocrine defects have been associated with decreased body size (Howard et al., 1981; Merimee et al., 1981; Eigenmann et al., 1984). Zn repletion has been associated with increased serum IGF-1 concentrations and rapid weight gain in humans and rats (Ghavami-Maibodi et al., 1983; Dorup et al., 1991; Nakamura et al. 1993). None of the above mentioned studies reported on the composition of the weight gained during Zn repletion. Muscle and fat tissue (with fat being the lighter tissue) are the major components of changes in body mass (Forbes, 1987). During a 33-day Zn repletion, the rats in the Dourp et al. (1991) study did not attain body masses similar to the control group. This leads one to ask the following question: Is the body composition of Zn repleted rats and humans like that of well nourished counterparts?

Muscle

Body Composition and Growth

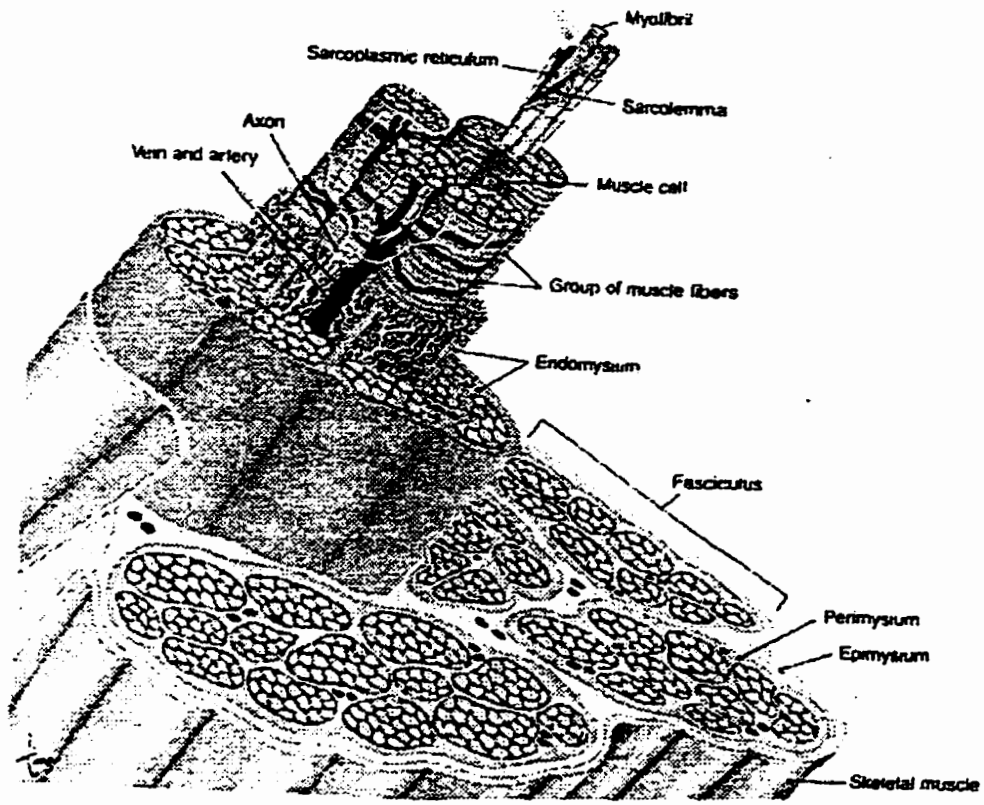
With growth, the percentage of body mass occupied by different types of tissues changes. Skeletal muscle accounts for a large percentage of body mass throughout the life cycle (Forbes, 1987). Changes in skeletal muscle mass can be documented in several ways, including percentage of total body weight and changes in muscle fiber area and diameter.

The human neonate is 25% skeletal muscle and 10-15% is adipose tissue. After 20-25 years of growth and development, the body mass of an adult male can be 40% skeletal muscle and 18% adipose tissue depending on activity, nutrition and health (Forbes, 1987). This increase in muscle mass is reflected in the histological structure of the muscle. The accretion of skeletal muscle mass in healthy humans and rats is associated with increases in muscle fiber area and diameter (Alanqeeb and Goldspink, 1986; Forbes, 1987).

Structure and Adenosine Triphosphatase Staining

A skeletal muscle fiber houses thousands of myofibrils and the contractile units (myosin and actin) that allow for the voluntary movement of the limbs, eyes and tongue (Gylys and Wedding, 1983). Each muscle fiber is jacketed by sarcoplasmic recticulum and a sarcolemma (See Figure 1). Groups of muscle fibers are bundled together into muscle fascicles that are surrounded by a perimysium and an epimysium (Krstić, 1984). Different numbers and lengths of muscle fibers are found in different skeletal muscles such as vastus lateralis, gastrocnemius and gluteus maxiumus (Krstić, 1984).

Histochemical staining of frozen skeletal muscle biopsy sample allows a pathologist to gather information about the health and/or pathology of the muscle (Dubowitz and Brooke, 1973; Samat, 1983). A routine histochemical stain, adenosine triphosphatase (ATPase), identifies the different muscle fiber types.



Adapted from Gylys & Wedding, 1983

Figure 1. Cross-sectional view of skeletal muscle structures

ATPase is an enzyme in muscle fibers that splits the terminal phosphate from the adenosine triphosphate (ATP) molecule. The first step in ATPase staining involves the exposure of a thin (10 μm) slice of frozen muscle to an acidic (pH 4.3 or 4.6) pre-incubation solution. The other muscle sample is not pre-incubated. Both muscle samples are incubated in an alkaline solution of calcium and ATP at 37°C. The ATPase enzyme splits the terminal phosphate from the ATP and the free phosphate is immediately bound by calcium creating calcium phosphate. The calcium phosphate is insoluble in the alkaline incubation solution and is deposited at the site of the ATPase activity. The muscle samples are then placed in a solution of cobalt chloride. The cobalt replaces the calcium, forming cobalt phosphate at the site of ATPase activity. When the muscle samples are exposed to a solution of ammonium sulphate, insoluble black cobaltous sulphide is formed. The black cobaltous sulphide marks the original site of ATPase activity (Dubowitz and Brooke, 1973; Sarnat, 1983). The pH of the pre-incubation solution or the omission of pre-incubation determines level of adenosine triphosphatase enzyme activity in each type of muscle fiber allowing for a color based differentiation of muscle fiber types (Sarnat, 1983).

An ATPase stain performed without pre-incubation (called ATPase pH9.4) results in the type 1 (slow twitch) muscle fibers appearing light in color (low ATPase activity) and the type 2 (fast twitch) muscle fibers appearing dark in color (high ATPase activity)(Sarnat, 1983)(See Figure 2). When the APTase stain is preformed with acidic pre-incubation at pH 4.3 (ATPase pH4.3) the type 1 muscle fibers are dark and the type 2 fibers are light in color (Sarnat, 1983). The ATPase stains allow the pathologist to assess the effects of growth, disease and malnutrition on the size, shape and distribution of the muscle fiber types.

Muscle Growth: Relationships with IGF-1 and Zn

i. IGF-1

As discussed earlier, both IGF-1 and its mRNA have been found in skeletal muscle (D'Ecrole et al., 1984; Turner et al., 1988; Issgarrd et al., 1989). As an anabolic peptide IGF-1 can potentially play many roles in skeletal muscle physiology.

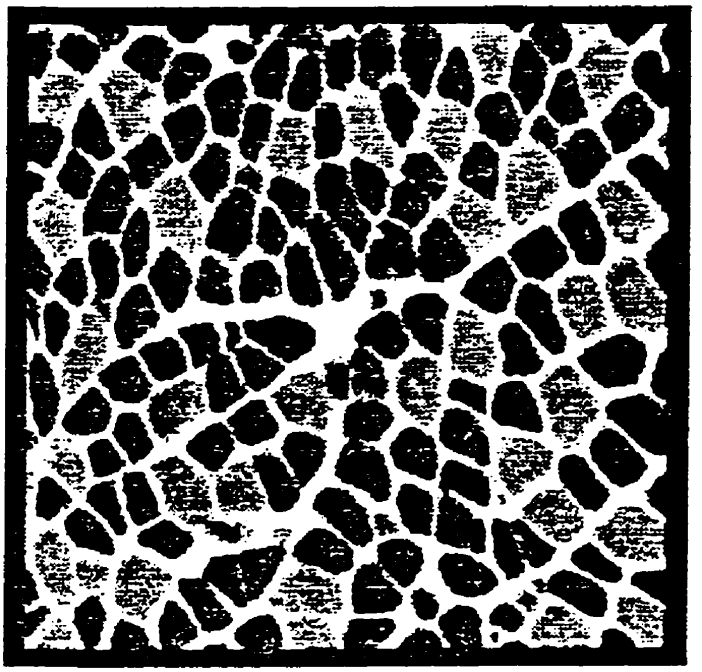


Figure 2. Example of ATPase pH9.4 differentiation of rat skeletal muscle fibers types. In this black and white photo, the type 1 muscle fibers are gray and the type 2 muscle fibers are black. In color, the type 1 muscle fibers are light brown and the type2 fibers are dark brown-black. This photo represents the size and shape of the muscle fibers from a well-fed 3-week-old male rat.

An immunohistochemical study by Jennische and Oliverorona (1987) demonstrated the presence of IGF-1 immunoreactivity during part of the normal post-natal growth of rat hind-limb skeletal muscle. The authors speculated that the IGF-1 detected was produced by immunoreactive muscle fibers and contributed to the muscle growth and development in an autocrine and paracrine fashion. Studies by Zanconato et al. (1994) and Adams and Haddad (1996) reported that rat skeletal muscle hypertrophy associated with exercise training and muscle overload was accompanied by increased IGF-1 mRNA expression. This increase in IGF-1 mRNA occurred with and without the presence of endogenous GH (Znaconato et al., 1994; Adams and Haddad, 1996). After irreversible ischemic damage, the extensor digitorum longus (EDL) muscle of rats demonstrated increased IGF-1 mRNA levels (Edwall et al., 1989) and increased IGF-1 peptide immunoreactivity (Jennishe et al., 1987). Meanwhile, the counterlateral muscle showed no increase in IGF-1 expression. Edwall et al. (1987) and Jenniche et al. (1987) noted that not all muscle fibers in the targeted muscle were damaged and that IGF-1 expression was increased only in the injured regenerating muscle fibers. Both groups of researchers stated that IGF-1 might act as a tropic factor during the regeneration of skeletal muscle after ischemic injury. In another study, Jennishe and Hanson (1987) injected a myotoxic snake venom, taipoxion, into the soleus muscle of mice. The taipoxion caused muscle necrosis that was associated with macrophage invasion. Two days after injection, increased IGF-1 immunoreactivity was observed in regenerating muscle fibers while no changes in IGF-1 immnuoreactivity were documented in the saline injected control mice. The authors also noted that the increase in IGF-1 immnoreactivity was limited to damaged muscle cells. Muscle cells unaffected by the taipoxion injection showed no changes in IGF-1 immnoreactivity.

These studies implicate IGF-1 as an important anabolic factor during the normal growth, hypertrophy and regeneration of skeletal muscle.

ii. Zinc

Many years of research have established that Zn is essential for optimal growth and development (Prasad, 1984; Golden, 1989; Cousins and Hempe, 1990) and that normal growth and development is accompanied by skeletal muscle accretion in rats and humans (Alnaqeeb and Goldspink, 1986; Forbes, 1987).

O'Leary et al. (1979) explored the effects of dietary Zn deficiency on skeletal muscle growth. The authors reported that the Zn concentration of the skeletal muscles of the Zn deficient rats was not significantly different from that of the pair fed and the control group. The Zn deficient rats had skeletal muscle weights that were significantly less ($P < 0.001$) than pair fed and control rats. If the reduction in muscle weight was not associated with decreased cellular Zn concentration, how did dietary Zn deficiency reduce muscle mass accretion? Giugliano and Millward (1987) explored the relationship between dietary Zn deficiency and reduced skeletal muscle mass accretion. The authors reported that the Zn deficient rats had decreased DNA and protein accumulation in gastrocnemius and soleus muscles. After 10 days, the protein synthesis (measured by muscle [^{3}H]phenylalanine incorporation) and RNA content were reduced in the Zn deficient rats compared to the pair fed rats. Muscle protein catabolism was reported to be faster in the Zn deficient rats versus pair fed rats. After 17 days, the gastrocnemius muscle of the Zn deficient rats had significantly less ($P<0.01$) total protein and DNA compared to the pair fed and control groups. The soleus muscle of the Zn deficient rats also had significantly less total protein ($P<0.01$) and total DNA ($P<0.05$). Eight days of Zn repletion resulted in a significant increase in gastrocnemius muscle weight ($P<0.01$) and in total protein ($P<0.01$) and total DNA ($P<0.05$) content. The weight of the soleus muscle also increased ($P<0.01$) with Zn repletion. Giugliano and Millward (1987) speculated that the reduction in skeletal muscle accretion was due to increased protein catabolism (associated with decreased feed intake) and decreased protein synthesis capacity. The authors hinted that the decreased protein synthesis could be associated with decreased IGF-1 bioactivity. An earlier study by Park et al. (1986) also found that the skeletal muscle of Zn deficient rats was significantly lighter ($P<0.05$) and contained significantly less

total DNA, RNA and protein. Dorup and Clausen (1991) reported that young rats fed a Zn deficient diet for 14 days had decreased soleus and EDL muscle weights ($P<0.001$) and no significant changes in muscle Zn concentration. [^{3}H]leucine incorporation in to muscles of the Zn deficient rats was significantly less ($P<0.001$) compared to pair fed rats. Dorup and Clausen (1991) utilized relative pair feeding which is less severe than the usual pair feeding described earlier, yet the growth and muscle accretion of Zn deficient rats were still significantly less than the pair-fed controls. The authors concluded that Zn deficiency has its own restrictive effect on protein synthesis independent of decreased feed intake and that the suppression of growth and protein synthesis occurred without significant changes in cellular Zn concentration.

The above studies documented that Zn deficiency impeded skeletal muscle accretion and maturation without changes in cellular Zn concentration. As discussed previously, Zn deficiency (a form of mirconutrient malnutrition) impeded the overall growth and development of children and rats. Inadequate dietary intakes of protein and energy (macronutrient malnutrition) also derail skeletal muscle and overall growth and development in young mammals.

Malnutrition

According to the 1998 World Health Organization (WHO) report on the state of the world's children, 67 million children suffer from malnutrition and 26 million die due to complications of malnutrition. It is estimated that 226 million children are shorter than and 183 million weigh less than same age controls (WHO, 1998).

In the face of decreased and/or unbalanced dietary intake, the primary physiological adaptations of the body are to change the mix of metabolic fuels utilized and to decrease overall energy expenditure (Hunt and Groff, 1990). At first the body tries to meet immediate glucose needs via glycogenolysis and gluconeogensis. The body's proteinaceous tissues (especially skeletal muscle) are catabolised to provide the amino acid substrates for gluconeogensis. If carbohydrate continues to be a scarce fuel, an increasing percentage of energy needs are met via lipolysis and fatty acid metabolism. Eventually, ketones become the primary metabolic fuel and glucose utilization is diminished. This reduces the amount of protein catabolism required to produce amino acid substrates for gluconeogensis. In addition to changing metabolic fuels, the body decreases energy expenditure. Energy is saved by the decrease in metabolically active tissue (weight loss), a decrease in the metabolic rate and by decreased physical activity (McLaren,1981). Since growth requires an energy output above maintenance levels, it is incompatible with the strict energy rationing of malnutrition.

Protein Energy Malnutrition

Protein Energy Malnutrition (PEM) is a macronutrient form of disordered nutrition brought on by various factors that can strike at any age. Inadequate dietary intake and disease are the immediate causes of PEM (WHO, 1998; Wardlaw and Insel, 1990). Children and the elderly are among the most vulnerable segments of the population to PEM due to their physiological situations of intensive growth and progressive aging, respectively (WHO,1998; Reuben et al., 1995). Kwashiorkor and marasmus are the most severe forms of PEM and are usually found in children of developing countries (Latham, 1990). Both forms of PEM have differential hallmarks. The general characteristics of kwashiorkor include edema, low serum albumin and fatty liver.

Marasmus's characteristics include a protuberant belly and a weight for age less than 60% of standards. The common characteristics of children suffering from PEM are marked muscle wasting and growth retardation (McLaren, 1981 and Latham, 1990). In short, childhood PEM is synonymous with growth failure (WHO, 1998).

PEM Recovery: Compensatory Growth

Nutritional rehabilitation following childhood PEM is characterized by rapid body mass accretion (Ashworth, 1969; Brooke and Wheeler, 1976; Payne-Robinson, 1980). This period of rapid body mass gain is called compensatory growth.

Ashworth (1969) described the growth rates of recovering PEM children during compensatory growth and after complete rehabilitation were achieved. In the Ashworth study and in other studies, complete rehabilitation was declared when PEM children had achieved expect weight for height (Montgomery, 1962 & Cheek et al., 1970. During compensatory growth the recovering children had growth rates that were 15 and 5 times greater than the expected growth rates of well-nourished children of similar age or height. Ashworth (1969) reported that the compensatory growth period was accompanied by significantly higher feed intakes and feed efficiency ($P<0.001$ and $P<0.05$, respectively) compared to values observed after weight for height was achieved. When the children achieved expected weight for height, feed intakes, growth rates and feed efficiency dropped.

MacLean and Graham (1980) reported that compensatory growth was associated with excessive adipose tissue accretion and poor muscle mass recovery. In short, recovered children at expected weight for height did not achieve expected body composition. The results of the above studies raise the following questions:

1. What happened to skeletal muscle mass during compensatory growth?

2. What prevented full recovery of skeletal muscle mass during compensatory growth?

Malnutrition to Recovery

Muscle

i. Children

PEM is associated with a loss of lean body mass (Reeds et al., 1978). This muscle wasting not only effects the skeletal muscles, but the smooth and cardiac muscles as well (Latham, 1990). The degree of skeletal muscle wasting is most evident in histological studies by Montgomery (1962) and Hansen-Smith et al. (1978). These studies documented diminished muscle fiber size and area of PEM children [8-16 months old]. Montgomery's (1962) histological notations described the sartorius muscles and the individual muscle fibers of the PEM children as extremely reduced in cross-sectional area compared to control subject data. Crowding of the sub-sarcolemmal nuclei was also noted in the 1962 Montgomery study. Hansen-Smith et al. (1978) studied the vastus lateralis muscle fiber areas of children (mean age 15 months) diagnosed with various types of PEM. No significant differences in muscle fiber area were found between the different types of PEM. All PEM subjects had muscle fiber areas that were significantly less than controls of similar age. This study included serial muscle biopsies that documented the changes in muscle fiber area throughout recovery. In the early phase of recovery the muscle fiber areas nearly doubled in size compared to the PEM values. Thereafter the muscle fiber areas increased in a steady fashion. By the time the PEM children had attained expected weight for height (mean age 13.8 months) their muscle fiber areas were still remarkably smaller than that of a 6-month-old control subject. Since muscle fiber area recovery did not happen when expected weight for height was attained, the authors speculated that there was excessive production of another type of tissue.

A study conducted by Graham et al. (1969) examined changes in body composition during and after nutritional rehabilitation in 5-30 month old PEM infants. Initial assessment of the PEM infants showed loss of muscle and visceral cell mass. With nutritional rehabilitation there was recovery of cell mass. In the older infants (>11 months) there was better recovery of muscle cell mass as compared to the younger infants. The younger subjects had greater gains of fat

mass. The authors concluded that the compensatory growth during recovery from PEM is not uniform and is not a resumption of normal growth.

Cheek et al. (1970) measured muscle protein concentration and RNA to DNA ratios before and after nutritional rehabilitation in order to gain insight into muscle chemistry. The rehabilitation data was taken 4-9 months after the initial assessment. The protein concentration and the RNA to DNA ratio of the subjects before and after rehabilitation were significantly lower ($P<0.01$) than well nourished controls. Although only 9 subjects were enrolled in this study, the documentation of abnormal muscle chemistry underlines the persistent alterations to muscle homeostasis caused by PEM despite rehabilitation and body mass accretion.

A retrospective study by MacLean and Graham (1980) compiled the results of nitrogen retention studies conducted on PEM children rehabilitated on diets with different protein sources and energy levels. The diets included in this study were isonitrogenous. The percentage of weight gained as nitrogen was calculated from nitrogen retention and weight change data. Overall, the authors found that the children studied had a tendency to regain more fat mass versus muscle mass and that the recovery of lean body mass was not as efficient at higher rates of weight gain.

ii. Rats

A review by Dastur et al. (1982) outlined several studies documenting the reduced muscle fiber size of PEM rats. Young rats subjected to protein deficiency had decreased muscle fiber size (Oldfors et al., 1983). The type 2B muscle fibers atrophied while the type 1 and type 2A fibers did not grow. The Oldfors et al. (1983) study did not include nutritional rehabilitation. A histochemical and morphometeric study lasting 180 days by Haltia et al. (1978) included nutritional rehabilitation of rats subjected to pre and post-natal PEM. Histochemical staining detected type 1 muscle fibers in the control rats at birth, but not in the PEM rats. This delayed muscle fiber differentiation was no longer notable 5 days after birth. Thereafter muscle fiber differentiation was reported to have proceeded normally. Haltia et al. (1978) reported that the PEM rats had reduced muscle fiber cross sectional areas in all muscle fiber types at all time

points studied. The reduced muscle fiber area of the PEM rats was significantly different from the control group during the PEM and recovery phases ($P<0.01$). Nutritional rehabilitation did not fully correct this difference.

Like Cheek et al. (1970), Howarth (1972) examined the muscle chemistry of protein deficient rats. The experimental rats were fed 6%, 12% or 18% protein diets for 14 days while the control group was fed a 24% protein diet. The protein deficiency resulted in reduced weight gains that corresponded to the protein content of the diet. The weights of the gastrocnemius from the protein deficient groups were significantly less than the control group ($P<0.01$). The increase in muscle protein and DNA content observed in the 12% protein group and in the 6% protein group was significantly less ($P<0.01$) than the control group. The muscle protein and DNA content of the 18% protein diet rats was not significantly different from the control group ($P<0.1$). The DNA content of muscle from the 6% protein diet rats was not significantly different from the baseline data obtained from 3 week-old animals terminated on day 0 of the study. The author concluded that DNA synthesis, the first event of cell growth, was more sensitive to the protein deficiency than protein synthesis. Howarth (1972) speculated that the imposed nutritional deficiency caused a shift in metabolic priorities with the synthesis of new muscle tissue falling to the wayside.

These studies and others document the poor recovery of muscle fiber size and overall muscle mass in rehabilitating PEM children and rats. Although the rehabilitation diets were adequate to produce rapid weight gains, they were unable to ensure normal body composition when expected body mass was achieved. These results raise the following question: What factor is limiting the metabolism of dietary nutrients into muscle mass during recovery from PEM?

Dietary Zinc

Recovery from PEM as noted by Graham et al. (1969) is not a simple resumption of normal growth. Poor muscle recovery and excessive gains of fat mass (Golden, 1989) characterize compensatory growth in PEM rehabilitation. Since normal growth is a time of high nutritional requirements and Zn is critical for growth and development (Prasad, 1984; Golden, 1989) compensatory growth can be viewed as a time of intensive nutritional requirements,

particularly for Zn (Morgan et al. 1988). Castillo-Duran et al. (1987) and Simmer et al. (1988) both found that Zn supplementation (2-10 mg Zn /kg body weight) during recovery lead to significantly improved weight gains versus the unsupplemented group. The 1987 Castillo-Duran study also found improved host defense with Zn supplementation. Infection is a major perpetuating factor of childhood malnutrition (WHO, 1998).

Golden and Golden (1981a) postulated that Zn requirements for tissue synthesis in compensatory growth could exceed the dietary supply. The researchers hypothesized that Zn could be the determining factor for the rate of weight gain and the type of new tissue synthesized. Golden and Golden (1981a) found that the plasma Zn concentrations were low in children throughout the high-energy nutritional rehabilitation period. The researchers noted that the fall in plasma Zn was more severe in the children fed a soy based diet versus a bovine milk diet. The authors found that a high rate of weight gain was associated with lower plasma Zn concentrations. This was a significantly negative relationship. The authors used energy cost of growth calculations developed by Jackson et al. (1977) to gain insight into the efficiency of recovery as well as the type of tissue synthesized. The cost of growth is the result of the energy available for tissue deposition (total energy intake minus energy required for weight maintenance) divided by the weight gained during recovery (Jackson et al., 1977). Jackson et al. (1977) found a significant correlation between the cost of growth and the percentage of weight gained as muscle mass. A low cost of growth was associated with a greater percentage of weight gained as muscle mass and a high cost of growth was associated with greater gains of fat mass. Golden and Golden (1981a) found that the cost of growth at the beginning of the recovery period was lower in the cow's milk fed subjects and relatively high in the soy fed subjects. However the low cost of growth seen in the cow's milk fed children was still high when compared to the theoretical costs of lean and fat mass accretion. This indicates that these children were gaining most of their body weight as adipose tissue. Overall, this study found that as plasma Zn dropped, the cost of growth increased. The authors speculated that the demand for Zn in new tissue synthesis exceeded supply and led to changes in the composition of weight gained.

To investigate this theory Golden and Golden (1981b) supplemented the rehabilitation diet of 16 PEM children with a Zn acetate solution to provide an additional 25-150 umol Zn /kg body weight. The Zn supplementation resulted in higher plasma Zn concentrations and a significant rise in the rate of weight gained in soy and cow's milk fed children. The Zn supplementation was also associated with a re-growth of the thymus and activation of the sodium pump in leukocytes. Both the changes in the rate of weight gain and in the energy cost of growth were significantly correlated to changes in plasma Zn concentrations. An increase in plasma Zn was associated with an increase in the rate of weight gain ($r = 0.74$, $P<0.01$). A drop in the cost of growth was associated with increases in plasma Zn concentration ($r = 0.77$, $P<0.001$). Based on the physiological responses observed in the children to Zn supplementation, the researchers concluded that the children studied were Zn deficient during the recovery period.

Golden and Golden (1992) further investigated the relationship between the cost of growth and plasma Zn by documenting the effect of Zn supplementation on net nitrogen absorption in recovering PEM children. Nitrogen balance and protein synthesis studies were used to indirectly estimate muscle protein accretion. The children studied had a mean age of 15 months and were fed a soy protein based diet. The children were divided into 3 Zn supplementation groups, low Zn (no supplementation), moderate Zn (76 umol Zn/kg diet) and high Zn (153 umol Zn per kg diet). The Zn supplemented children ingested the same amount of diet as the unsupplemented children. Net nitrogen absorption as a percentage of nitrogen intake increased in the Zn supplemented groups during the recovery period, but not in the unsupplemented group. For this parameter, repeated measures ANOVA revealed that there was a significant independent effect of Zn ($P<0.01$), time ($P<0.05$) as well as an interaction between Zn and time ($P<0.05$). Nitrogen retention increased in all groups with rehabilitation. The nitrogen retention per gram of weight gain continued to increase during mid and late recovery in the supplemented groups, but not in the unsupplemented group. Repeated ANOVA showed that only time was significant for this parameter. The authors concluded that Zn supplementation influenced intermediary nitrogen metabolism and thus influenced the type of tissue synthesized.

Morgan et al. (1988) conducted a study where the offspring of undernourished female mice rehabilitated on a low (5 ppm Zn), marginal (10 ppm Zn) or high Zn diet (40 ppm Zn). The control group mice were fed a 25% protein diet containing 30 ppm Zn. The pups recovered on the marginal Zn diet had body weights that were similar to the control group, but had smaller lean mass gains and the male pup had larger fat mass gains. The mice rehabilitated on the low Zn diet did not achieve control group body weight. This group of mice also had significantly smaller protein mass gains compared to the control group and the mice recovering on the high (40 ppm) and marginal (10 ppm) Zn diets. Mice fed the high Zn recovery diet attained control body weights and normal body composition. Morgan et al. (1988) concluded that the low Zn diet was unable to meet the Zn requirements of compensatory growth and resulted in limited weight and protein recovery meanwhile the marginal Zn diet limited protein mass recovery.

The above human and animal studies implicate Zn as an important nutritional factor in PEM recovery diets. The Zn supplementation of the recovery diets led to better weight gains as well as better recovery of lean body mass.

Insulin-like Growth Factor-1

i. Children

As documented before, IGF-1 bioactivity is regulated by nutritional status. Therefore it is not surprising that decreased IGF-1 bioactivity and concentration accompany the clinical diagnosis of PEM. Hintz et al. (1978) and Mohan and Rao (1979) found that children diagnosed with PEM had significantly lower serum and plasma IGF activity ($P<0.01$ and $P<0.001$, respectively) compared to well nourished age matched controls. Serum and plasma IGF activity was assessed by porcine and rat cartilage ^{35}S incorporation assay. Both teams of researchers reported that the PEM children had normal or elevated circulating GH concentrations. With normal or high circulating GH concentrations one might expect somewhat normal GH activity including IGF-1 expression and bioactivity. In the PEM state GH peptide concentration was not representative of GH activity. Since these studies reported circulating IGF 'activity', it is important

to assess if there was a coordinate change in IGF-1 concentration. Later studies by Soliman et al. (1986) and Smith et al. (1989) utilized IGF-1 radioimmunoassays and reported that PEM children had significantly lower circulating concentrations of IGF-1. These IGF-1 radioimmunoassay studies documented that childhood PEM is associated with decreased circulating IGF activity and IGF-1 concentrations. Soliman et al. (1986) and Smith et al. (1989) also reported elevated GH concentrations.

Hintz et al (1978) described the inverse relationship between GH concentration and IGF activity in PEM as a physiological block in the synthesis and/or release of IGF. Mohan and Rao (1979) reported that when plasma albumin concentrations dropped below 36.3 μmol/L, plasma GH concentrations increased and plasma IGF activity decreased. Nutritional rehabilitation in all of these studies was reported to increase circulating IGF activity and IGF-1 concentration as well as decrease GH concentrations to normal. It is interesting to note that Soliman et al. (1986) reported that serum concentration of IGF-1 in PEM children was significantly correlated with the percent weight deficit ($r = -0.521$, $P<0.001$), expected height ($r=0.42$, $P<0.001$) and weight for $(height)^2$ index ($r=0.34$, $P<0.01$). Percent weight deficit, expected height and weight for $height^2$ index are parameters that are frequently used to assess growth and nutritional status.

These studies documented that childhood PEM is associated with significant reductions in circulating IGF activity and concentration and that nutritional rehabilitation increased both circulating IGF-1 activity and concentration.

ii. Rats

Like children with PEM, protein and energy deprived rats also have decreased circulating IGF-1 concentrations (Prewitt et al., 1982; VandeHaar et al., 1990; Yahya et al., 1990). Prewitt et al. (1982) randomly assigned 4 week-old rats to one of 9 diets that consisted of 3 levels of energy (ad libitum, 75% and 50% of ad libitum intake) and 3 levels of protein (5, 10 and 15% lactalbumin). Serum IGF-1 concentrations were assayed when the rats were 5, 6, 9 and 12 weeks of age. In the 5 week-old rats, dietary protein level had the greatest impact on serum IGF-1

concentrations. Serum IGF-1 concentrations increased linearly as the level of dietary protein increased. Energy intake was reported to only have a significant impact in the 15% protein diet groups at this time point. Increasing the energy intake from 50% to 75% in the 15% protein group resulted in a significant increase in serum IGF-1 concentration ($P<0.05$). In the 6, 9 and 12 week old animals both protein and energy level significantly influenced serum IGF-1 concentrations ($P<0.05$). Increasing the dietary protein level increased serum IGF-1 concentrations, but the level of energy intake modulated the magnitude of the increase. Prewitt et al. (1982) also found that serum IGF-1 concentration was significantly correlated with body weight ($r= 0.84$, $P<0.001$) and tail length ($r=0.74$, $P<0.001$) at all time points. The authors concluded that serum IGF-1 concentrations were able to reflect both growth and nutritional status.

VandeHaar et al. (1990) and Yahya et al. (1990) reported that 5% and 0.5% protein isocaloric diets, respectively, resulted in significantly reduced plasma and serum IGF-1 concentrations compared to rats fed 15% and 20% protein diets. These authors went one step further and examined the effects of protein deficiency on the skeletal muscle expression of IGF-1. VandeHaar et al. (1990) reported that 2 groups of weanling rats fed a 5% protein diet for 1 week and 3 weeks had a 34% ($P<0.001$) and 24% ($P<0.01$) reduction in skeletal muscle IGF-1 mRNA abundance compared to control rats. Yahya et al. (1990) found that skeletal muscle IGF-1 concentrations of rats fed a 0.5% protein diet for 1 week and 3 weeks were 39.7% and 35.3%, respectively, of control rats.

These studies demonstrated that rats subjected to dietary protein and energy deficiencies also have reduced circulating IGF-1 concentrations and impaired growth like children with PEM. The studies by VandeHaar et al. (1990) and Yahya et al. (1990) demonstrated that the effects of protein deficiency on IGF-1 expression and concentrations extended to the skeletal muscle. However, these studies did not include nutritional rehabilitation.

Cossack (1988) subjected growing rats to a 72 hour fast followed by nutritional rehabilitation on diets of different Zn concentrations (0.9, 30, 90 and 140 ppm Zn). The 30, 90 and 140 ppm Zn rats were pair fed to the 0.9 ppm Zn group to ensure that refeeding was isocaloric and isonitrogenous. Eight rats were terminated before and after the 72 hour fast to serve as time

0 (t_0) and time 3 (t_3) control groups. Another group of eight rats was fed a nutritionally complete diet throughout the study. Cossack reported that serum IGF-1 concentrations dropped significantly after the 72 hour fast compared to the well nourished group ($P<0.05$). In the fist 24 hour of nutritional rehabilitation, there was a significant increase in serum IGF-1 concentration regardless of dietary Zn concentration ($P<0.005$). After 48 hours of refeeding, the serum IGF-1 concentrations of the rats fed the 0.9 and 30 ppm Zn diets dropped significantly ($P<0.05$). In the 90 and 140 ppm Zn groups, serum IGF-1 concentrations continued to increase and reached control levels after 8 days of refeeding. The serum IGF-1 concentrations of the 0.9 and 30 ppm Zn groups were still significantly less than the other groups after 8 days of refeeding. Cossack (1988) concluded that the Zn content of the refeeding diet was an important factor in the normalization of serum IGF-1 concentration following starvation in rats and that careful consideration should be given to dietary Zn content when planning PEM recovery diets.

II. STUDY RATIONALE

Millions of children suffer from Zn deficiency and PEM. Both Zn deficiency and PEM are associated with decreased serum IGF-1 concentration and retarded growth and development. Nutritional rehabilitation leads to compensatory growth, but recovery of muscle mass is incomplete and adipose accretion is excessive. Zn status during compensatory growth is tenuous. The addition of Zn to the rehabilitation diet enhances the recovery of skeletal muscle mass. IGF-1 is an anabolic peptide associated with skeletal muscle hypertrophy and regeneration. The circulating concentration of IGF-1 is strongly influenced by nutritional state, including Zn, protein and energy status.

It is speculated that sub-optimal Zn status during compensatory growth could result in decreased serum and tissue IGF-1 concentrations, which could lead to poor recovery of skeletal muscle mass and fiber diameter. Since this speculation would be difficult to explore in a single study, the hypothesis of this study is that the full recovery of muscle fiber diameter following Zn, protein and energy deficiencies is dependent upon the full recovery of serum IGF-1 concentration and Zn status (as determined by serum and femur Zn concentrations).

The objectives of this study were:

1. To investigate the effects of Zn, protein and energy deficiencies on skeletal muscle mass and fiber diameter in relation to serum IGF-1 concentration in young rats
2. To examine the effects of a standard 30 ppm Zn diet on compensatory growth, the recovery of skeletal muscle mass and fiber diameter and serum IGF-1 concentrations following Zn, protein and energy deficiencies.

In order to meet the objectives of the study, weanling rats were subjected to either Zn, protein, energy or combined Zn and protein deficiency via dietary manipulation. Weanling rats were chosen for this study, as they are poised for rapid growth and development like many children who suffer from Zn deficiency and PEM. Studies concerning IGF-1, malnutrition and recovery from malnutrition frequently use the rat model. The weanling rats were fed the deficient

diets for 3 weeks (deficiency phase). Then rats from each dietary group were rehabilitated on a 30 ppm Zn diet for 3 weeks (recovery phase). Each phase of the study was 3 weeks long to ensure that the rats entered the desired state of deficiency and that the rats would have an opportunity to complete compensatory growth.

Previous studies have examined changes in IGF-1 concentrations or changes in skeletal muscle mass before and after Zn, protein and energy deficiencies. An extensive review of the available literature has not revealed any studies that have examined the effects of, and recovery from, Zn, protein, energy and combined Zn and protein deficiencies in relation to skeletal muscle fiber histology and serum IGF-1 concentrations. By examining these nutritional deficiencies from this standpoint this thesis project will assist in the characterization of Zn deficiency and skeletal muscle recovery in compensatory growth.

III. Materials and Methods

Experimental Design

Eighty-eight three week-old weanling Sprague Dawley rats (Charles River Laboratories, St Constant, PQ) were used to conduct a six week study. Eight rats per group were used as most studies regarding Zn deficiency use six rats per group and there was an extra two rats in case of mortality. The weanling rats were randomly assigned to either the baseline (W) group and terminated on day zero or to one the following five dietary treatment groups: Zinc + protein deficient (ZP, 2% protein + < 1ppm Zn), zinc deficient (Z, 20% protein + <1ppm Zn), protein deficient (P, 2% protein + 30 ppm Zn), energy deficient (E, 20% protein + 30 ppm Zn) or control (C, 20% protein + 30 ppm Zn). All diets were based on the AIN-93G diet for growing rats (see Table 1 for diet composition). The ZP, Z, P and C groups were fed ad libitum while the E group's access to the nutritionally complete control diet was limited. The quantity of control diet given to each E rat was determined by the amount of diet consumed by its Z group counterpart on the previous day. The E group acted as the pair fed controls for the Z group as well as a model for energy deficiency. The rats were fed in this manner during the 3 weeks of the deficiency phase. At the end of the deficiency phase, 8 animals from each group were terminated. The remaining 40 animals entered the 3 week recovery phase and were fed the control diet ad libitum. At the end of the recovery phase, the remaining 8 animals per group were terminated (see Figure 3).

Great care was taken during the mixing of the diets to avoid zinc contamination. The work area was washed before ingredients were opened as were the Hobart mixer, stainless steel bowl and paddle used to prepare the diet. The surface of containers were washed before opening, and hands were washed before and after the addition of each ingredient. The double deficient diet was mixed first followed by the single deficient and the control diets. The diets were mixed for 10 minutes and checked visibly for homogeneity. The diets were mixed for as short a time as possible to avoid heating the diet and compromising the integrity of the soybean oil. The diets were stored in polyethylene bags in plastic containers at 4°C. The diets were fed to the animals in glass jars that were weighed daily to estimate feed intake.

Table 1.

Diet Formulation and Nutritional Information

Ingredients[1]	Diet Formulation (g/kg)			
	ZP[2]	Z[2]	P[2]	C[2]
Dextrose	851.3	664.6	841.3	654.6
Egg white	25	212.5	25	212.5
Soybean oil	70	70	70	70
Mineral Mix[3]	35	35	35	35
Potassium phosphate	6.2	5.4	6.2	5.3
Vitamin mix[4]	10	10	10	10
Choline	2.5	2.5	2.5	2.5
Zinc pre-mix[5]	0	0	10	10
Nutrition Information [6]				
Kcal/gram diet	3.67	3.64	3.67	3.64
Protein (%kcal)	2.18	18.69	2.18	18.69
Carbohydrate (%kcal)	80.67	63.99	80.67	63.99
Fat (%kcal)	17.15	17.32	17.15	17.32
Zinc (ppm)	1.95	0.76	30.00	28.71

[1] Diet ingredients were purchased from Harlan Teklad (Madison, WI) with the exception of the soybean oil (Vita Health, Winnipeg, MB) and the dextrose (Moonshiners, Winnipeg, MB).
[2] Zinc + protein deficient (ZP), zinc deficient (Z), protein deficient (P) and control (C) diets were based on the AIN-93G diet for growing rats.
[3] AIN-93 zinc free mineral mix (Harlan Teklad).
[4] AIN-93 vitamin mix (Harlan Teklad).
[5] Zinc premix was composed of 5.77 g of Zinc carbonate in 1 kg of dextrose.
[6] The nutritional value of the diets was calculated with the exception of zinc which was determined by flame atomic absorption spectrophotometry.

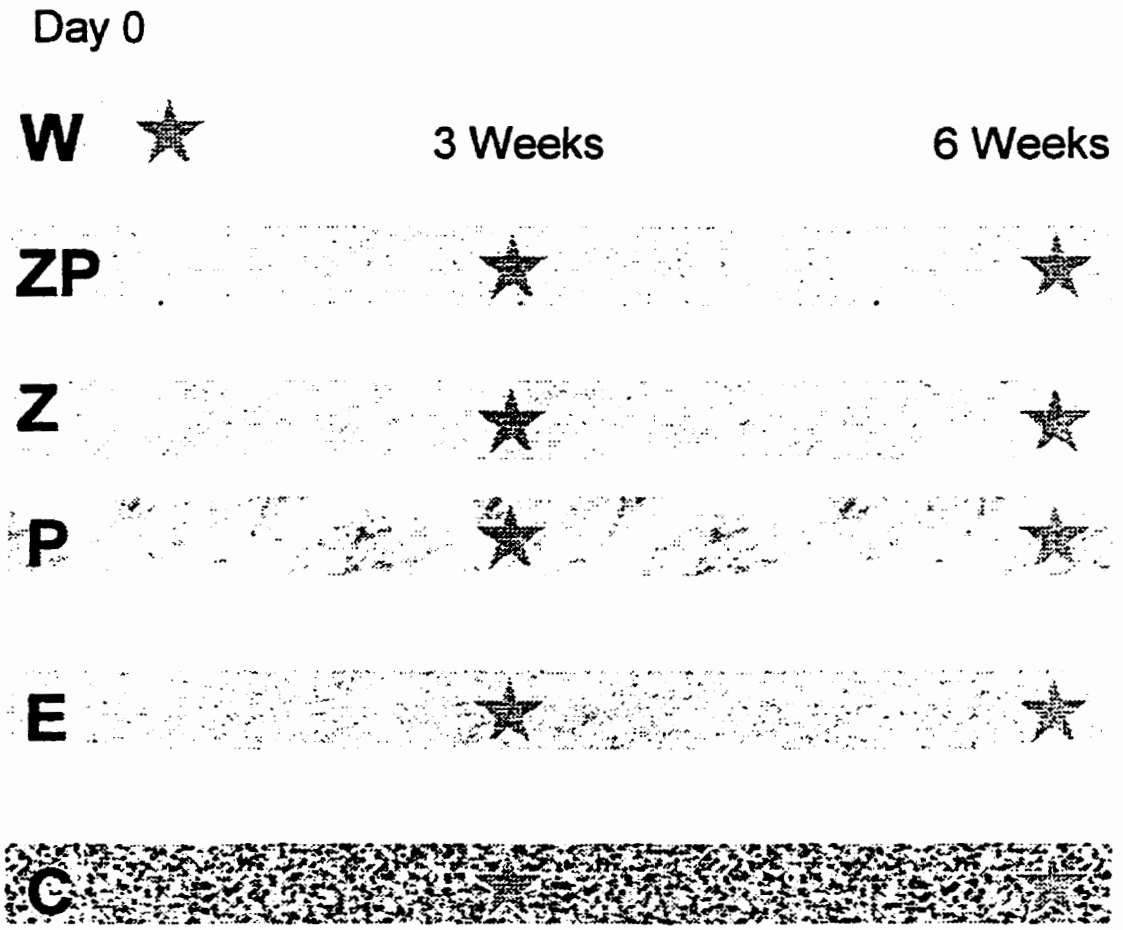


Figure 3. Experimental Design. At each termination 8 rats were killed [★]. The baseline group (W) was terminated on day 0. The remaining 80 rats were randomly assigned to either a zinc+protien deficient (ZP) diet, zinc deficient (Z), protein deficient (P), energy restricted (E) or control diet (C) for 3 weeks. Eight rats from each group were killed and the remaining rats were fed the control diet for 3 weeks. At the end of the study (6 weeks) the finial 40 rats were terminated.

Animal Care & Assessment of Growth

The animals were housed in stainless steel wire-bottom cages in a room (21-23 °C and 55% relative humidity) with a 14-hour light and 10-hour dark cycle. The rats had free access to double distilled water from a polyethylene bottle with a stainless steel sipper tube, but exact fluid intake was not measured. The animals were weighed weekly in order to document changes in body weight. Using a ruler, nose to anus and anus to tip of tail measurements were recorded at termination as part of the assessment of growth.

Termination

At the end of deficiency and recovery phases, the rats were terminated in blocks of five (one animal from each treatment group). The rats were asphyxiated with carbon dioxide, weighed, measured and decapitated by guillotine for the collection of trunk blood. The blood was collected in ice chilled polyethylene test tubes. The tubes were centrifuged (Beckman TJ-6R) at 2400 RPM for 15 minutes. The serum was removed and allocated into four microcentrifuge tubes using autoclaved pipette tips. The serum for the IGF-1 analysis was stored at -80°C. The remaining samples were stored at –20°C.

Liver, kidneys and spleen were removed, weighed and immediately frozen in liquid nitrogen. All organs were stored at –80°C. The upper torso was separated from the lower limbs using the guillotine. The skin was removed from the lower limbs and the inguinal fat pads were excised and weighed. One gastrocnemius muscle was carefully excised, mounted in gum tragacanth on cork and frozen in -150°C isopentane using methods adapted from Dubowtiz and Brooke (1973) and Estes and Goss (1996) for freezing human skeletal muscle. Isopentane cooled to –150°C was employed to minimize freeing time and the potential for ice crystal artifact formation. The countra-lateral muscle was excised, weighed and frozen in liquid nitrogen For analysis of muscle IGF-1 concentration. The gastrocnemius muscle was selected for biopsy as it is easy to excised and is used regularly by the rat in daily activities. The lower limbs were frozen in liquid nitrogen and stored at –20°C for femur mineral analysis.

Assessment of Zinc and Femur Mineral Status

Serum and femur zinc concentrations were used to assess zinc status. The zinc content of the experimental diets was also assessed. Zinc concentration was determined by flame atomic absorption spectrophotometry. Bone mineral status was determined using ICP vacuum emission spectrometry.

Serum Zn Concentration

Microcentrifuge tubes containing serum for Zn analysis were kept on ice until shortly before analysis by flame atomic absorption spectrophotometry (Spectra AA-30 Spectrophotometer, Varian Canada, Georgetown, ON). Each microcentrifuge tube was gently inverted until completely thawed. Aliquots of serum ranging from 140μl to 220μl were mixed with double deionized water to make a final solution of 1 ml. The diluted serum sample was vortexed to ensure homogeneity and aspirated into the nebulizer of the flame atomic absorption spectrophotometer. The detected Zn concentrations (μg/ml) were multiplied by the appropriate dilution factor to yield serum Zn concentration (μg/ml).

Femur Zinc and Femur Mineral Analysis

The frozen lower limbs were defrosted overnight at 4°C. The tendon and tissue were carefully removed from the femur using a scalpel. The femur was excised and its wet weight was recorded. The femur was then placed in a drying oven preheated to 85°C for 48 hours. The dry bone weight was documented upon removal from the oven. The dried bone was stored at 4°C in a sealed petri dish. The femurs were prepared in the above described fashion in eight blocks of eleven femurs each.

The wet-ash methods of Clegg et al. (1981) were used to digest the bone samples. All glassware and equipment were acid washed with a 21% nitric acid solution. Each femur was placed in a Pyrex glass test tube containing 2 ml of 70% nitric acid solution and covered with a marble. Two Pyrex test tubes containing powdered bovine liver (No. 1577B, U.S. Department of Commerce, National Institute of Standards and Technology, Gaithersburg, MD) were processed as quality controls. The bovine liver had a reading of 12.11 ppm Zn. Four blanks containing deionized water and nitric acid were also processed with the bone

samples. The blanks had an average reading of 0.016 ppm. The test tubes were left at room temperature for 2 hours and then place in an 85°C test tube heater for two days. The digested bone-acid mixture was placed in a 10-ml volumetric flask and diluted with Millipore water. To ensure that the zinc concentration of the solution would be within the standard curve, additional dilutions based on the femur wet weight and treatment group were made. The zinc concentration was determined via flame atomic absorption spectrophotometry. Since a Varian Liberty 200 ICP vacuum emission Spectrometer (Mississauga, ON) was available, the acid-bone mixtures were also user to determine femur calcium and phosphorus concentrations. Examining the effects of the experimental diets on the major components of bone (calcium and phosphorus) would allow for the further characterization of Zn, protein and energy deficiencies and the degree of recovery from these deficiencies. The mineral concentration was calculated with the following formula:

$$\frac{\text{Mineral concentration } (\mu g/ml) \text{ X } 10 \text{ ml X dilution factor}}{\text{Femur weight (mg)}}$$

Dietary Zinc Content

The Zn content of the experimental diets was determined following wet ashing (Clegg et al, 1981). One gram of the Zn deficient diets and 0.2g of the Zn adquate diets were added to an acid washed Pyrex test tube containing 2 ml 70% nitric acid. The diet samples were prepared for Zn analysis in the same fashion as the bone samples.

Assessment of Protein Status

The serum albumin and liver lipid concentrations were assessed in order to determine protein status. Low serum albumin and elevated liver lipid concentrations are hallmarks of protein deficiency (Latham, 1990).

Serum Albumin Concentration

The serum albumin concentration was determined using Sigma Diagnostics (St. Louis, MO) kit #631, which is a modification of the methods of Dumas (1971). The kit contained a yellow-green solution of bromcresol green (BCG) that turned blue-green upon

binding with albumin. The intensity of the blue-green color was directly proportional to the albumin concentration of the serum sample. Ten micro-liters of each serum sample was diltued with 10 μl of double deionized water and mixed with 2 ml of BCG solution in a cuvet by gentle inversion. The cuvet was immediately placed in a Milton Roy Spectronic 3000 Spectrophotometer (Fisher Scientific, Nepean, ON) for duplicate absorbance readings at 628 nm. The mean absorbance reading was used to calculate serum albumin concentrations (g/dl) with the following formula:

$$\frac{\text{Sample absorbance} - \text{Blank absorbance}}{\text{Standard absorbance} - \text{Blank absorbance}} \times \text{Concentration of Standard} \quad [X\ 2]$$

Liver Lipid Concentration

The concentration of liver lipid was determined using the principles of Folch et al. (1956). Briefly, a Polytron homogenizer (model PT 1020 3500, Brinkmannn Instruments) was used to homogenize 1 gram of liver in 22 ml solution of 2 parts chloroform and one part methanol. The homogenate was filtered though a Whatman number 1 filter paper into a graduated cylinder. The volume of elutate was recorded, 20% of the elutate volume was added as water and the cylinder was shaken. The cloudy suspension was left to separate overnight. Sodium chloride was added to some samples to ensure full separation of the lower lipid layer containing chloroform and the upper methanol layer. The volume of the chloroform layer was recorded and the methanol layer was removed. 10 ml of the chloroform layer was placed in a 25 g glass vial. The chloroform was evaporated using a heated water bath (OA-SYS heating system, Organomation Associates, Berlin, MA) and nitrogen air (0.7 Kg/cm^2) for one hour. After chloroform evaporation the vials were cooled in a desiccator and then weighed to determine lipid content.

Serum Insulin-like Growth Factor-1 Concentration

Serum IGF-1 concentration was determined using a rat serum IGF-1 double antibody radioimmunoassay (RIA) kit (DSL-2900) produced by Diagnostic Systems Laboratories Inc. (Webster, TX). The RIA kit included 6 rat IGF-1 standards and 2 rat IGF-1 controls. The kit employed the RIA principle of competition between radioactive and non-radioactive antigens for a set number of antibody binding sites (Edwards, 1985). The amount of radioactive IGF-1 bound to antibody sites was inversely proportional to the concentration of unlabelled IGF-1 in the serum sample. The free and bound IGF-1s were separated via a double antibody system made up of a goat anti-rat IGF-1 serum and a donkey anti-goat gamma globulin. All samples, controls and standards were assayed in duplicate. The kit instructions were followed for the assay as well as for the calculation of IGF-1 concentration.

Muscle Histology

The cork-mounted muscle biopsy samples were prepared for histochemical staining using a microtome in a cryostat. The cork-mounted samples were oriented perpendicular to the microtome knife so that cross sectional slices measuring 10 μm in thickness were obtained. The slices were placed on glass microscopes sides and stored at –80°C overnight. All histochemical staining was based on the methods of Estes and Goss (1996) and those used routinely by the neuropathology lab at the Health Sciences Center (Winnipeg, MB). All muscle samples were subjected to ATPase pH4.3 and 9.4 reactions as described earlier (p.12). In addition to the ATPase reactions, the muscle samples were subjected to haematoxylin and eosin (H & E), modified gomori trichrome, oil red O, periodic acid schiff (PAS) with and without diastase histochemical stains. H & E is a traditional stain that adds color to the tissue sample for morphological assessment of the muscle. In muscle samples H & E stains the nuclei a purple-blue color while the cytoplasm of muscle fibers stain pink. The modified Gomori trichrome stains muscle fibers dark green, connective tissue light green and nuclei and mitochondria red. The oil red O stain dectects the presence of triglycerides and neutral fat in muscle. The lipids appear bright red or orange-red and the nuclei appear blue. PAS stains muscle glycogen purple-pink and the nuclei blue. In order to confirm the presence

of glycogen, a second sample is incubated with salivary amylase at 37°C and the salivary amylase breaks-down the glycogen. This is called the diastase procedure. The presence of glycogen is confirmed if tissue that appeared purple-pink in the PAS sample is clear in the diastase sample.

Muscle Fiber Diameter Analysis

Photographs of the ATPase pH 9.4 muscle slides were taken with a Leica DMRB (Leica Mikroskopie, Germany) photographic microscope. Photos of a microscopic calibrated micron side were also taken at various levels of magnifications. The color film was processed without color adjustments by a local photography lab. The photos were scanned to create digital images onto 512 pixels by 512 pixels black background. The images were analyzed using IBAS software (version 2.0, Kontron Electronics, Germany). Sergio Mejia (Department of Geological Sciences, University of Manitoba) designed an IBAS program specifically for the analysis of muscle fiber diameter. The photos of the calibrated micron scale along with information about the magnification level were used to calibrate the image analysis program. Thus, the results of the image analysis were actual measurements and not an assessment of relative size. The utilization of IBAS technology allowed for the analysis of more muscle fiber diameters per treatment group (>580 fibers per group) and limited the potential for error with visual measurements. The computer program determined the largest and smallest diameter, the circularity and smoothness of each muscle fiber. Only the smallest diameter (D-min) data was used in the statistical analysis.

Statistical Analysis

The main effects of diet, time and interactions between diet and time were analyzed by ANOVA using SAS software version 6.04 (SAS institute, Cary, NC). Duncan's multiple range testing was used to identify significant differences between treatment group means. Differences were deemed significant at $P<0.05$. Repeated measures analysis was performed on the feed intake and body weight. Excel 97 (Microsoft Corporation, Roselle, IL) was used to construct histograms of the muscle fiber data.

IV. Results

The results of the study are presented in chronological order, beginning with the baseline and deficiency phase data followed by the recovery phase data. The data is presented in Figures 4-32 beginning on page 54. The focus of this section will be on the results of the Duncan's and repeated measures means testing as the ANOVA analysis revealed little variation in the main effects of diet, time and the interaction of diet and time on the variables measured. The P values for the main effects are presented in Table 2. In brief, the effects of diet and time were significant for all variables except for the spleen weight to body weight and kidney weight to body weight ratios, respectively. The interaction of diet and time was significant for all parameters except for body length, liver mass, kidney weight to body weight ratio, spleen weight to body weight ratio and type 2 muscle fiber minimum diameter.

Body Weights

Weekly weights and weight gains

Figure 4 depicts the weekly weights and Figure 5 shows the weekly weight gains of the rats during the deficiency and recovery phases. Throughout the deficiency phase (0-3 weeks), the body weights of the C group were significantly greater than the deficient groups (Figure 4). The body weights of the ZP and P groups were both 34% of the mean C group weight at the end of deficiency phase. The Z and E groups had body weights that were 50 % and 51% of the C group mean body weight and were not significantly different from each other. During the first two weeks of the deficiency phase, the control group had a rapid weight gain (g/week), which was significantly greater than the other groups (Figure 5). The weight gain of the Z and E groups was 46% and 40% of that observed in the C group. The ZP and P groups exhibited weight loss and gain during the same time period. Between the second and third weeks the C, Z and E experienced decreased rates of weight gain and the protein deficient groups maintained their body weight.

Table 2. The main effects of diet, time and the interaction of diet and time on study parameters as determined by ANOVA.

Parameter Measured	P values for Main Effects:		
	Diet	Time	Diet * Time
Cumulative feed intakes	0.0001	0.0001	0.0002
Feed efficiency	0.0001	0.0007	0.0001
Tail length	0.0001	0.0001	0.0001
Body length	0.0001	0.0001	0.9902
Liver mass	0.0001	0.0001	0.1608
Liver weight: Body weigh ratio	0.0004	0.0001	0.0032
Kidney mass	0.0001	0.0001	0.0485
Kidney weight: Body weight ratio	0.6505	0.0001	0.8134
Spleen mass	0.0001	0.0001	0.0013
Spleen weight: Body weight ratio	0.0482	0.1576	0.2458
Inguinal fat pad mass	0.0001	0.0001	0.0038
Liver lipid concentration	0.0001	0.0001	0.0001
Serum albumin concentration	0.0001	0.0001	0.0001
Serum zinc concentration	0.0001	0.0001	0.0001
Bone zinc concentration	0.0001	0.0001	0.0001
Bone calcium concentration	0.0001	0.0001	0.0001
Bone phosphate concentration	0.0001	0.0001	0.0001
Bone calcium: phosphate ratio	0.0001	0.0483	0.0002
Serum IGF-1 concentration	0.0001	0.0001	0.0001
Gastrocnemius muscle weight	0.0001	0.0001	0.0056
Type 1 muscle fiber minimum diameter	0.0001	0.0001	0.0147
Type 2 muscle fiber minimum diameter	0.0001	0.0001	0.0683

During the recovery period (4-6 weeks), the deficient rats gained weight rapidly, but their weekly body weights were always significantly less than the control group (Figure 4). The pattern of significance observed in the body weight data of the deficiency phase (ZP & P < Z & E< C), continued in the recovery phase. Between weeks 3 and 4, the deficient groups showed a sharp increase in weight gain followed by a more tapered weight gain in weeks 5 through 6 (Figure 5). The C group also experienced increased weight gain, but in a less dramatic fashion. During the last week of the study, the C and E groups had weight gains that were significantly less than the Z, ZP and P groups. At the end of the study, the body weights of the deficient rats were only 66% to 80% of the C group. During the recovery phase, the deficient groups grew rapidly, but were unable to achieve body weights that were similar to the C group by the end of the study.

Feed intakes

Weekly feed intakes

The C group had the largest weekly feed intake throughout the 6-week study (Figure 6). As expected the weekly intakes of the Z and E (pair-fed control) groups were not significantly different during the deficiency phase. It is interesting to note that the Z and E weekly intakes continued to be similar throughout the recovery phase. The weekly intakes of the protein deficient groups were significantly different from each other during the first week of the deficiency phase. Thereafter, the protein deficient groups had similar weekly intakes. The weekly intake of the deficient rats was the lowest during the third week of the deficiency phase (30-48% of control). The weekly intakes of all groups increased between weeks 4 through 6. The protein deficient groups had a significantly smaller intake of recovery diet in week 4 compared to the Z and E groups. During week 6, the deficient groups had similar feed intakes (80-88% of control).

Change in weekly feed intakes

Figure 7 depicts the changes in feed intake throughout the study. Between the first and second weeks of the deficiency phase, the overall intakes of the ZP, E and Z groups increased without significant differences among the groups. The C and P groups had changes in weekly

intakes that were significantly different from all other groups. The C group's intake increased by 37 grams and the P groups intake decreased by 4 grams. The feed intakes of all groups decreased between weeks 2 and 3. The feed intake of the ZP and P groups was significantly decreased compared to the other groups. During the changeover point from deficiency phase to recovery phase (weeks 3-4) the weekly feed intake of all groups increased. The C group had significantly smaller increase in feed intake compared to the recovering deficient groups. The Z group had the largest increase in feed intake followed by the E, ZP and P groups. The increased feed intake by the Z group was significantly greater than the P group, but was not significantly different from the E and ZP groups. There were no significantly different changes in feed intake among the study groups between weeks 4-5 and weeks 5-6.

Cumulative feed intakes

The total quantity of diet consumed by each of the deficiency groups (in grams) during the deficiency phase was not significantly different from each other (Figure 8). By the end of the deficiency phase, the C group had consumed 50% more diet than the other groups. By the end of the study, the C group had consumed significantly more diet than all other groups. The Z group had the second largest feed intake during the 6-week study followed by the E, P and ZP groups. The total intakes of the E, P and ZP groups were not significantly different from each other. The total amount of diet consumed by the Z group (73% of C group intake) was not significantly different from that of the E group (68% of C group intake).

Feed efficiency: Cumulative feed intake to final body weight ratio

To gain insight into how readily the feed consumed by the rats was transferred into weight gain, the cumulative feed intake was divided final body weight. During the 3 week deficiency phase, the feed intake: body weight ratio of the P group was significantly greater than all other groups indicating an inefficient use of feed for body mass accretion (Figure 9). The feed intake: body weight ratio of the ZP group was the second largest and significantly different from that of the Z, C and E groups. The ratio of the C group was not significantly different from that of the Z and E groups, but the ratio for the Z group was significantly greater than that of the E group.

During the 3 week recovery phase, the C group had the largest intake: body weight ratio followed by the P, Z, ZP and E groups. The ratio of the C group was not significantly different from that of the P and Z groups. However, the C groups intake: body weight ratio was significantly greater than that of the ZP and E groups. There were no significant differences among the feed intake: body weight ratios of the deficient groups. It is interesting to note that the feed intake: body weight ratio of the P group after the three week deficiency phase was significantly greater than that of all other groups at both 3 and 6 weeks.

Linear Growth

Tail length

After the deficiency phase, all groups had tail lengths that were significantly greater than those of the W baseline group (Figure 10). The deficient groups had tail lengths that were significantly shorter than the C group. The tail length for the Z group was16% shorter than the C group, followed by the E group at 18%, ZP group at 27% and the P group at 29%.

After the recovery period, the pattern of recovery and significant changes observed in the weight data was evident in the tail length data. The C group had the longest tails followed by the Z and E groups and finally the protein deficient groups. At this time point, the mean tail length of the P and ZP groups were 21% shorter than the C group followed by the E and Z groups at 15% and 12%, respectively.

Body length

After the deficiency phase, all 6-week old rats had mean nose to anus measurements that were significantly greater than the W group (Figure 11). The body lengths of the deficient groups were all significantly shorter at 70-80% of the C group. The mean length of the E group (80% of C) was significantly greater than that of the Z group (76% of C). The body lengths of the protein deficient groups were similar to each other and significantly shorter than the other groups during the deficiency phase.

At the end of the recovery period, the C group had the longest mean body length at 21.3 cm. The nose to anus measurements of the ZP and P groups were still significantly less than the

other groups (88% and 90% of C, respectively). The body lengths of the Z and E groups (94% and 93 % of C, respectively) were significantly greater than those of the protein deficient groups, but were significantly less than the C group.

Organ Weights and Organ Weight to Body Weight Ratios

In addition to recording the liver, kidney and spleen weights at termination, the organ weight to final body weight ratio was calculated. This ratio provided insight into how the experimental diets may influence the proportion of body mass occupied by certain tissues.

Liver mass

After the deficiency phase, the C group had liver weights that were significantly greater than the W baseline and deficient rats (Figure 12). The liver weights of the deficient groups were 50-62% lighter than the control value and were not significantly different from each other or the W group.

At the end of the recovery phase, the liver mass of the C group was significantly heavier than all other groups. The Z, E, ZP and P groups had mean liver weights that were 90%, 84%, 80% and 70% of the C group, respectively. The liver weight of the Z group was significantly greater than the protein deficient groups, but it was not significantly different from the E group. There were no significant differences between the liver weights of the E and ZP groups. The liver weight of the P group was significantly less than the C, Z and E groups.

Liver weight: Body weight ratio

The liver weight to body weight ratios (l: b) of the W and the 3 and 6 week C groups were not significantly different from each other (Figure 13). The liver weight was consistently 4.5% of total body weight in well-fed rats. The l:b ratio of the Z group (3.9%) was similar to the C group (4.5%), but the l:b ratio of the E group (3.6%) was significantly less than the C group. The ratio for the P group (4.6%) was significantly greater than the deficiency phase C group. The l:b ratio of the ZP group(%) was not significantly different from of the W or C group ratios.

After the recovery phase, the livers of the E and P groups were 4.9% and 4.8% of total body weight and were not significantly different from the ratio of the C group (4.5%). The l: b

ratios of the ZP and Z groups (5.2% and 5.1% of total body weight, respectively) were significantly greater than the C group.

Kidney Weights

At the end of the deficiency phase, the kidney weight of the C group was significantly greater than the deficient groups (Figure 14). The kidneys of the ZP and P groups were the lightest (35% C group) and were not significantly different from each other or the W group. The E and Z groups had kidneys that were heavier than the protein deficient groups, but were not significantly different from each other. The kidney weights of the E and Z groups were 61% and 53% of the C group.

By the end of the recovery phase, the C group had significantly heavier kidneys than the other groups. The kidney weights of the Z and E groups were 85% and 80% of the C group, but were not significantly different from each other. The kidneys of the ZP and P groups were 27% and 31% lighter than the kidneys of the C group, but there was no significant difference between the protein deficient groups.

Kidney weight: Body weight ratios

There were no significant differences in the kidney weight to body weight (k: b) ratios among the 6-week old deficiency phase groups nor with the W group (Figure 15). On average, the kidneys of the 3 to 6-week old animals contributed 1.2% of total body weight. The 9-week old recovery phase animals all had similar k: b ratios. It is interesting to note that the k: b ratios of the 9-week old animals are significantly less than those of the 6-week old deficiency phase animals. Overall, less than one percent of the total body weight of the 9-week old animals was renal tissue.

Spleen weights

After the deficiency phase, the spleen weight of the C group was significantly greater than that of the deficient and W groups (Figure 16). The spleen weight of the C group increased 37.9% from the baseline value. There were no significant differences among the spleen weights of the W, E and Z groups. The P and ZP groups had mean spleen weights that were significantly

less than the W and C groups. The spleen weight of the ZP and P groups decreased 51.2% and 38.2%, respectively, from baseline.

By the end of the recovery phase, there were no significant differences among the C, ZP, E and P groups. Only the spleen weight of the Z group was significantly less than the C group at 84.2% of control.

Spleen weight: Body weight ratio

The W group had a significantly greater spleen weight to body weight (s: b) ratio than the deficiency and recovery phase animals (Figure 17). The W rat spleens contributed to 0.44% of total body weight. There were no significant differences among the s: b ratios of the deficiency phase rats. In general, 0.23% of total body weight was spleen tissue. The only significant difference in s: b ratio after the recovery phase was between the ZP and C groups. The ZP group had a significantly greater s: b ratio than C group (0.30% of total body weight). The spleen comprised 0.22% of total body weight in 9 week-old C rats.

Inguinal fat pad weight

The inguinal fat pad was removed at termination as it was the easiest to remove as a whole unit and it provided information about how the different types of dietary deficiency may affect fat stores. By the end of the deficiency phase, the C group had an inguinal fat pad weight that was significantly greater than the other groups including the W group (Figure 18). The inguinal fat pad of the C had increased by 144% from baseline. There were no significant differences among the mean fat pad weights of the W, ZP, P and Z groups. The protein deficient groups had inguinal fat pads that were significantly greater than the E group. Although, no fat pads could be excised from the E group, the fat pads removed from the Z and W groups were not heavy enough to be deemed significantly different from the E group via Duncan's mean testing.

After the recovery phase, there were no significant differences in inguinal fat pad weight among the C, E and P groups. The Z and ZP groups had inguinal fat pad weights that were significantly less than C rats. The inguinal fat pad weights of the ZP and Z groups were 74.8% and 69.9 % of C group.

Protein Status

Protein status was assessed by liver lipid and serum albumin concentrations. During protein deficiency, liver lipid concentration increases and serum albumin concentration decreases (Latham, 1990).

Liver lipid concentration

During the deficiency phase, the ZP and P groups had liver lipid concentrations that were significantly greater than all other groups (Figure 19). The liver lipid concentration of the ZP and P groups were 2.4 and 2.3 times greater than the C group concentration. By the end of the recovery phase all animals had similar liver lipid concentrations.

Serum albumin concentration

After the deficiency phase, the ZP and P groups had serum albumin concentrations that were significantly less than the other groups (Figure 20). The serum albumin concentrations of the ZP and P groups were 58% and 54% of baseline, respectively and 43% and 41% of C, respectively. The E, C and Z groups all had similar serum albumin concentrations. Following the recovery phase, all groups had serum albumin concentrations similar to the C group.

Zinc Status and Femur Mineral Concentrations

Serum zinc concentration

Following the deficiency phase, the P, ZP and Z groups had serum Zn concentrations that were similar and significantly less than the W, E and C groups (Figure 21). The protein deficient and Z groups had serum Zn concentrations that ranged from 30% to 35% of the baseline value and from 22% to 26% of the control value. The C group had a serum Zn concentration of 1.63 μg/ml. The E group had a serum Zn concentration that was significantly greater than all other groups at 1.9 μg/ml. After the recovery phase, all groups had similar serum Zn concentrations.

Femur Zn concentration

After the deficiency phase, the C and E had femur Zn concentrations that were significantly greater than the other groups (Figure 22). The femur Zn concentrations of the P and Z groups were significantly less than the W group and only 46% and 35% of the C group. The femur Zn concentration of the ZP group was not significantly different from the W value. Following the recovery period, the femur Zn concentrations of the deficient groups were similar to that of the C group.

Femur calcium concentration

The W group had the lowest femur calcium (Ca) concentration (Figure 23). The Ca concentration of the C group increased 1.35 times from the baseline value. The femur Ca concentration of the C group was significantly greater than all other groups at the end of the deficiency phase. The E group had the second highest femur Ca concentration followed by the Z, ZP and P groups. The Z group femur Ca concentration was significantly less than the E group. There were no significant differences between the femur Ca concentrations of the ZP and P groups. After the recovery phase, all groups had femur Ca concentrations that were similar to the C group.

Femur phosphate concentration

The C group had a femur phosphate concentration than was significantly greater than all groups at the end of the deficiency phase (Figure 24). The E group had a femur phosphate concentration than was significantly less than the C group and significantly greater than the Z group. The femur phosphate concentration of the Z group was 82% of the C group. The P and ZP groups had phosphate concentrations that were similar to each other and not significantly different from the W group (77% and 74% the C group).

There were no significant differences in femur phosphate concentrations among the E, Z, P and ZP groups after the recovery phase. The femur phosphate concentrations of the P and ZP groups (93.4% and 92.9% of control, respectively) were significantly less than the C group.

Femur calcium: Phosphate ratio

After the deficiency period, the calcium to phosphate ratios (Ca:P) of the C and W groups were not significantly different from each other (1.34 and 1.33, respectively). The E group Ca:P ratio of 1.35 was significantly greater than the baseline value, but not significantly different from the C group. The Z and P groups had Ca:P ratios (1.44 and 1.39, respectively) that were significantly greater than the C group. The Z group Ca:P ratio was significantly greater than the P group ratio.

At the end of the recovery phase, the E and C groups had similar Ca:P ratios of 1.38 and 1.39, respectively, that were significantly less than the other groups. The Ca:P ratio of the P and Z groups were similar at 1.42 and 1.40, respectively. The ZP group Ca:P ratio (1.45) was significantly greater than the other groups.

Serum insulin-like growth factor-1 concentration

At the end of the deficiency phase, the E and Z groups had serum IGF-1 concentrations that were significantly less than all groups including the W group (Figure 25). The serum IGF-1 concentrations of the E and Z groups were 51% and 25%, respectively, of the baseline group and were 22% & 11%, respectively, of the C group. The W and ZP groups had similar IGF-1 concentrations. The P group had a higher IGF-1 concentration (58.9% of C group), but it was significantly less than the C group. Following the recovery phase, all groups had similar serum IGF-1 concentrations.

Muscle Biopsy

Gastrocnemius muscle weights

The gastrocnemius muscle weights of the P and ZP rats were significantly less than the other 6-week old rats (Figure 26). The muscle weights of the P and ZP groups were 32% and 39%, respectively, of the C group. The E and Z muscle weights were significantly greater than that of the protein deficient groups, but significantly less than the C group. The muscle weights of the E and Z groups were 59% and 61% of the C group.

After the recovery phase, the ZP and P gastrocnemius muscle weights were still significantly less than the other groups and recovered to 61% and 68% of the C group. The

muscles of the Z group (88% of C) were significantly heavier than the muscles of the E group (79% of C). The C group had the heaviest gastrocnemius muscles at the end of the study.

Muscle histology

A light microscopic assessment of the muscle samples studied with the H&E, modified Gomori trichrome, oil red O, PAS with and without diastase procedures did not reveal histochemical differences between the study groups. Figures 27 and 28 are examples of the ATPase pH 9.4 reaction photos used for the analysis of muscle fiber diameter.

Type 1 muscle fiber minimum diameter

After the deficiency phase, the type 1 muscle fiber minimal diameter (T1-MFD) of the C group increased 1.34 times from baseline measurements (Figure 29). The P, Z and ZP groups had T1-MFD values that were not significantly different from the 3-week old W group. The T1-MFD of the P, Z and ZP groups were 80%, 81 and 82% of the C group, respectively. The C and E groups had similar T1-MFD values of 31.54 μm and 31.47 μm, respectively that were significantly greater than the other groups.

By the end of the recovery phase, the T1-MFD of the C group had increased 1.17 times from the 6-week old C group value/measurement. The P, E , ZP and Z groups had T1-MFD values that were 84%, 91%, 91% and 94% , respectively of the control value. The T1-MFD of the P and E groups were significantly less than the C group. The Z and ZP T1-MFD values recovered to the C group value.

Type 2 muscle fiber minimum diameter

At the end of the deficiency phase, the type 2 muscle fiber minimal diameter (T2-MFD) of the C group had increased by 1.47 times from the baseline measurement and it was significantly higher than the other groups (Figure 30). The 6-week old P, ZP and Z groups had T2-MFDs that were not significantly different from the 3-week old W group. The T2-MFDs of the P, ZP and Z groups were 69%, 72% and 78% of the C group, respectively. The Z and E groups had similar T2-MFD values that were significantly less than the C group.

After the recovery phase, the C, Z, ZP and E groups had similar T2-MFD values. The P group had a significantly smaller T2-MFD at 85% of the C group.

Muscle fiber histograms

Constructing histograms from the muscle fiber measurements are another way of examining muscle biopsies for changes in muscle fiber size (Dubowitz and Brooke, 1973). Histograms also provide information about the skewness and the distribution of the data (Olson, 1987).

Figure 31 depicts histograms constructed from the T1 and T2 MFD data of the baseline group on day 0 and the deficient and control groups after the deficiency phase. In Figure 31, type 2 muscle fibers are the dominant fiber type in the gastrocnemius as evidenced by the larger frequency and distribution of the T2-MFD data versus the T1-MFD. However, the type 1 muscle fibers tended to have the larger diameter. In all of the histograms in Figure 31, the mode (the observation that occurs the most frequently) of the T2-MFD data is left of the mode of the T1-MFD data. The distribution of the baseline (W) MFD data had a leptokurtic shaped curve. After 3 weeks of normal growth, the C group T1-MFD had an almost symmetrical distribution and the T2-MFD data had a right skewed distribution. In the C group histogram, the T1-MFD distribution data fell completely within that of the T2-MFD distribution curve. The T2-MFD distribution curve of the deficient rats also had a tendency to be right skewed, but with a smaller spread than the C group data. The T1-MFD of the deficient rats had more symmetrical distributions that tended to fall to the right and outside the distribution curve of the T2-MFD data. The Z and E group histograms had distinctive bimodal distributions with the T1-MFD distribution falling further to the right than in the other groups. Although the ZP and P groups had similar T2-MFDs, the distribution of the T2-MFD distribution of the P group was more reminiscent of the W group distribution than that of the ZP group.

At the end of the recovery phase, the spread of the MFD distributions of all groups moved to the right (Figure 32). This reflects the increase in muscle fiber size. The T2-MFD data of all the groups showed increased right skewness. The T2-MFD modes of all groups were either along

side or to the right of the T1-MFD mode. This indicated that the diameter of most of the type 2 muscle fibers tended to be either the same size or larger than most the type 1 fibers. It is interesting to note that the Z group T1-MFD distribution curve was an different shape with a plateau between 30 μm and 35μm.

Key to figures

The majority of the data for this study is presented in the form of graphs.

The following codes were used to identify the different experimental groups:

W = Baseline

ZP = Zinc + protein deficiency

P = Protein deficiency

Z = Zinc deficiency

E = Energy restriction

C = Control

Please note that different colors were used to identify data collected at the end of the deficiency and recovery phases. The baseline (W) data, collected on day 0, was plotted in the same data series as the data collected at the end of deficiency phase. Therefore the W group data has the same color as the end of deficiency phase data.

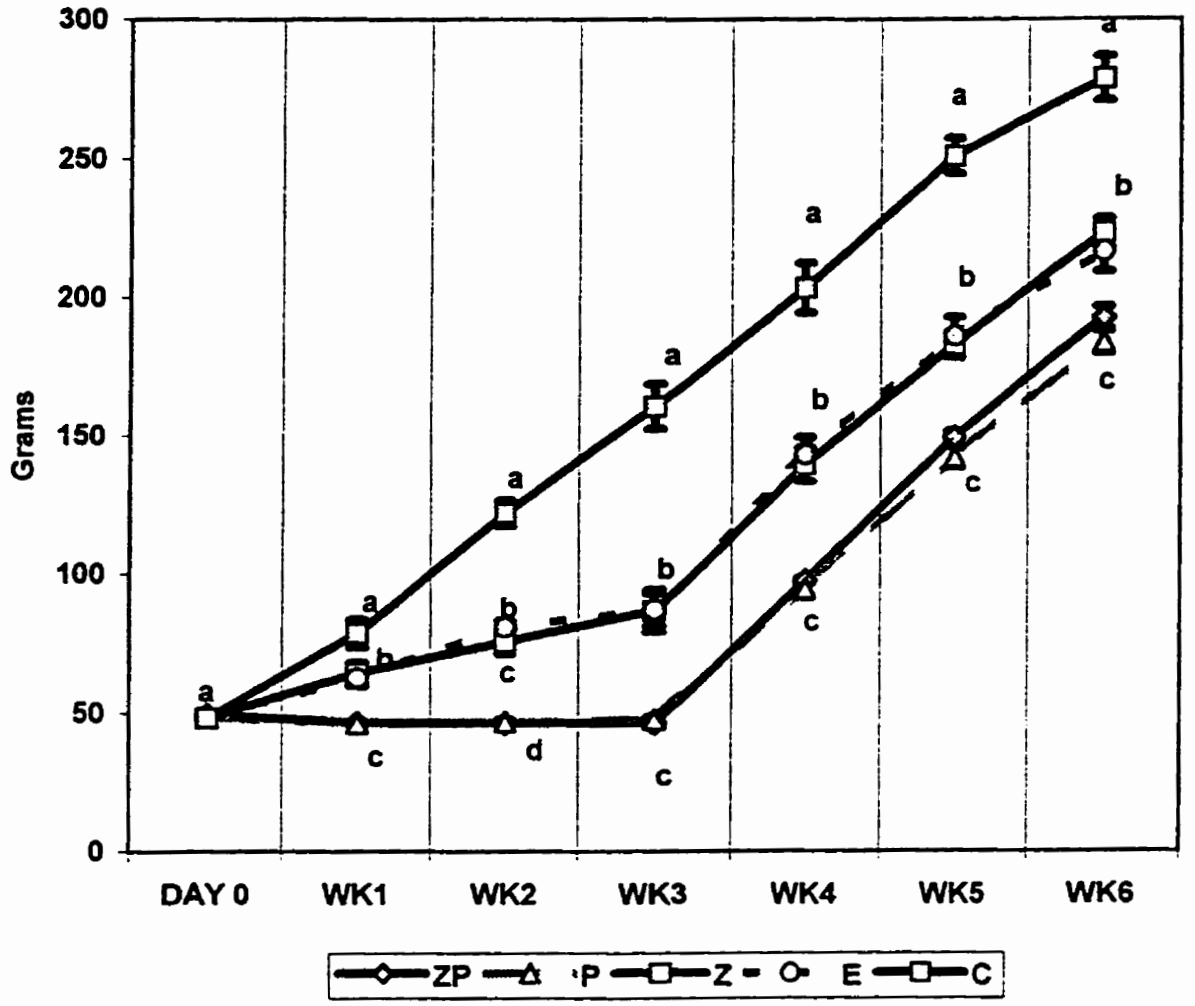


Figure 4. Weekly weights of the rats during the deficiency and recovery phases. The data for the ZP and P groups overlap each other as does the data for the Z and E groups. Each symbol represents group means +/- SEM, n=8. Symbols with different lower case letters are significantly different within the same week ($P<0.05$) as determined by repeated measures testing. Since the data points overlap each other at several points, one letter of significant was used for two data points to indicate that the data points were not significantly different from each other.

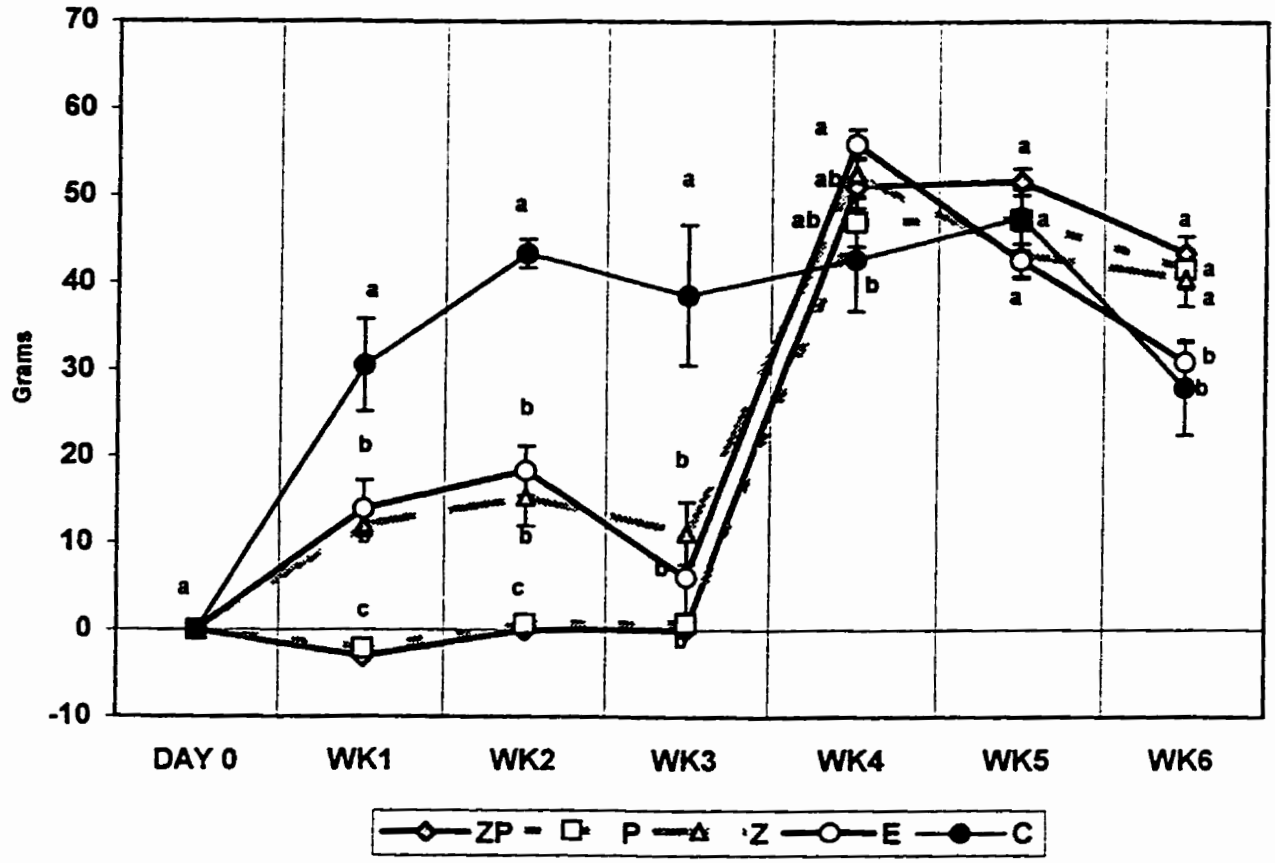


Figure 5. Weight gain (or loss) during the deficiency and recovery phases. Each symbol represents group means +/- Sem, n=8. Symbols with different lower case letters are significantly different within the same week ($P<0.05$) as determined by repeated measures testing. Since the data points frequently overlap, one letter of significance was used to indicate no significant difference between one or more data points.

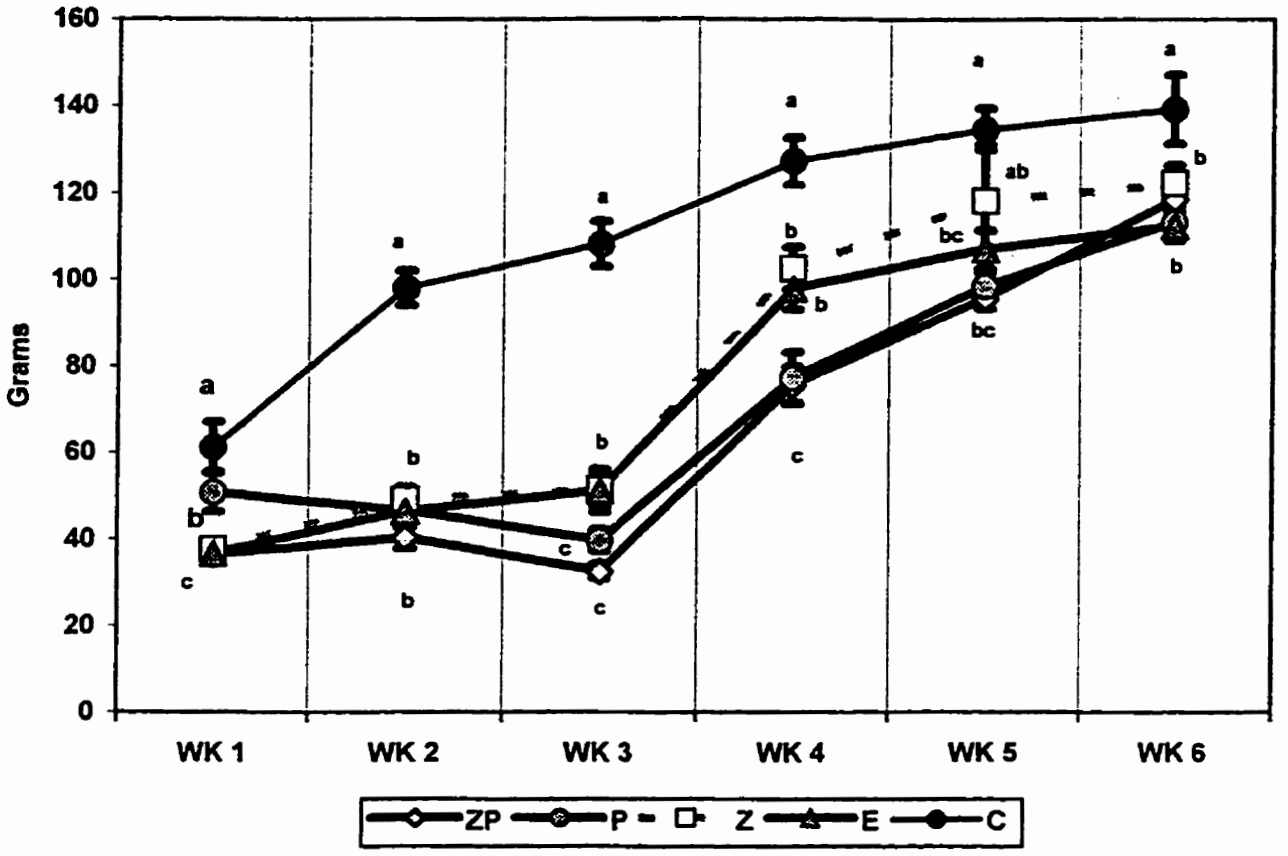


Figure 6. Weekly feed intakes during the deficiency and recovery phases. Each symbol represents group means +/- SEM, n=8. Symbols with different lower case letters are significantly different within the same week ($P<0.05$) as determined by repeated measures testing.

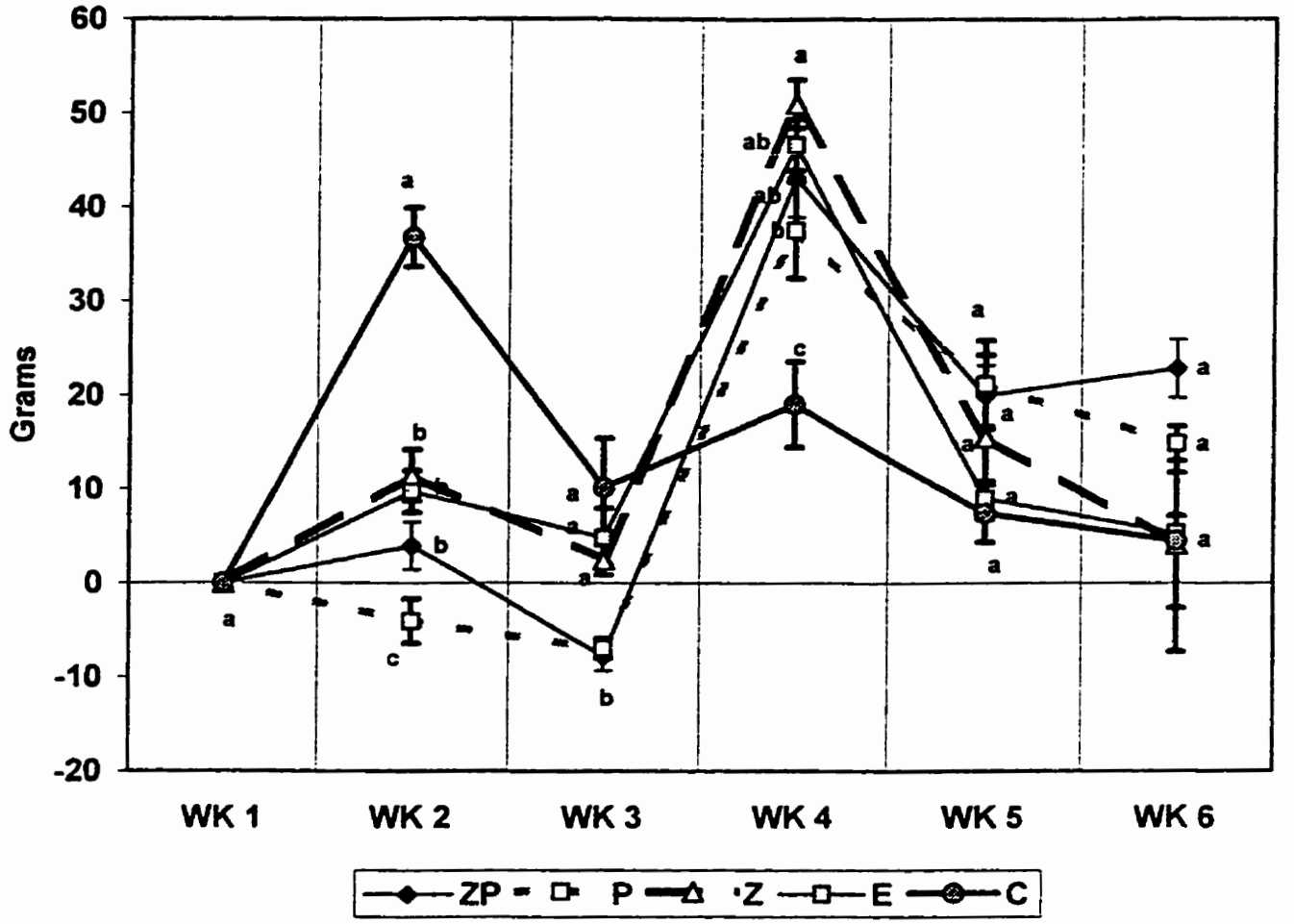


Figure 7. Changes in weekly feed intake during the deficiency and recovery phases. Each symbol represents group means +/- SEM, n=8. Symbols with different lower case letters are significantly different within the same week ($P<0.05$) as determined by repeated measures testing.

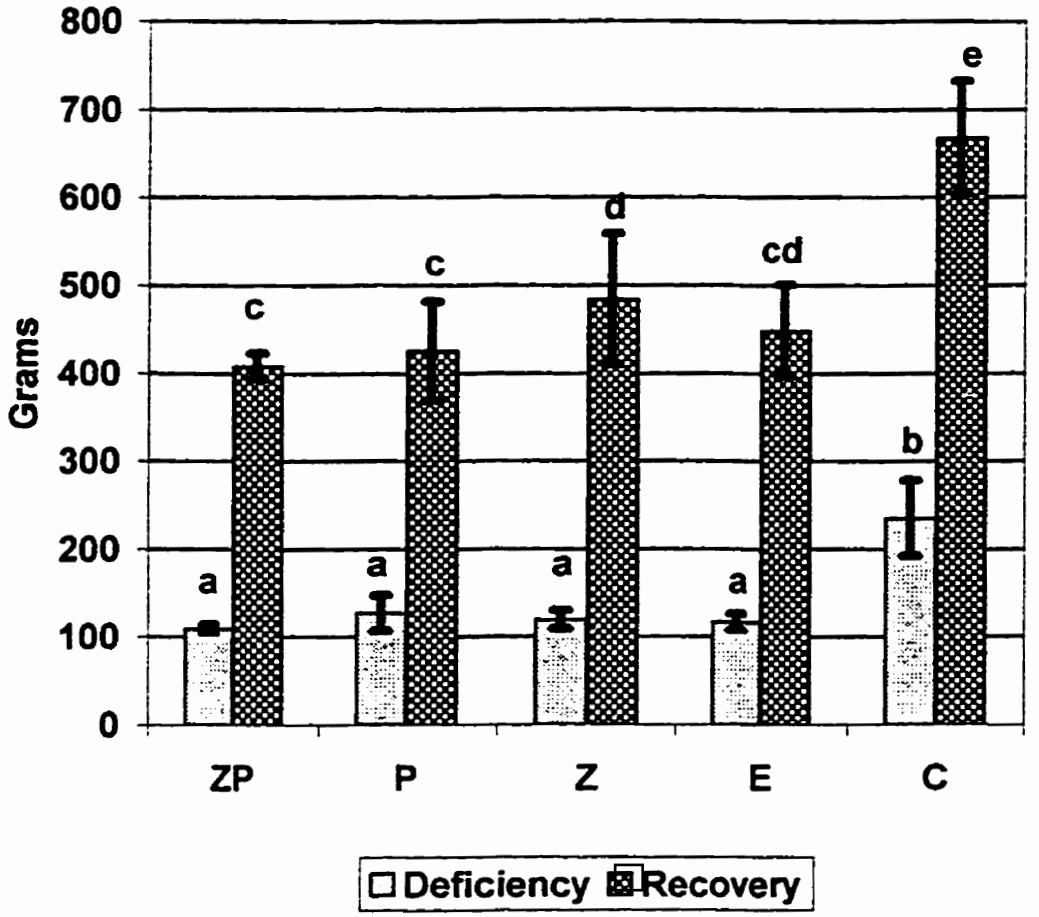


Figure 8. Cumulative feed intakes during the deficiency phase and through the recovery phase. The columns represent group means +/- SEM, n= 8. Columns with different lower case letters are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

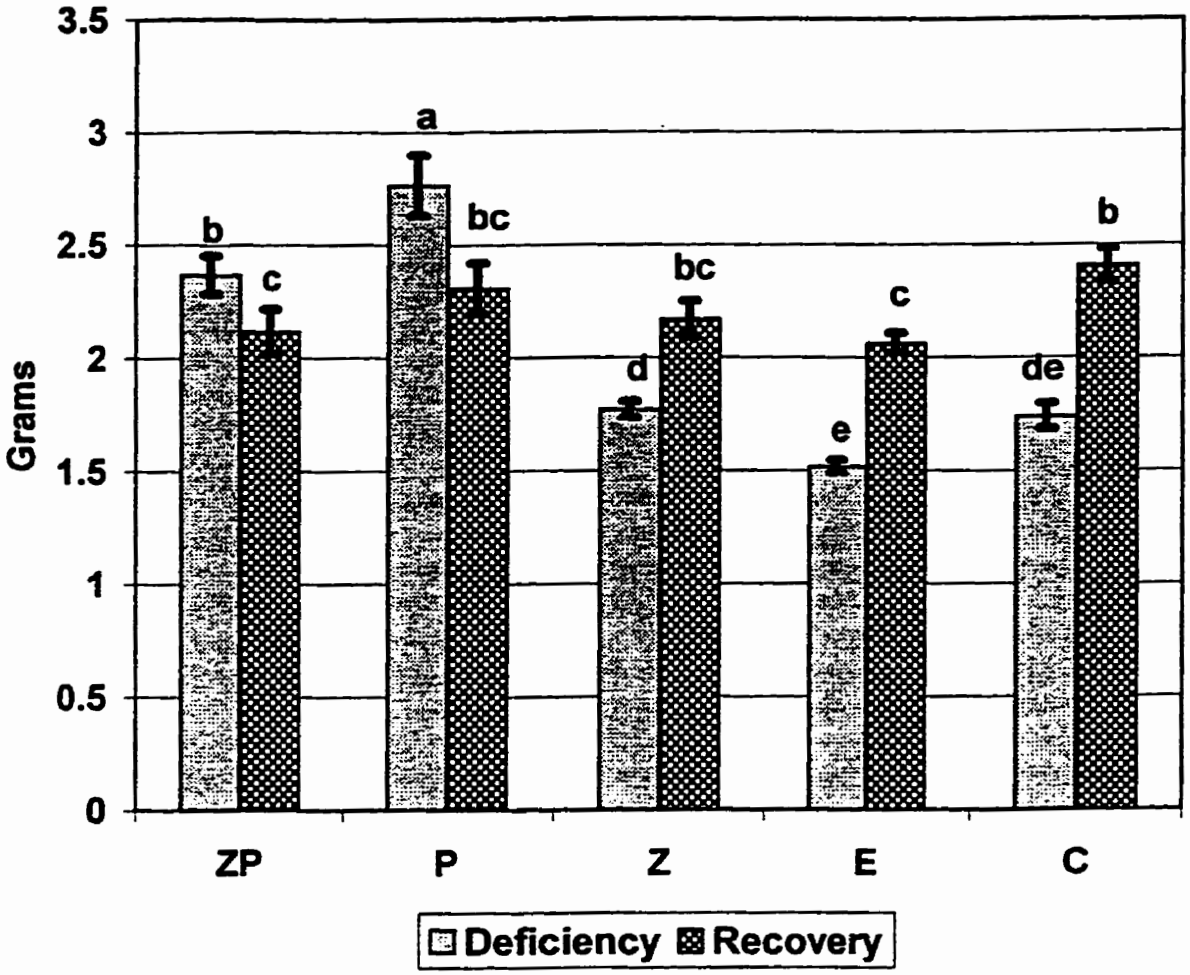


Figure 9. Feed efficiency during the deficiency and recovery phases. Feed efficiency was calculated by dividing the cumulative feed intake by body weight at termination. The columns represent group means +/- SEM, n= 8. Columns with different lower case letters are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

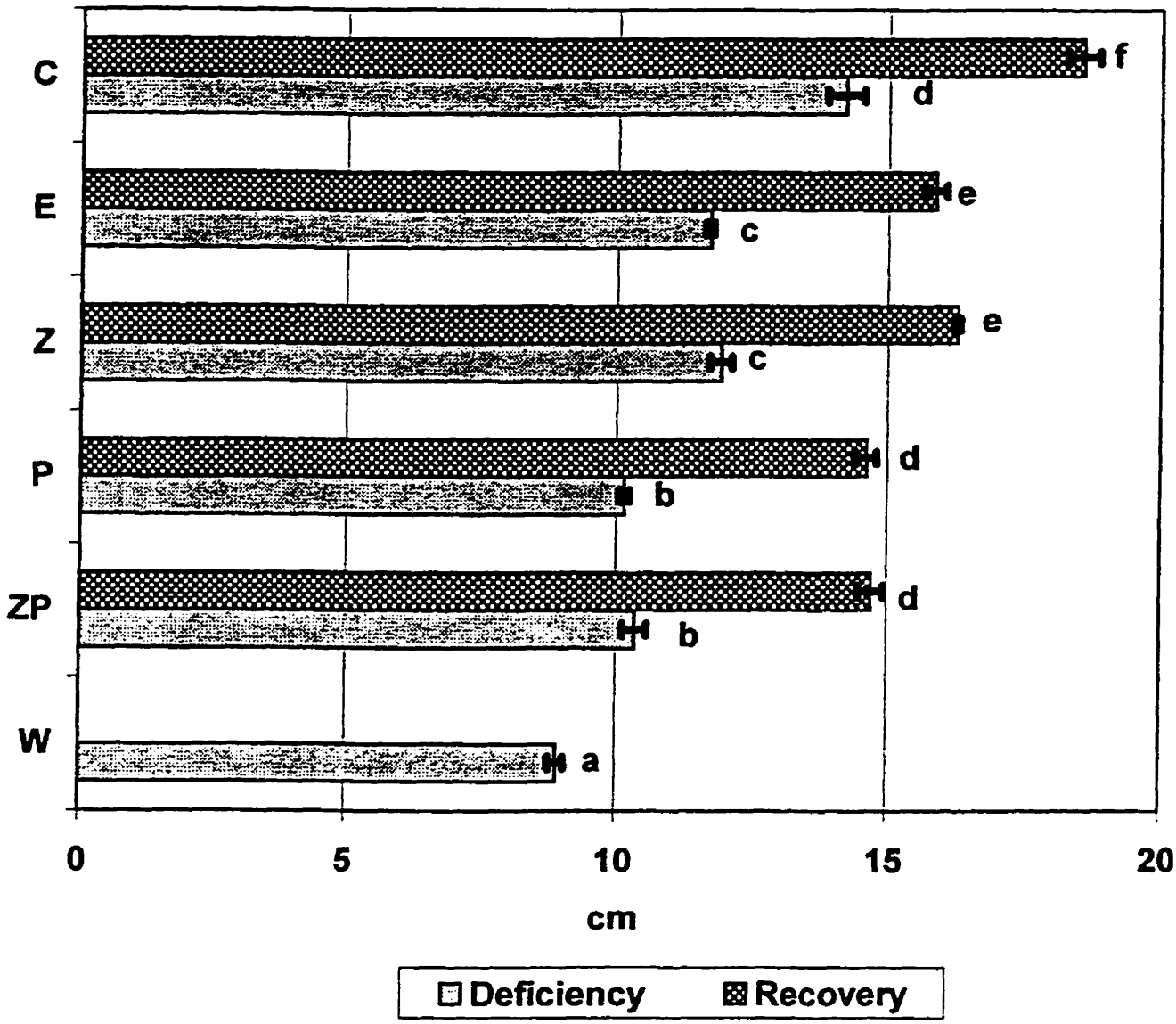


Figure 10. Tail length after the deficiency and recovery phases. The bars represent group means +/- SEM, n= 8. Bars with different lower case letters are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

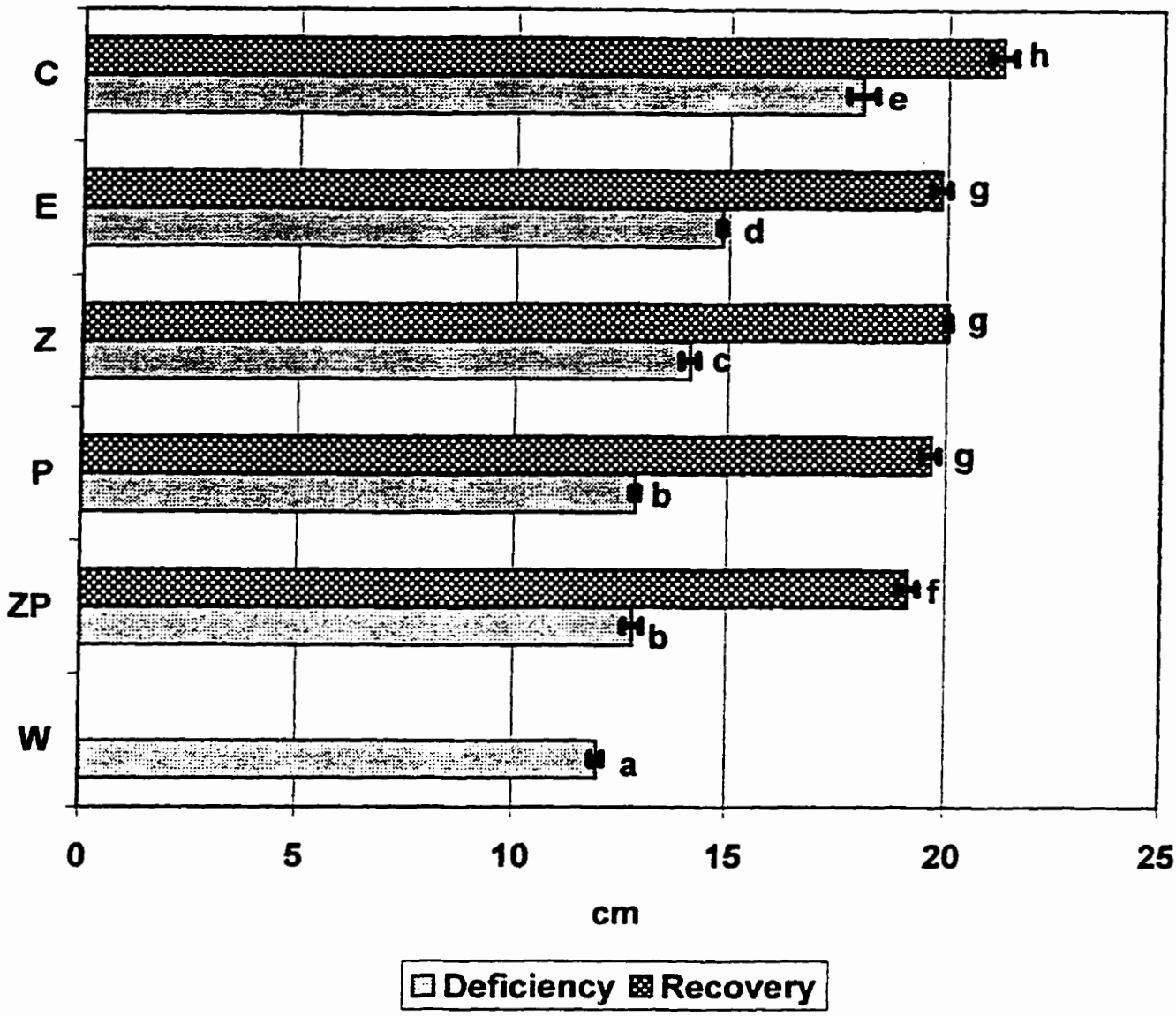


Figure 11. Body lengths after the deficiency and recovery phases. The bars represent group means +/- SEM, n= 8. Bars with different lower case letters are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

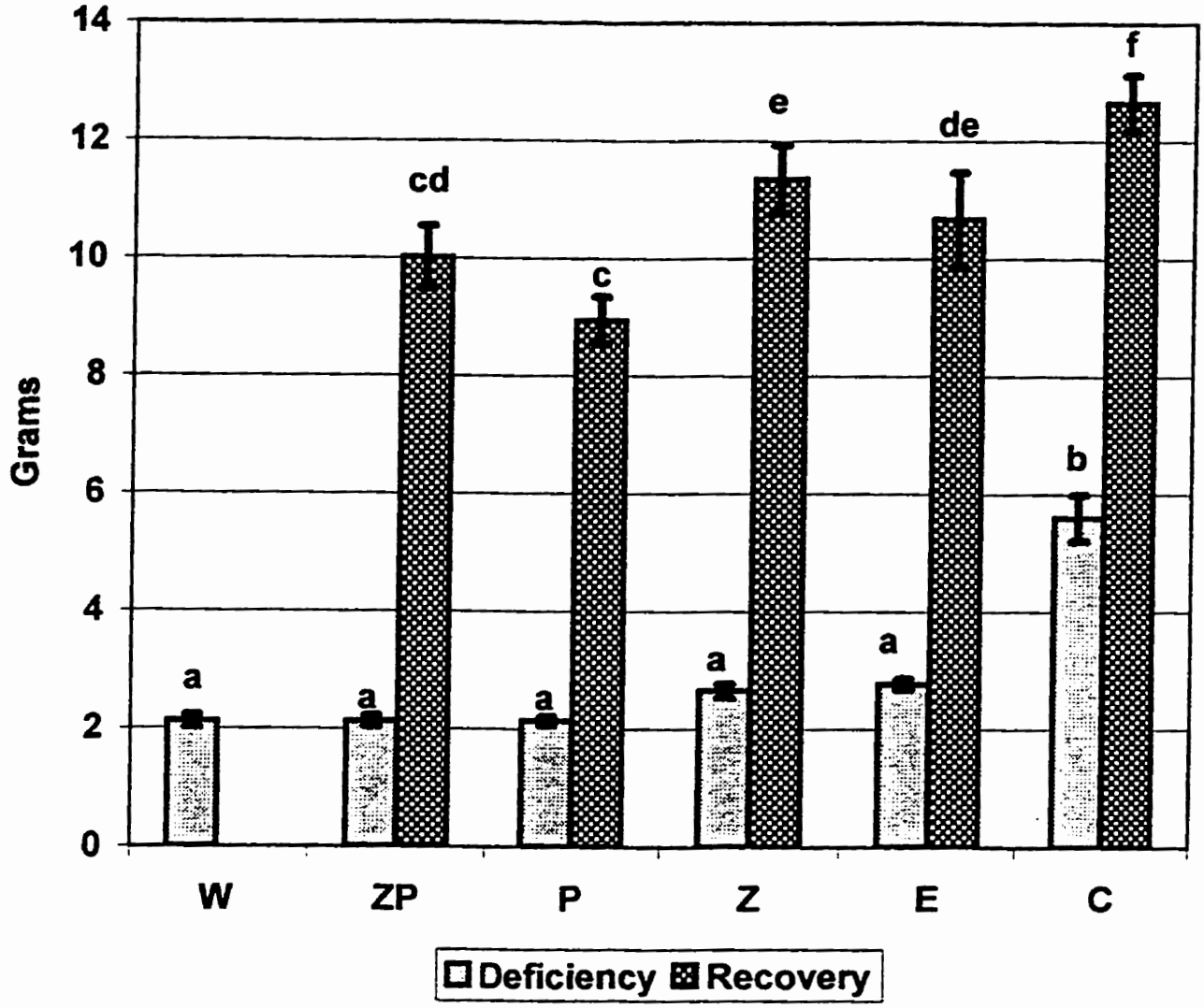


Figure 12. Liver mass after the deficiency and recovery phases. The columns represent group mean +/- SEM, n=8. Columns with different lower case letters are significantly different as determined by Duncan's multiple range test.

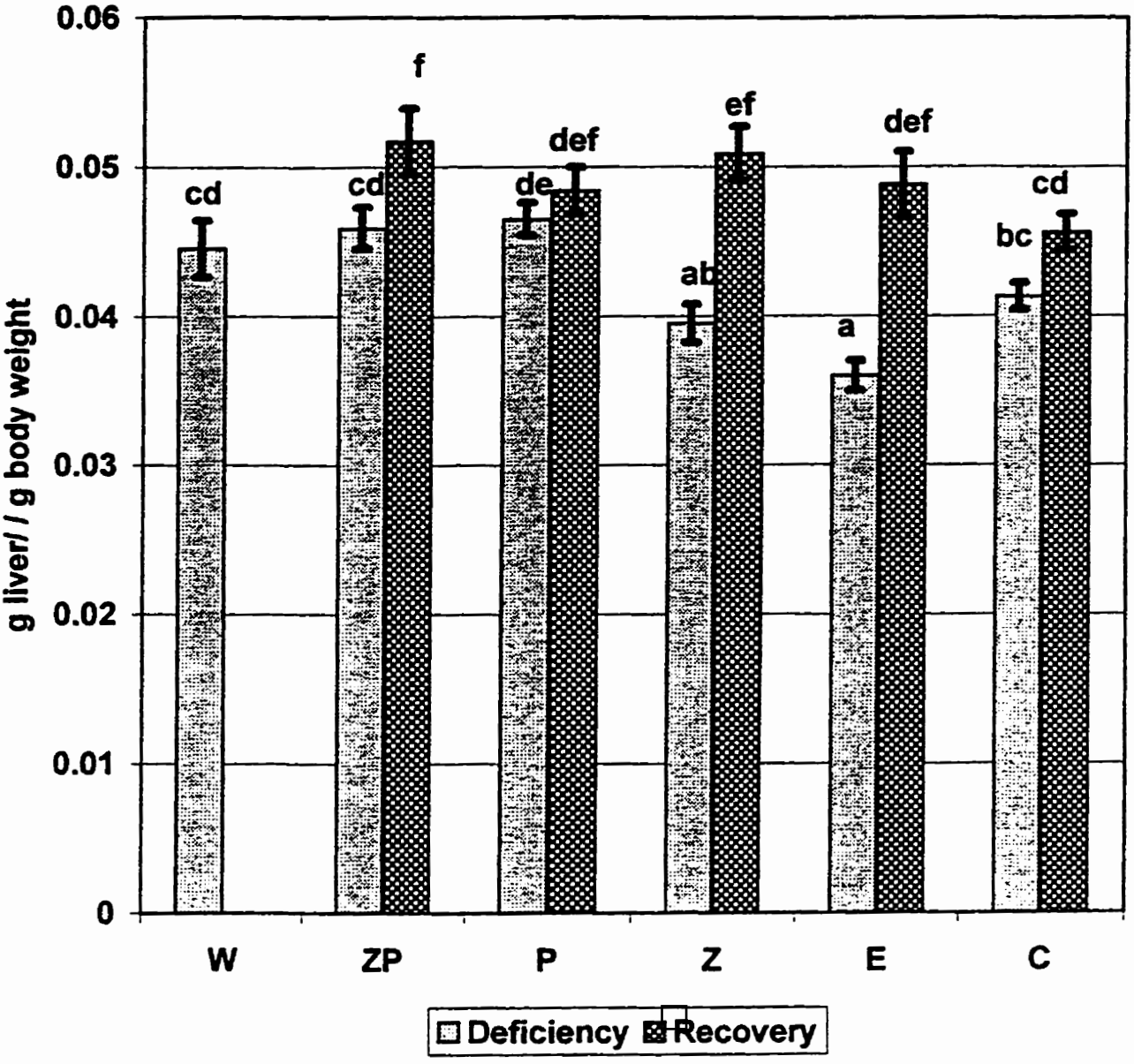


Figure 13. Liver weight to body weight ratio after the deficiency and recovery phases. The columns represent group mean +/- SEM, n=8. Columns with different lower case letters are significantly different as determined by Duncan's multiple range test.

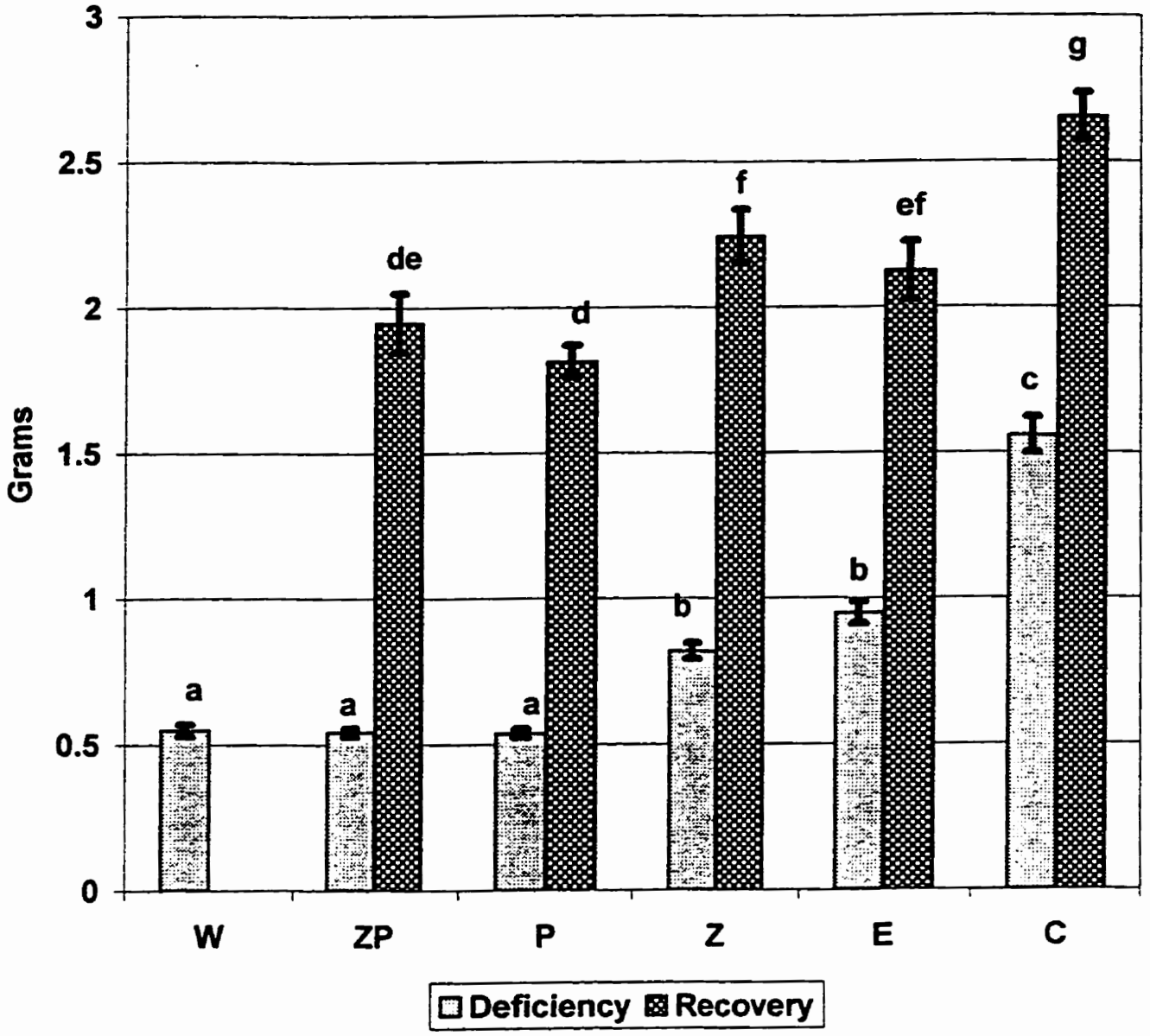


Figure 14. Kidney mass after the deficiency and recovery phases. The columns represent group mean +/- SEM, n=8. Columns with different lower case letters are significantly different as determined by Duncan's multiple range test.

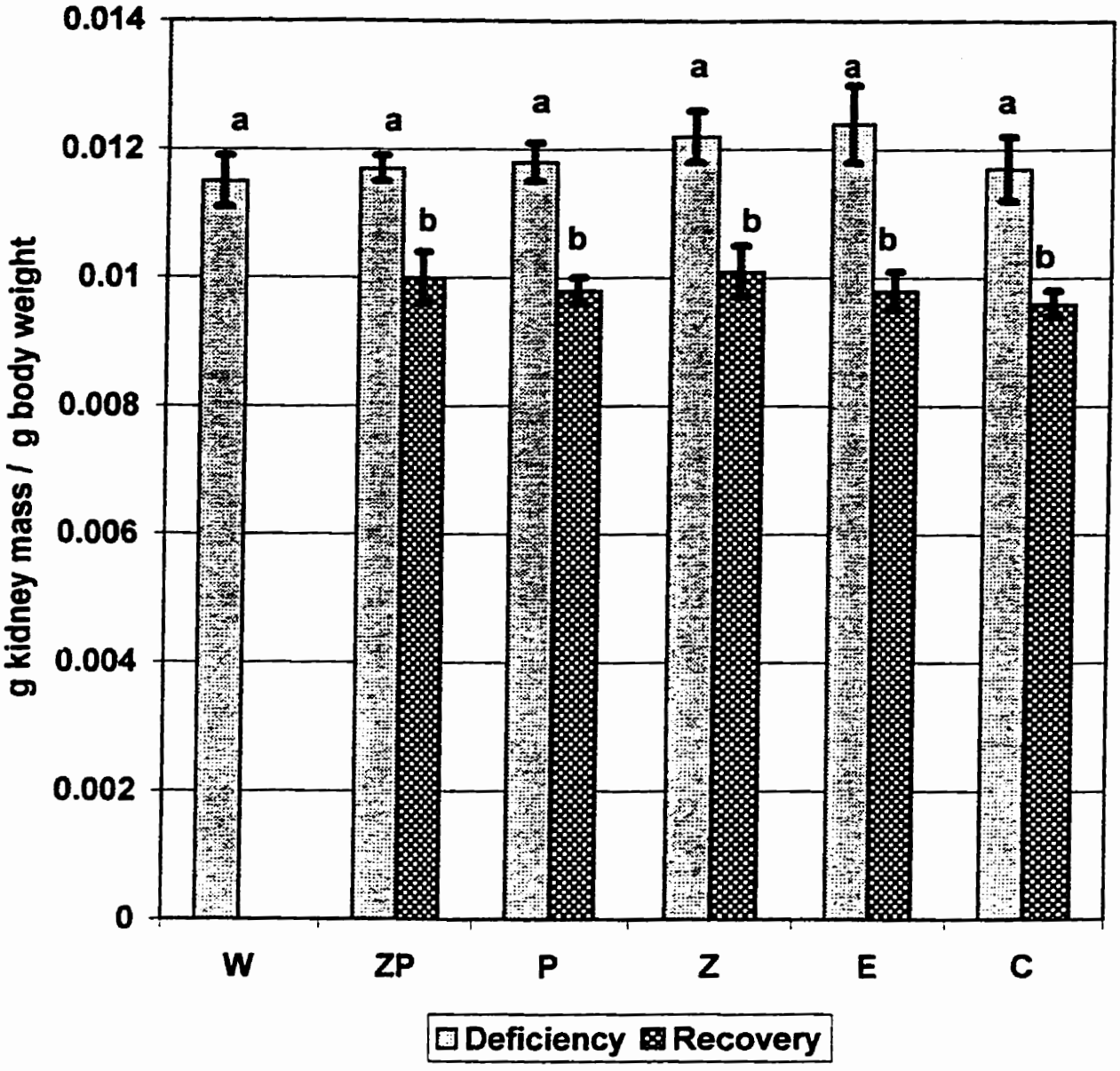


Figure 15. Kidney weight to body weight ratio after the deficiency and recovery phases. The columns represent group mean +/- SEM, n=8. Columns with different lower case letters are significantly different as determined by Duncan's multiple range test.

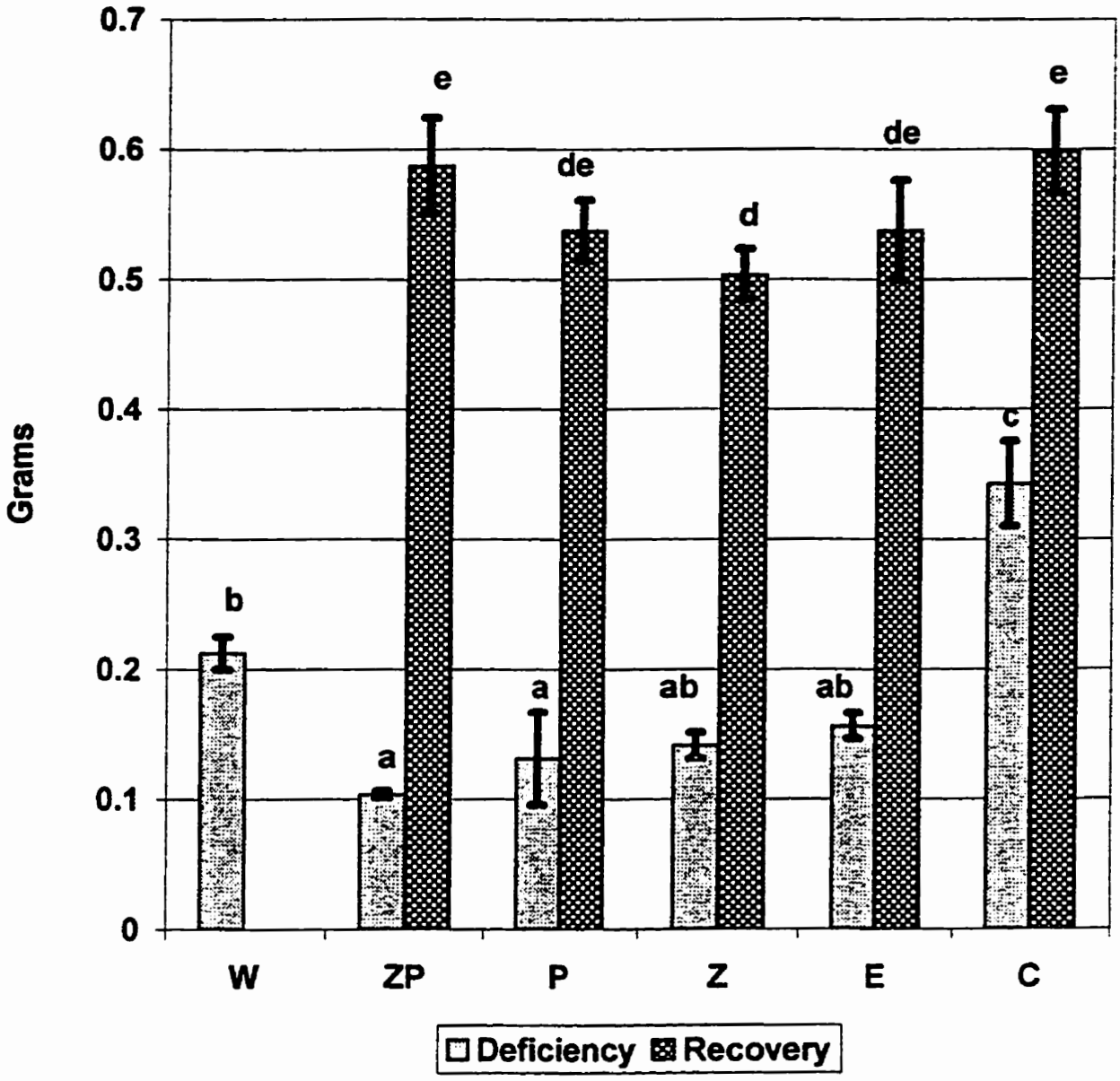


Figure 16. Spleen mass after the deficiency and recovery phases. The columns represent group mean +/- SEM, n=8. Columns with different lower case letters are significantly different as determined by Duncan's multiple range test.

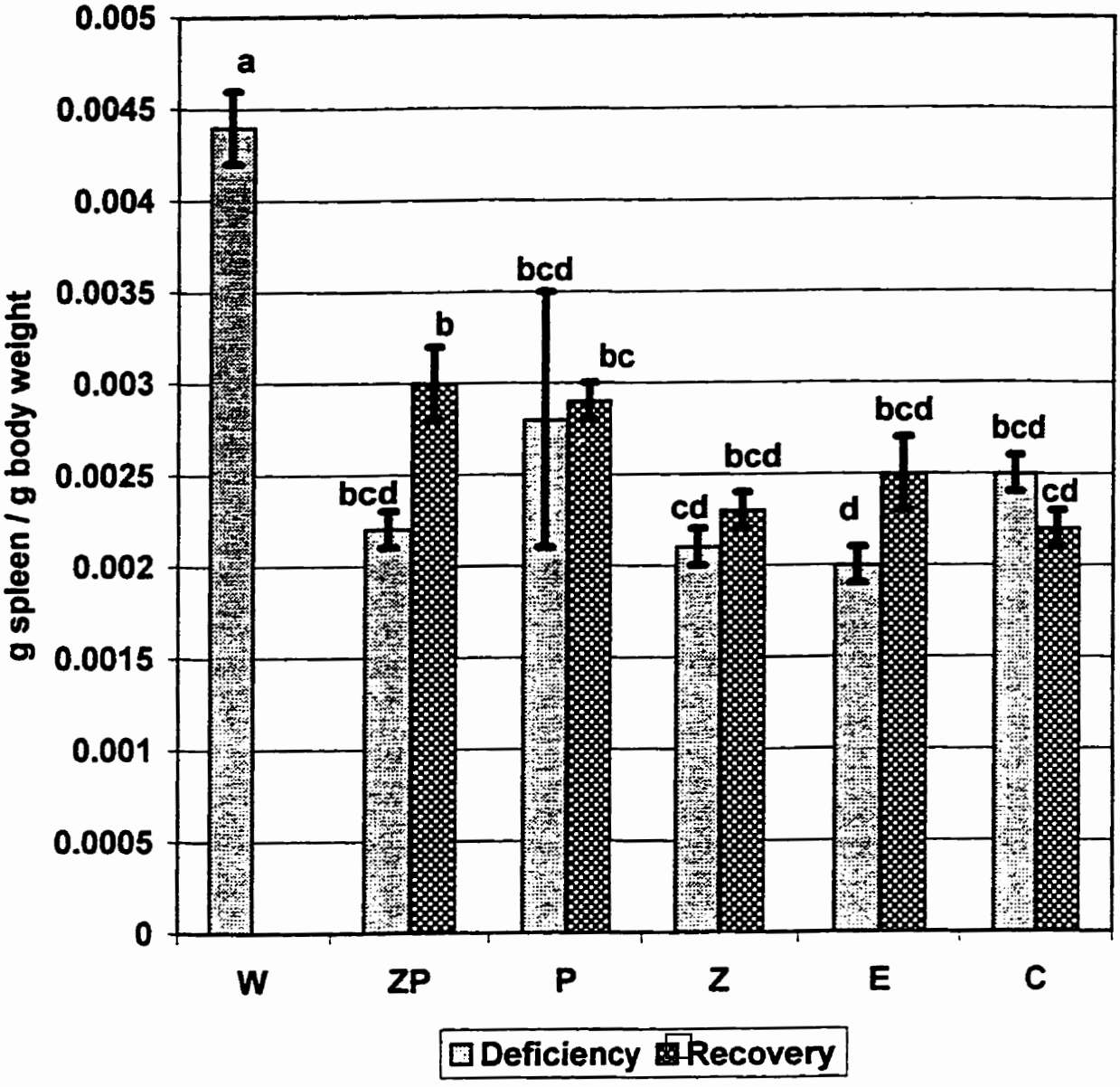


Figure 17. Spleen weight to body weight ratio after the deficiency and recovery phases. The columns represent group mean +/- SEM, n=8. Columns with different lower case letters are significantly different as determined by Duncan's multiple range test.

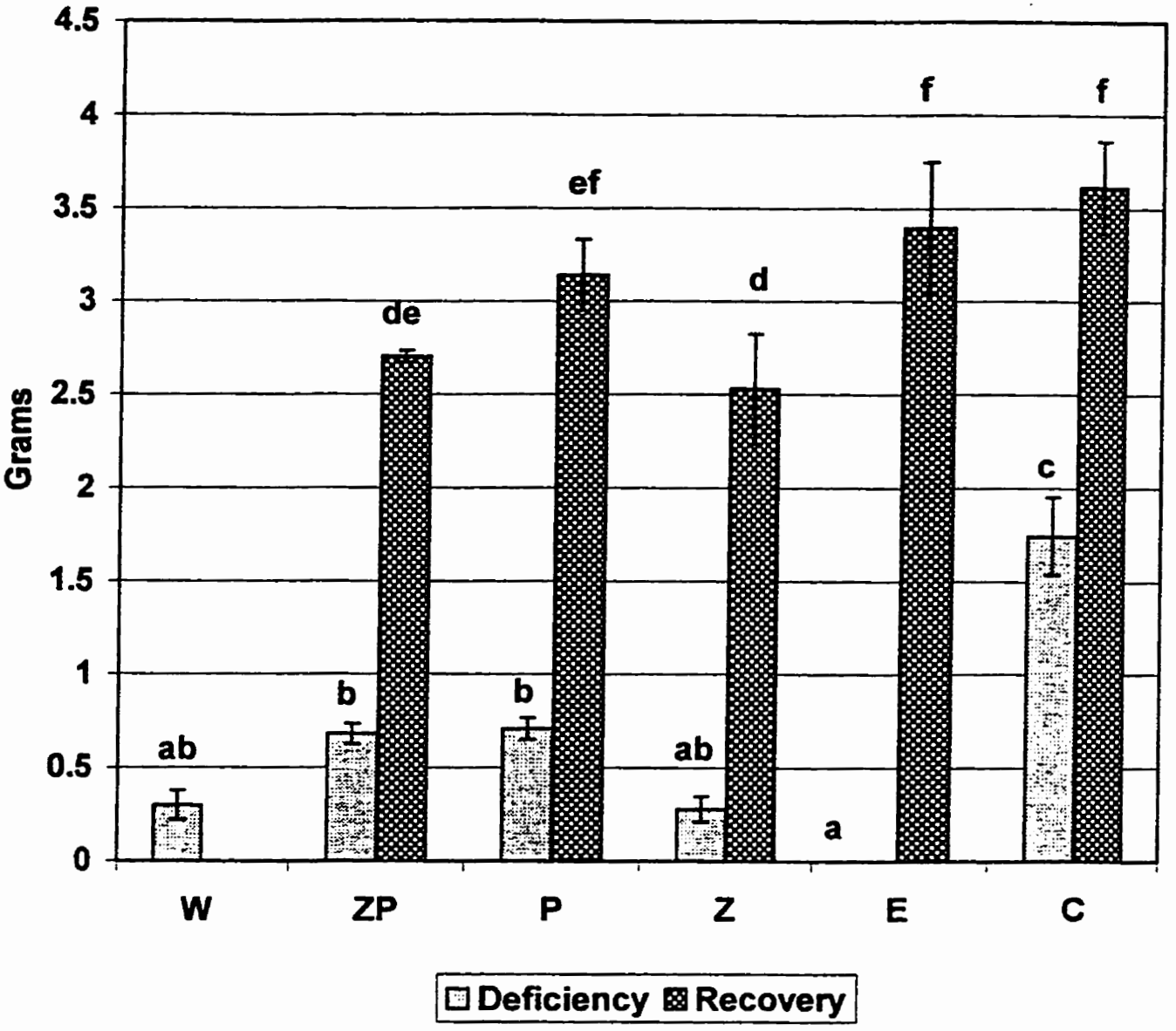


Figure18. Inguinal fat pad weights after deficiency and recovery phases. The columns represent group means +/- SEM, n= 8. Columns with different lower case letters are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

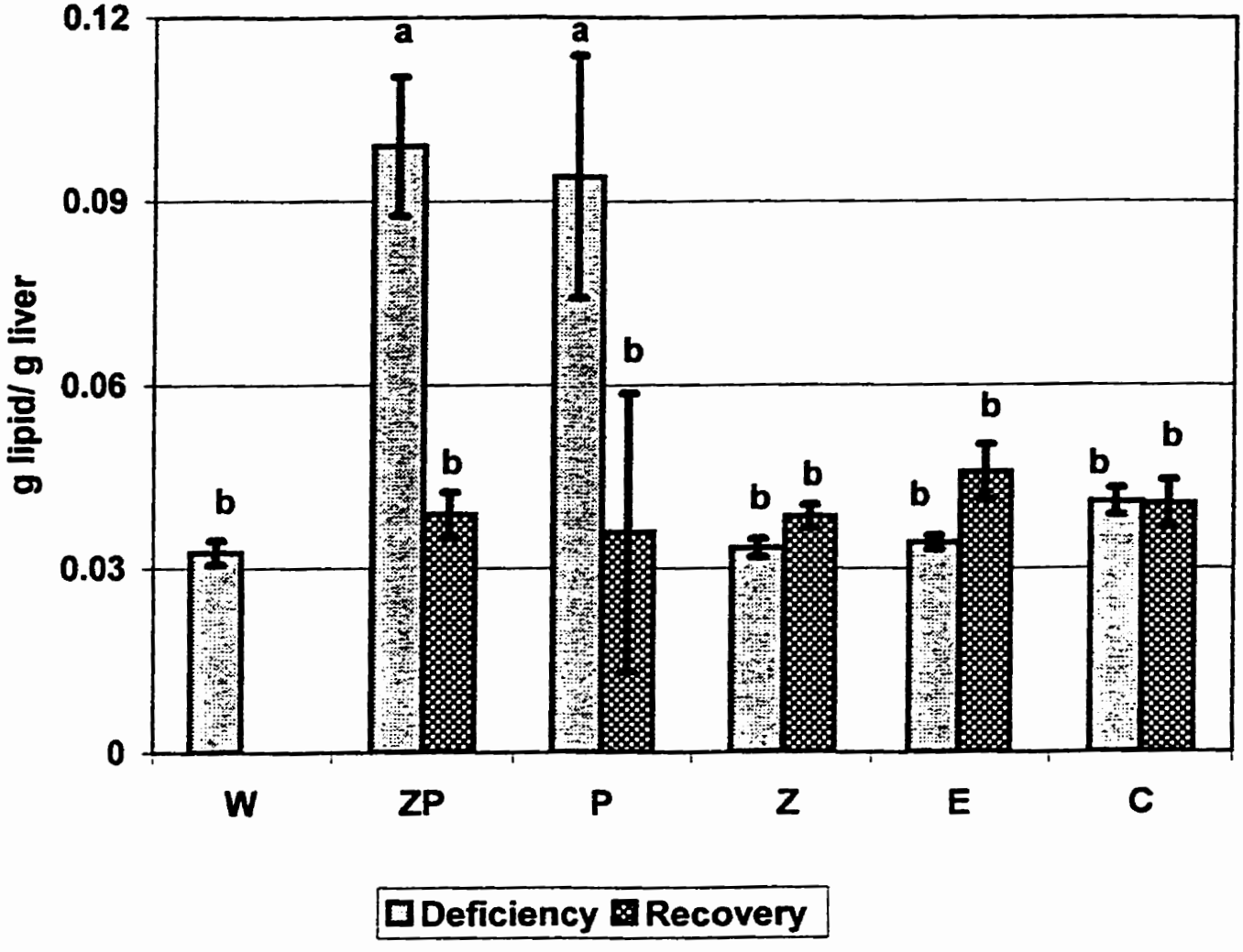


Figure 19. Liver lipid concentration after the deficiency and recovery phases. The columns represent group means +/- SEM, n= 5. Columns with different lower case letters are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

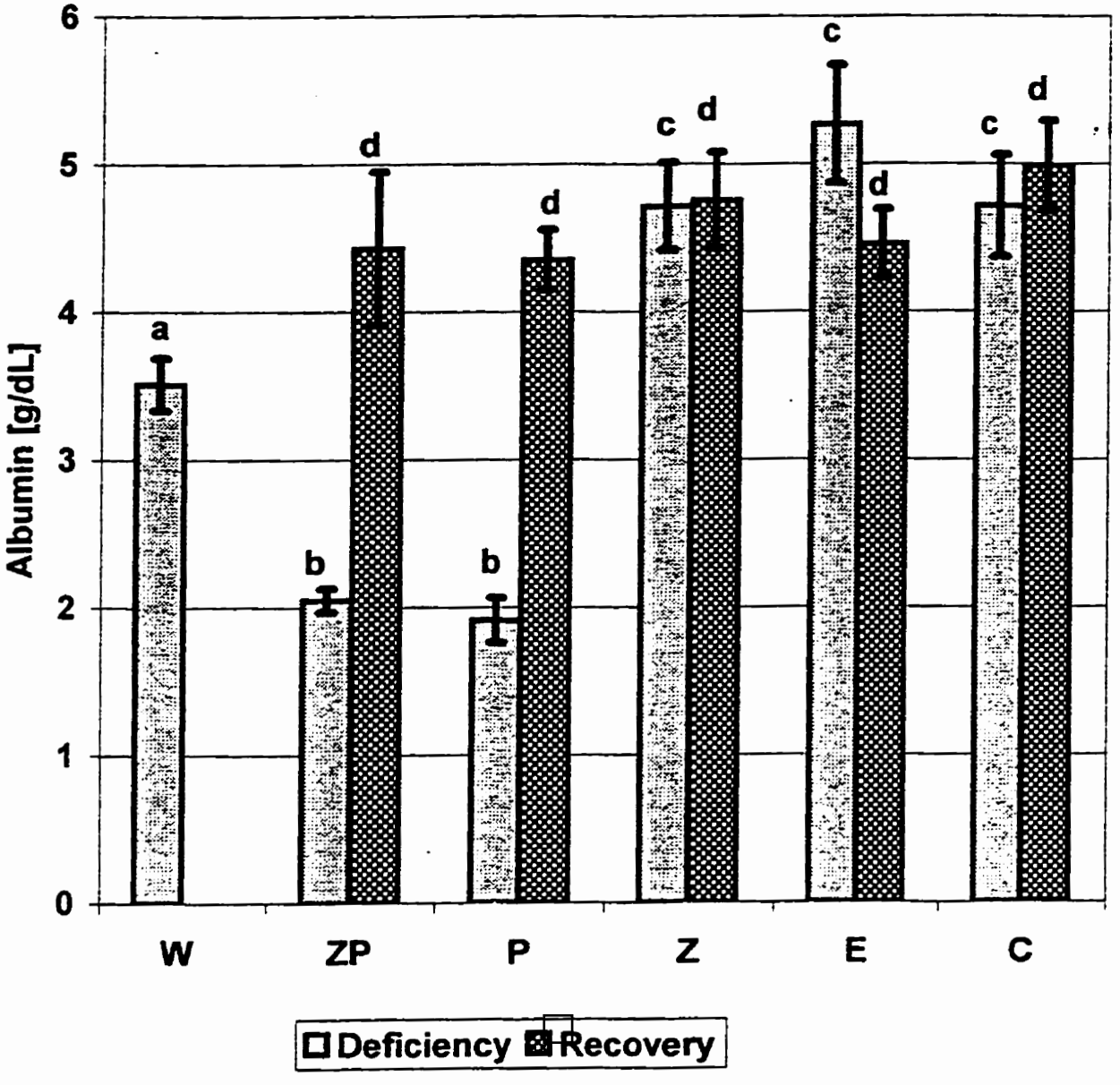


Figure 20. Serum albumin concentration after the deficiency and recovery phases. The columns represent group mean +/- SEM, n=8 except for the W group (n=6) and the ZP group (n= 7) at the end of the deficiency phase. Columns with different lower case letters are significantly different as determined by Duncan's multiple range test.

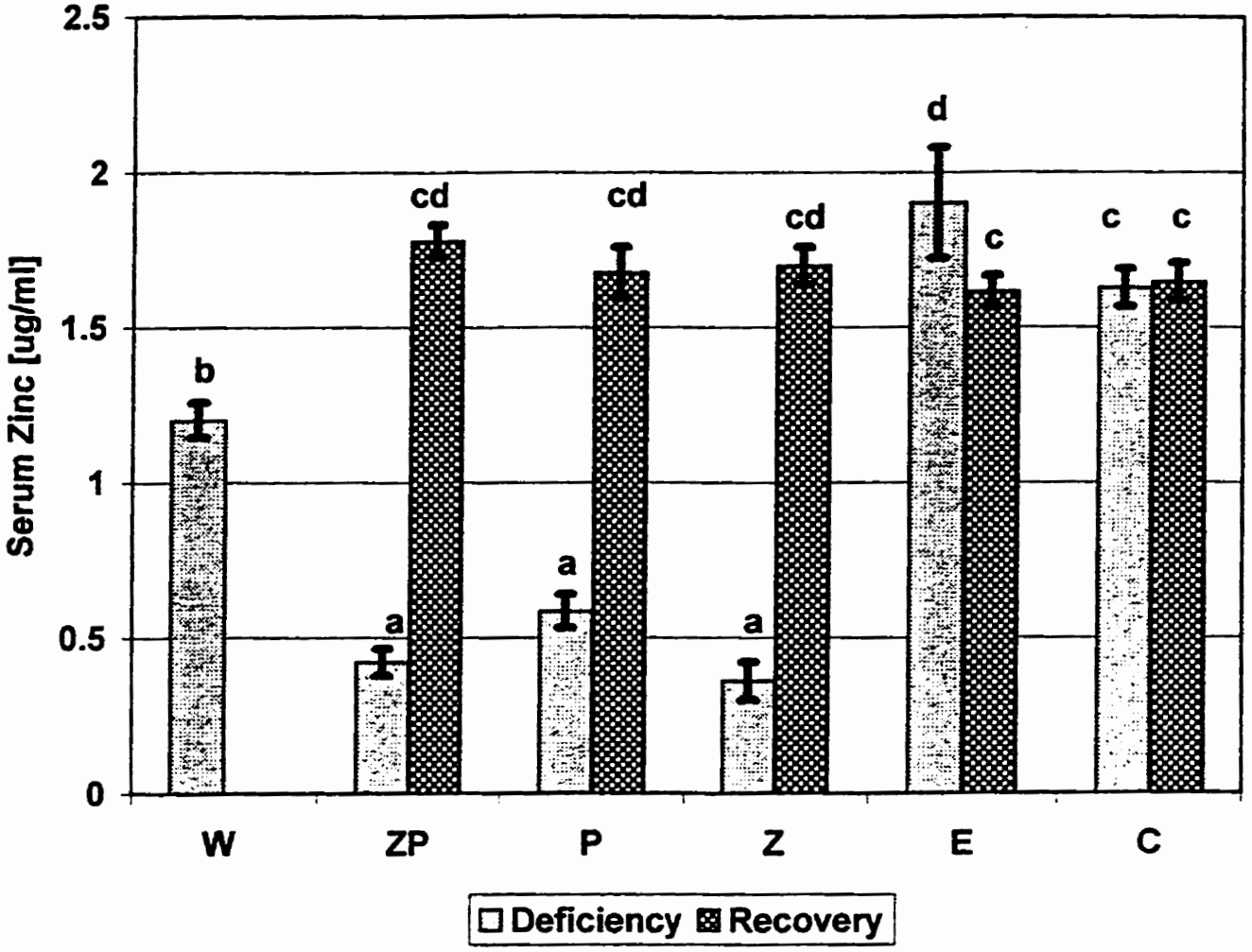


Figure 21. Serum zinc concentration after the deficiency and recovery phases. The columns represent group means +/- SEM, n= 8 with the exception of the C group at the end of the recovery phase (n=7). Columns with different lower case letters are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

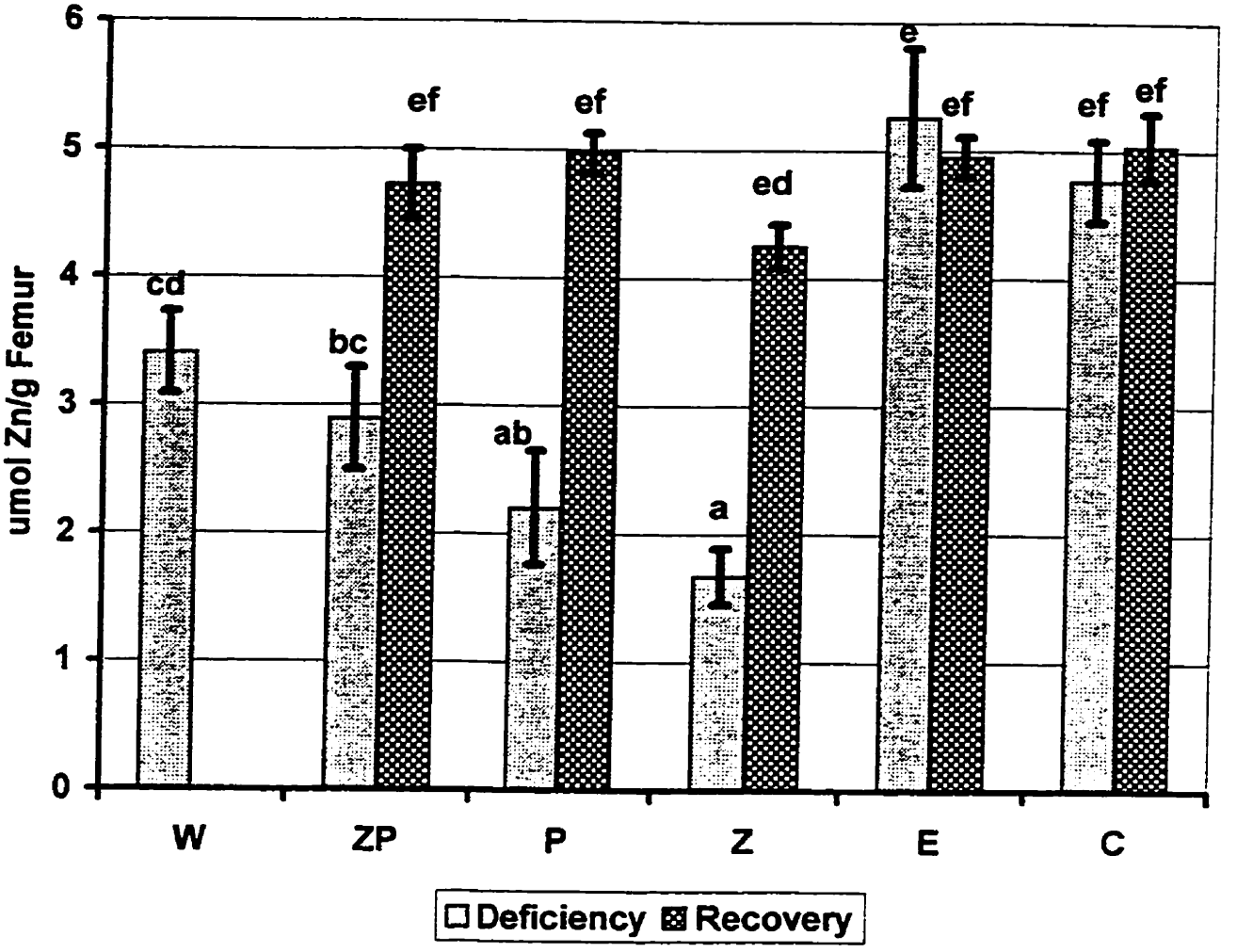


Figure 22. Femur zinc concentration after the deficiency and recovery phases. The columns represent group means +/- SEM, n= 8. Columns with different lower case letters are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

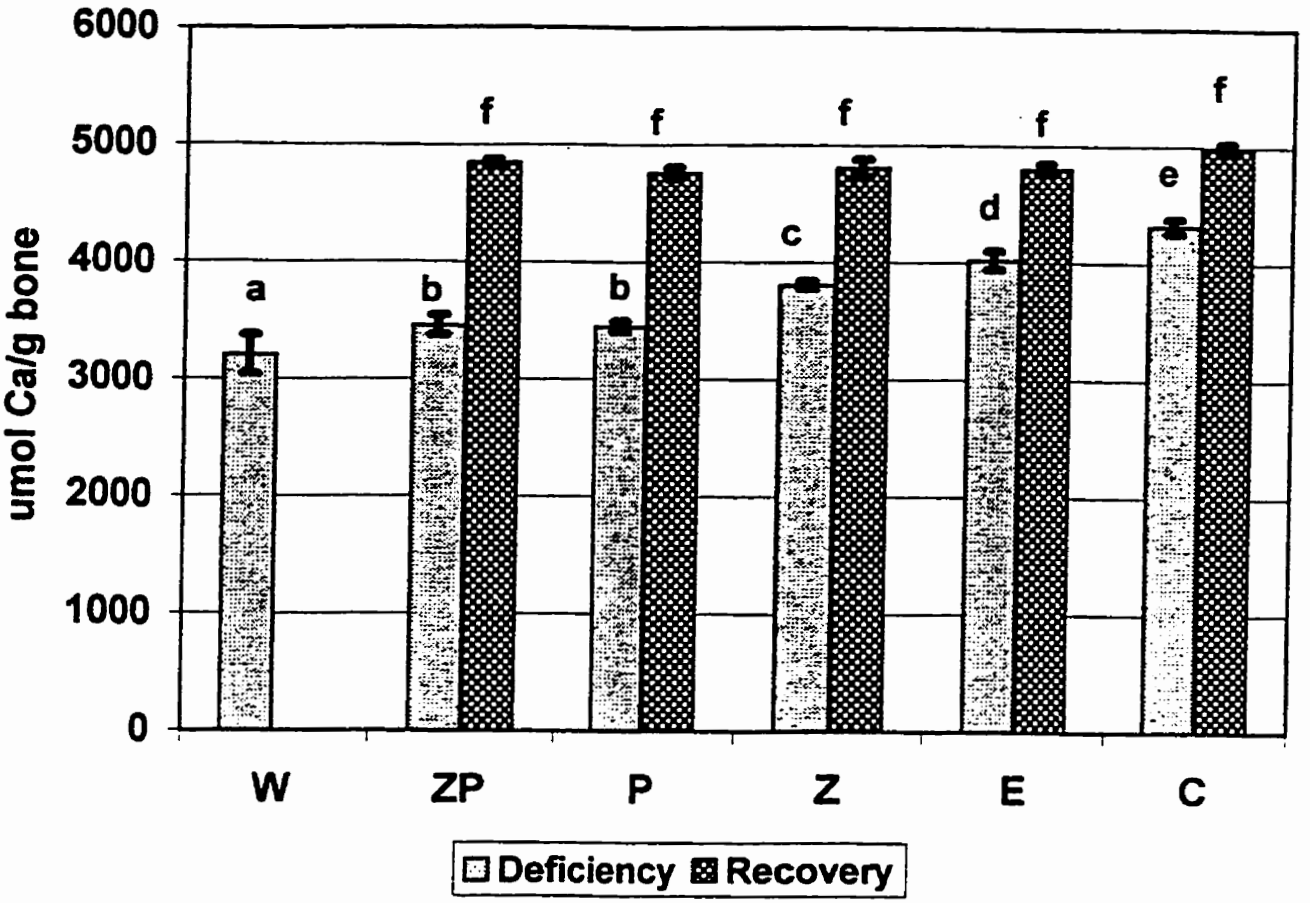


Figure 23. Femur calcium concentration after the deficiency and recovery phases. The columns represent group means +/- SEM, n= 8. Columns with different lower case letters are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

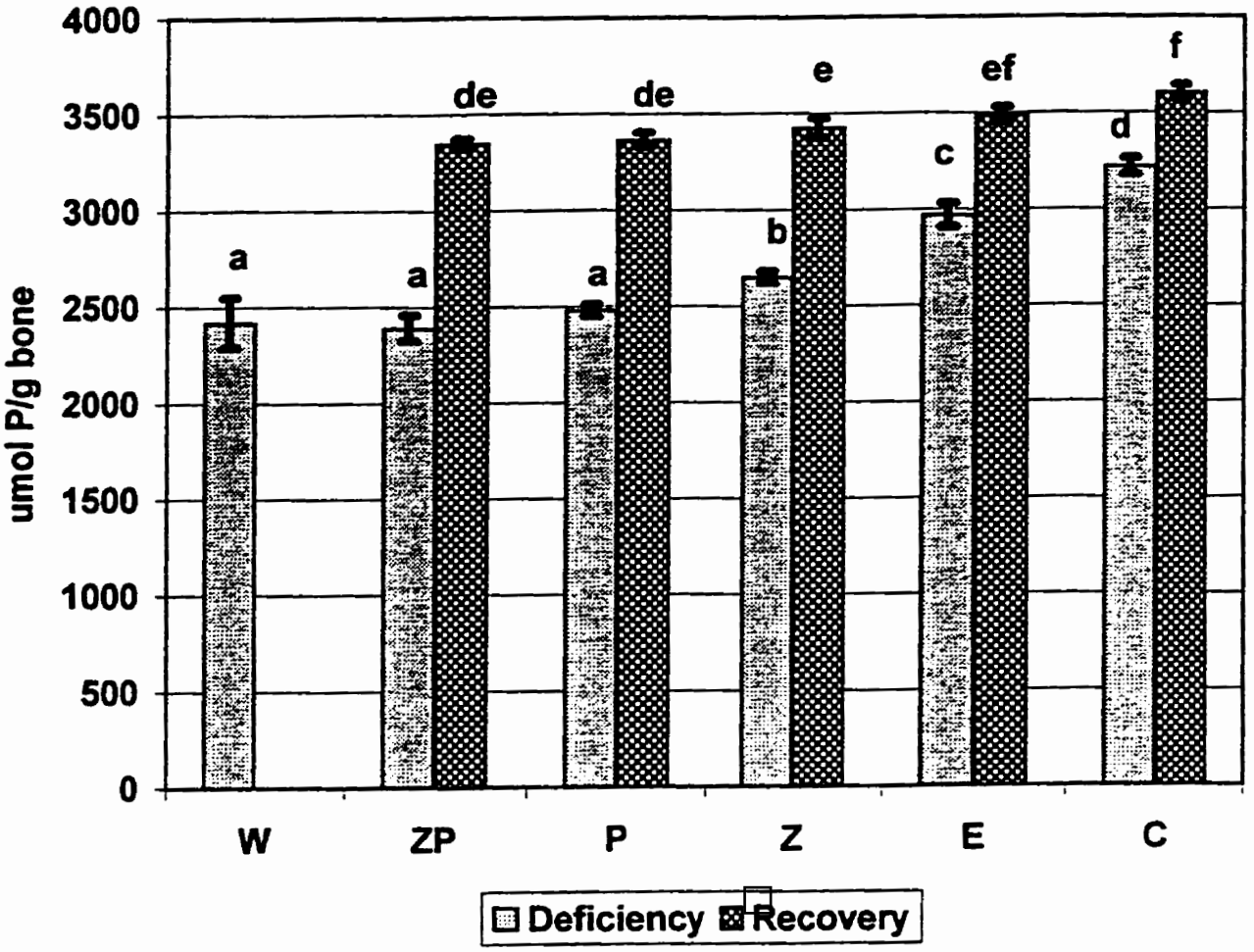


Figure 24. Femur phosphorous concentration after the deficiency and recovery phases. The columns represent group means +/- SEM, n= 8. Columns with different lower case letters are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

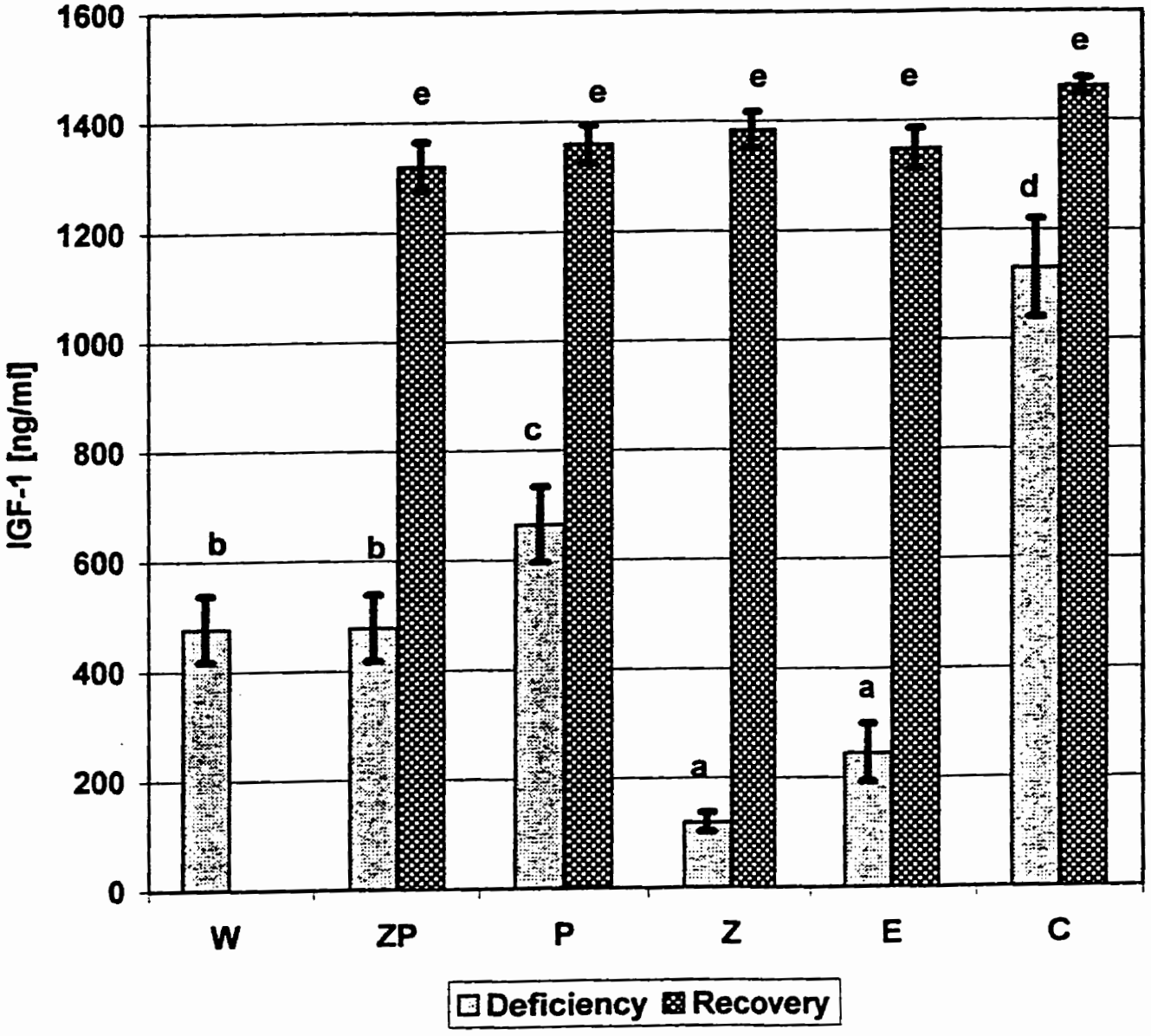


Figure 25. Serum IGF-1 concentrations after the deficiency and recovery phases. The columns represent group means +/- SEM, n= 8 with the exceptions of C (n=7), ZP (n=6) and E (n=7) groups after the deficiency phase. Columns with different lower case letters are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

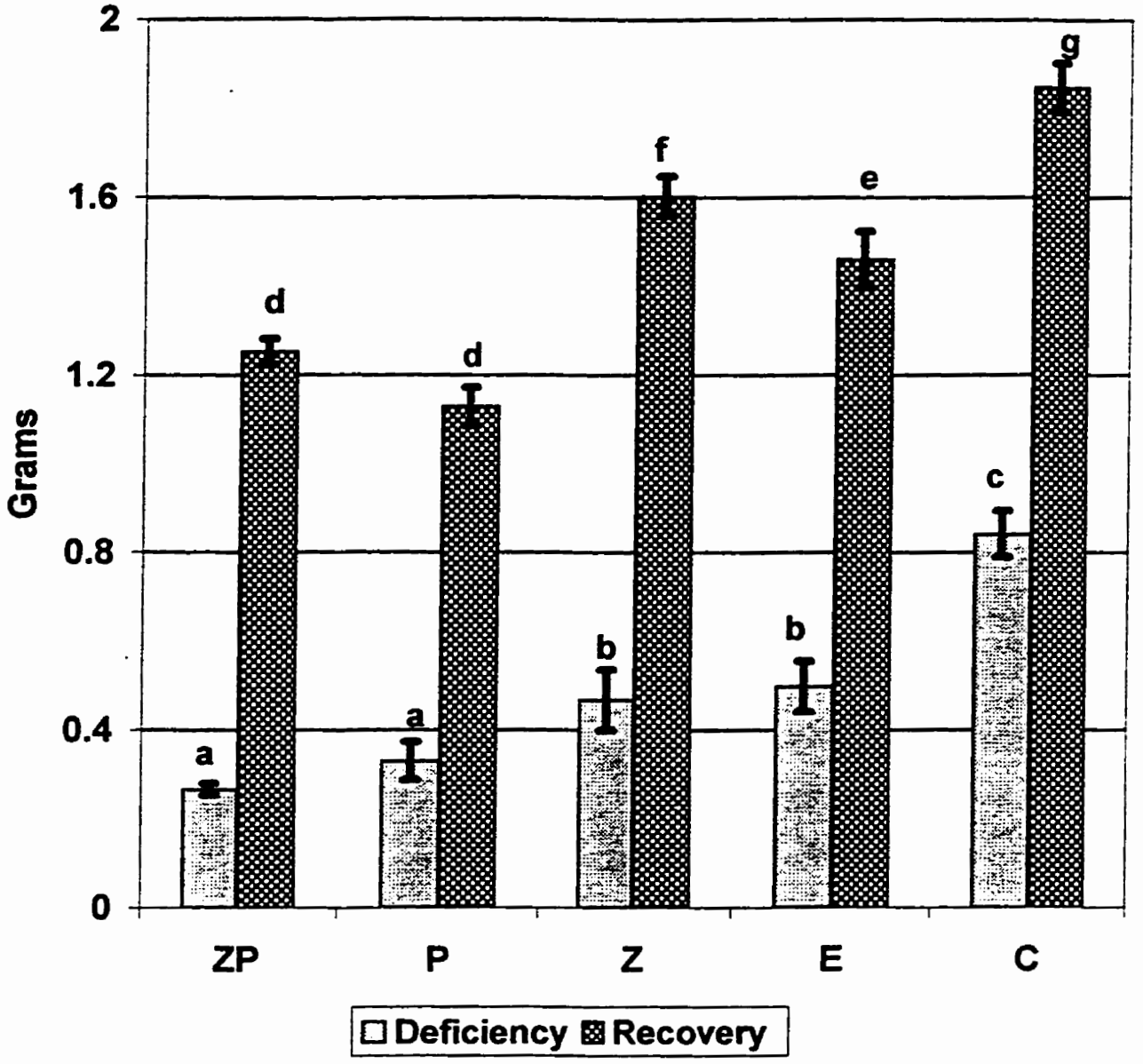


Figure 26. Gastrocnemius muscle mass after the deficiency and recovery phases. The columns represent group mean +/- SEM, n=8. Columns with different lower case letters are significantly different as determined by Duncan's multiple range test.

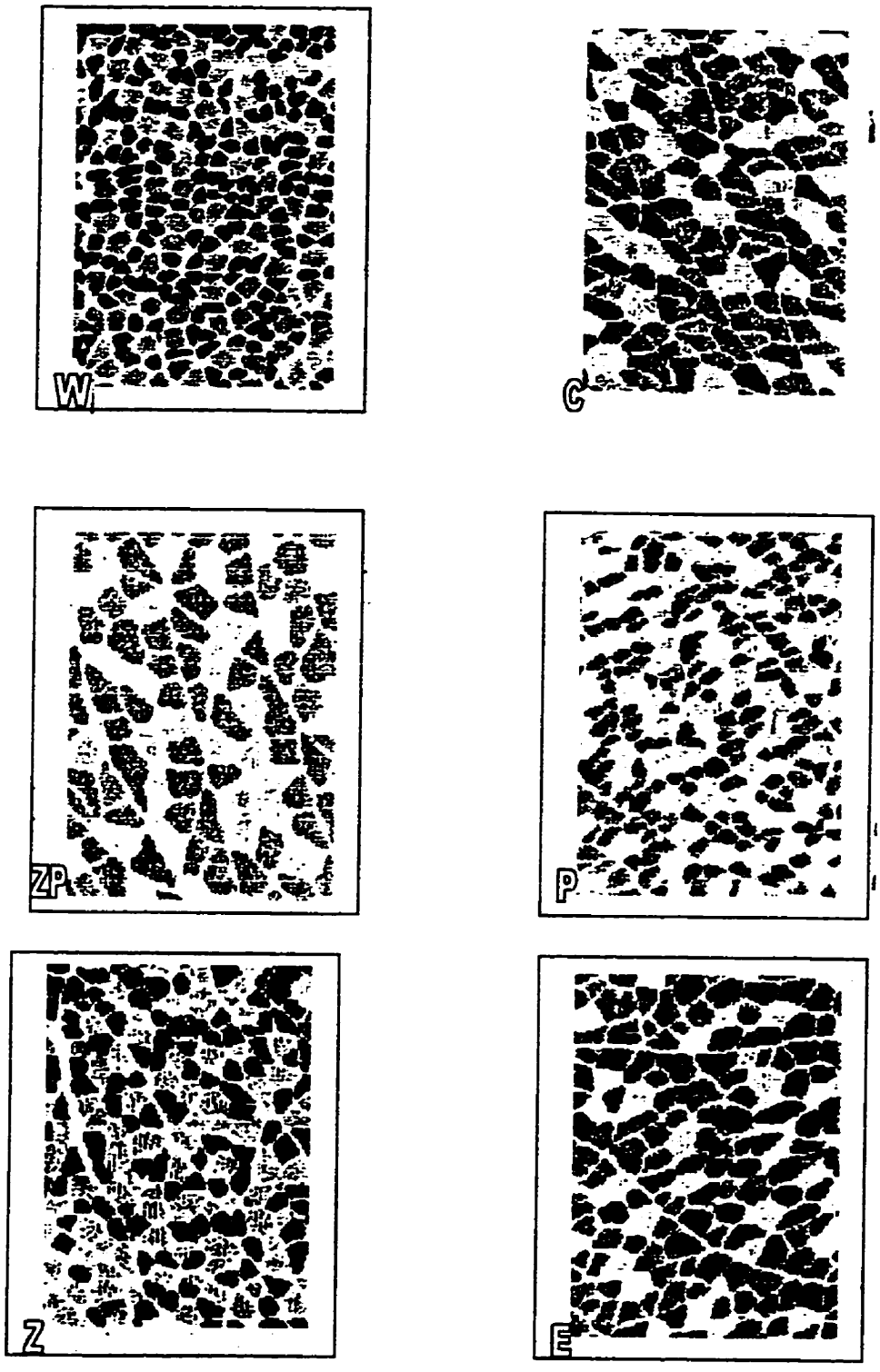


Figure 27. Digital images of gastrocnemius muscle subjected to ATPase 9.4 staining. The muscle samples were taken at day 0 (W) and after the deficiency phase.

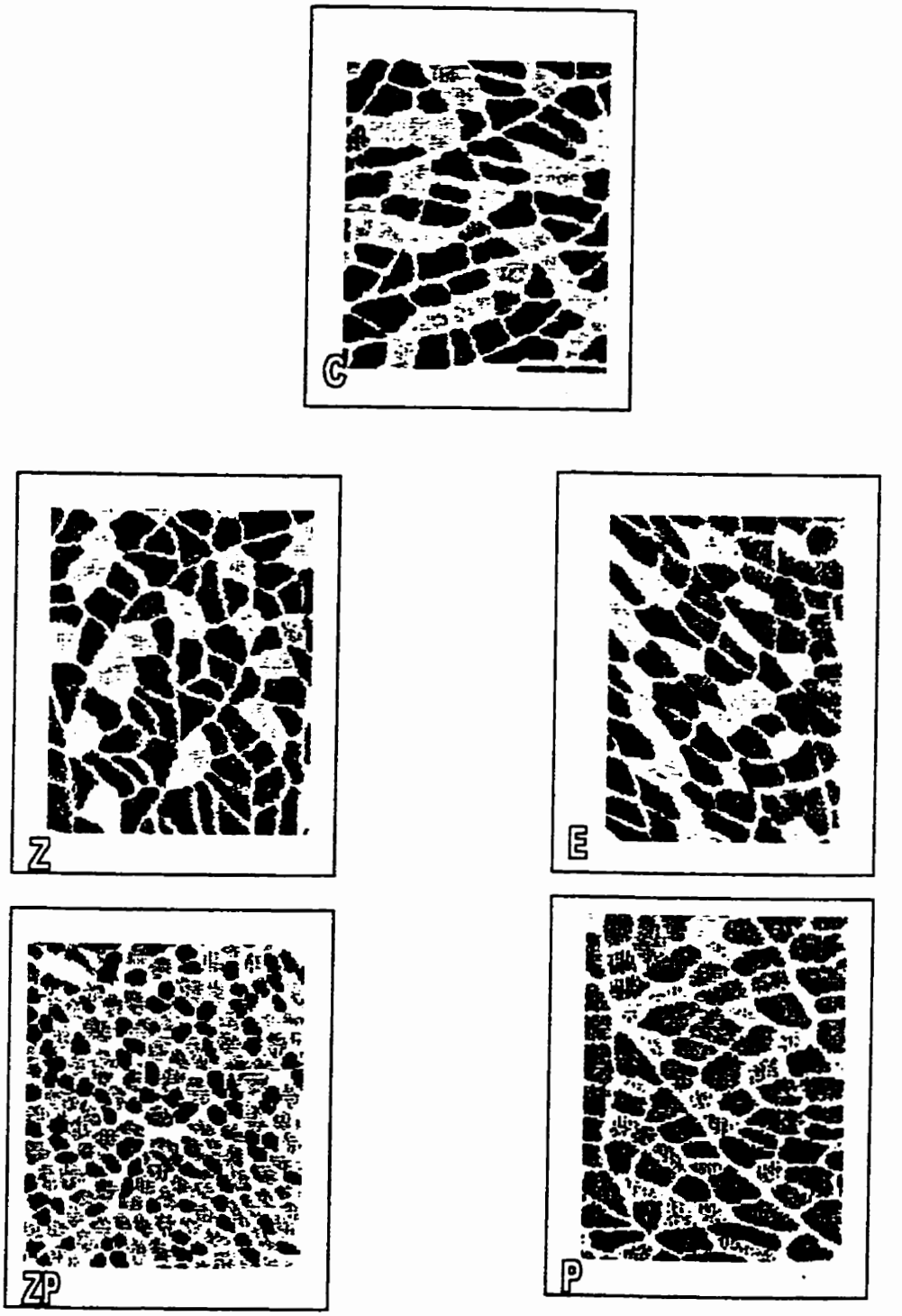


Figure 28. Digital images of gastrocnemius muscle subjected to ATPase 9.4 staining. The muscle samples were taken after the recovery phase.

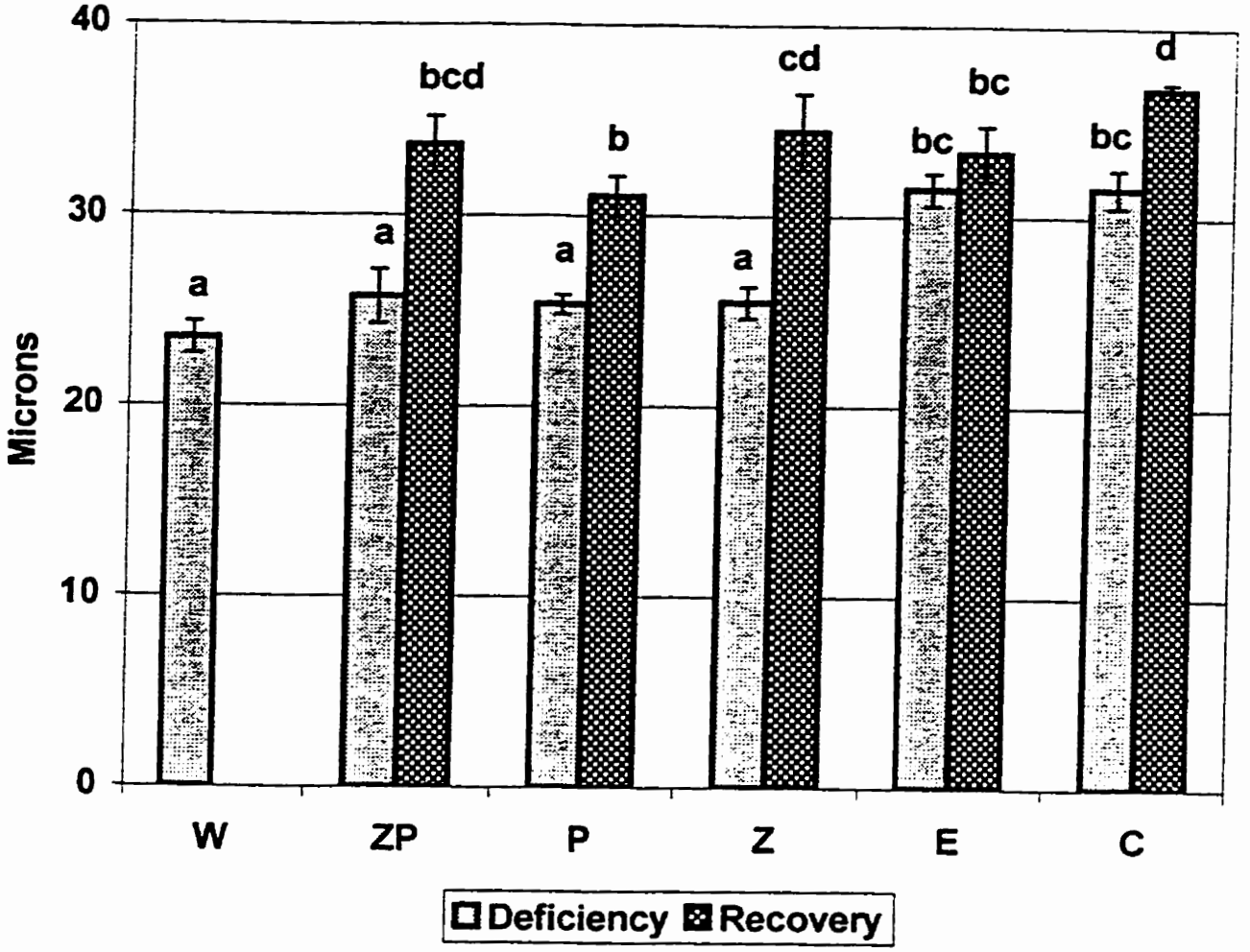


Figure 29. Type 1 muscle fiber minimum diameter after the deficiency and recovery phases. The columns represent group means +/- SEM, n= 6. Columns with different lower case letters are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

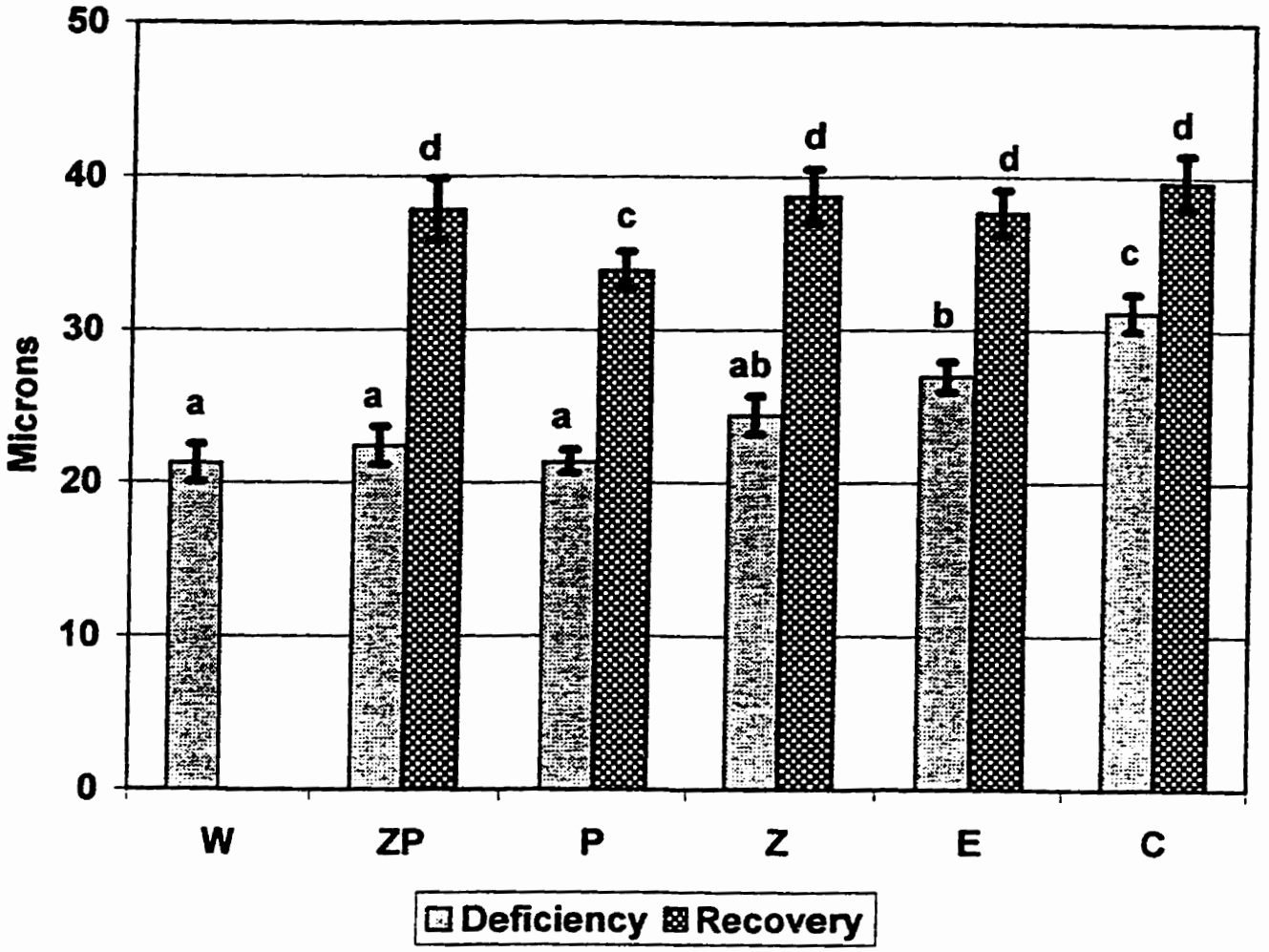


Figure 30. Type 2 muscle fiber minimum diameter after the deficiency and recovery phases. The columns represent group means +/- SEM, n= 6. Columns with different lower case letters are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

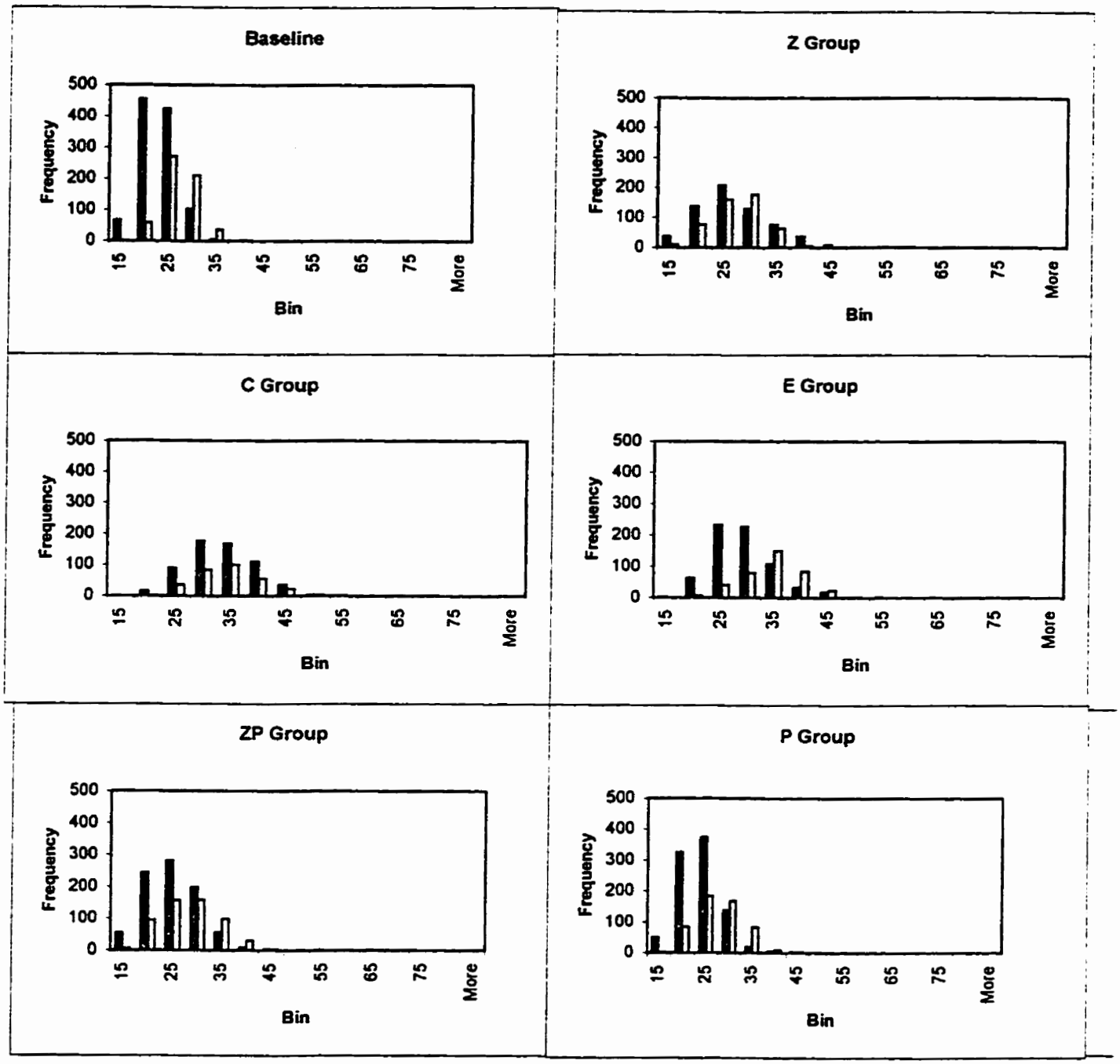


Figure 31.Frequency histograms of the Type1 (□)and Type 2 (■) MFDs at day 0 and after the deficiency phase. Each histogram depicts n = 6 rats.

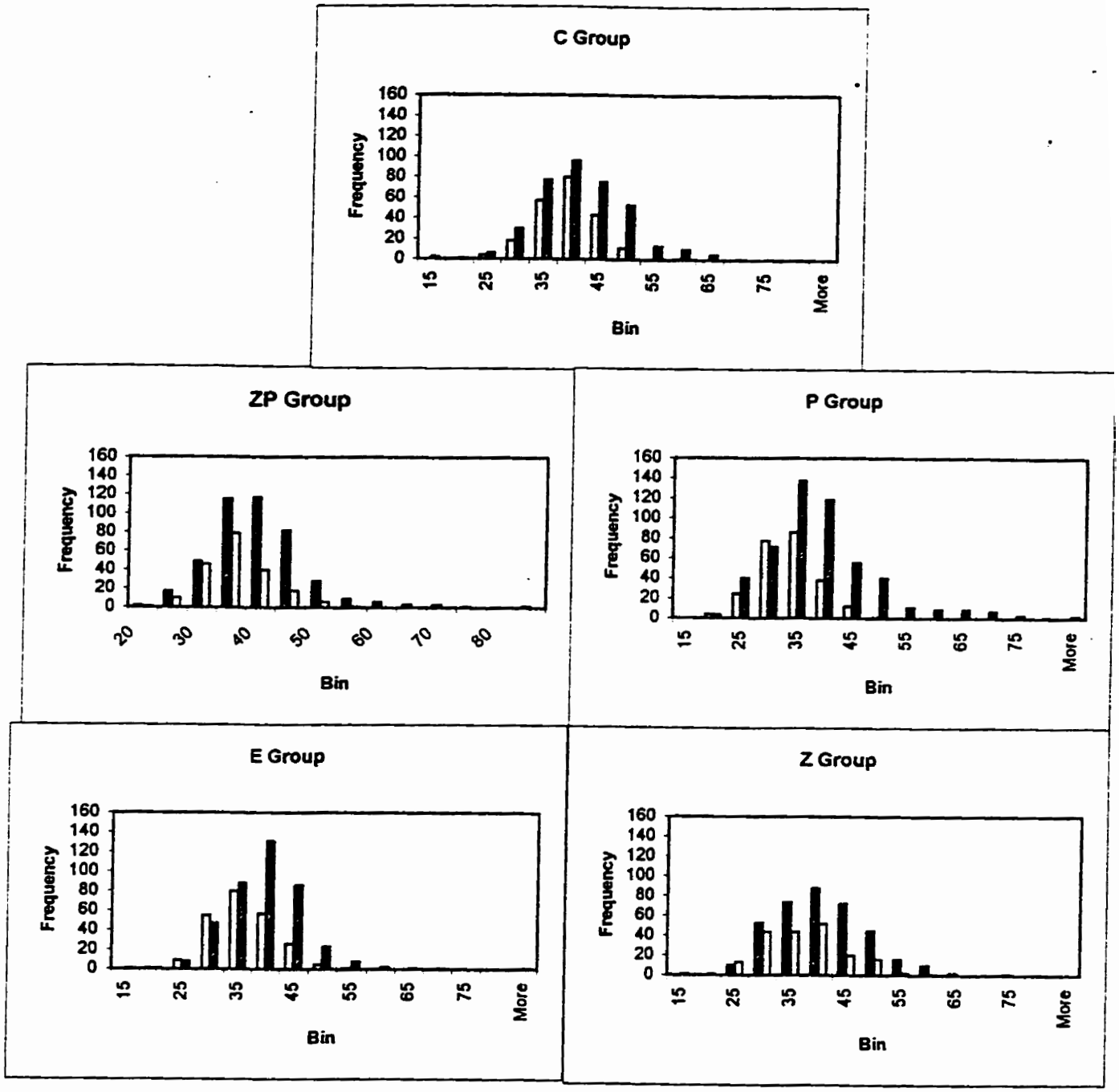


Figure 32. Frequency histograms of the Type1 (□) and Type 2 (■) MFDs after the recovery phase. Each histogram depicts n = 6 rats.

V. Discussion

This study included two important features. Firstly, the study examined the effects of zinc, protein, energy and combined zinc and protein deficiencies on the whole body, tissue, cellular and biochemical levels of organization in young growing rats. Secondly, the study included a recovery period, which allowed for observations regarding the degree of recovery from the nutritional deficiencies. This combination of study features allowed for further characterization of Zn, protein and energy deficiencies and their effects on muscle tissue and histology.

Normal Growth

Between 3 weeks and 9 weeks of age, the male weanling rat experienced a period of intensive growth and development as evidenced by the C group data. The body weight gains (Figure 5), organ (Figures 12,14 & 16) and inguinal fat pad weights (Figure 17), tail and body lengths (Figures 10 and 11), serum IGF-1 concentrations (Figure 25), T1-MFD and T2-MFD (Figures 29 and 30) all increased over the length of the study indicating growth. This intensive growth was accompanied by a steady increase in feed intake (Figure 7) and a relatively efficient utilization of dietary nutrients for growth (Figure 9).

Growth during Nutritional Deficiency

Zinc, protein, energy and combined Zn + protein deficiencies derailed the growth and development of the young rats. The Z and E groups experienced suppressed growth during the deficiency period as evidenced by their lower weight gains (Figure 4) and body weights (Figure 5), diminished serum IGF-1 concentrations (Figure 25) and T2-MFD (Figure 29) and shorter tail and body lengths (Figures 10 and 11) as compared to the C group. This depressed growth was associated with significantly diminished feed intakes (Figure 6) versus the C group. At the same time, the Z and E groups had feed efficiencies (Figure 9) that were similar to the C group. Despite similar feed efficiencies, the Z and E groups experienced significantly less growth compared to the C group. It is interesting to note that the E group had a significantly greater feed efficiency (a smaller feed intake to body weight ratio) than the Z group, yet both groups had similar body

weights, lengths and serum IGF-1 concentrations at the end of the deficiency phase. The Z and E groups were able to significantly increase their body length and mass above baseline measurements during the deficiency phase. The protein deficient groups experienced a greater degree of growth suppression. The body weights and T2-MFD of the ZP and P groups did not increase significantly above baseline during the 3-week deficiency phase. These rats also had the smallest increases in tail and body length, feed intake and organ weights. The P group required significantly more grams of feed per gram of body weight gained and had significantly higher serum IGF-1 concentration versus the ZP group. At the end of the deficiency phase, the ZP and P groups had similar body weights and lengths. The protein deficient rats were able to significantly increase their linear growth from baseline measurements, but they were unable to significantly increase their body mass from baseline.

In summary, the Z, E and protein deficient rats had reduced body weight and length, which were typical of rats and children suffering from macro and micro nutrient deficiencies (Hansen-Smith , 1978; Glore and Layman, 1987; Smith et al., 1989; Glore et al. 1993). The deficient rats in this study also exhibited reduced serum IGF-1 concentrations and altered muscle fiber development. These findings will be discussed in detail in later sections.

Glore et al. (1993) reported that young rats (75-100 g) fed a 3% protein diet for 21 days experienced a 10% weight loss while well-fed control rats had a 129% weight gain. The body lengths of the 3% protein rats did not significantly increase from baseline and the control rats did experience significant linear growth. Unfortunately, the authors did not state the age of the rats nor did they provide data on the actual amount and velocity of somatic and linear growth. Without this information one could postulate that the rats began the Glore et al. (1993) study at 5 to 6 weeks of age which was twice the age of the rats in the present study. With this in mind it is stunning that the ZP and P (2% protein) diets produced a similar degree of retarded body weight gain (66%) as the Glore et al. (1993) 3% protein diet (61%). The Glore et al. (1993) and the current study differ in terms of the effect of protein deficiency on linear growth. The ZP and P rats were able to significantly increase their linear measurements above baseline while the Glore et al.

(1993) were unable to do so. One could speculate that the linear growth in the younger ZP and P rats merited a greater priority than in the more mature rats of the Glore et al. (1993) study.

In children, the magnitudes of somatic and linear growth are frequently used as part of the assessment of nutritional status (Hohenbrink and Nicol, 1993). Although different researchers utilized different standards of 'normal' or 'expected' weight for age and height, children diagnosed with PEM failed to meet the standards employed (Montgomery, 1962; Ashworth, 1969; Brooke and Wheeler, 1976; Hansen-Smith, 1978; Payne-Robinson, 1980; Simmer, 1988; Golden and Golden, 1992). Indeed the percentage of expected weight deficit for age has been used to create the Wellcome classifications of PEM (Lathan, 1990). Therefore, detailed weight, height and age information is important to the assessment of nutritional status, the degree of malnutrition and growth retardation as well as the assessment of the nutritional rehabilitation regime (Hohenbrink and Nicol, 1993).

Growth during Nutritional Rehabilitation

During the first week of the recovery phase (week 4), the deficient rats experienced a rapid increase in weight gain and feed intake (Figures 4 and 5). The large weight gain in week 4 was associated with high feed efficiency (Figure 9)(a small weekly feed intake to weight gain ratio). The body weights and feed intakes of the recovering rats continued to increase during weeks 5 and 6, but in a less dramatic fashion. In week 6, the ZP, P and Z groups had significantly greater weight gains than the E group, but there were no significant differences in feed intakes among the deficient groups. The feed efficiencies of the P and ZP (2.3 and 2.1, respectively) were similar to the E group (2.1), yet the final body weights of the protein deficient groups (66%-69% of the C group) were significantly less than the E and Z groups (78% and 80% of the C group, respectively). The serum IGF-1 concentrations fully recovered during the rehabilitation period. Despite the rapid weight gains, increased feed intakes, relatively high feed efficiencies and increased serum IGF-1 concentrations, the deficient rats did not have a full and complete recovery. The deficient rats had an incomplete recovery of body weight, liver and kidney weights, body and tail lengths. The T1-MFD of the P and E groups and the T2-MFD of the P group also did

not fully recover. Due to the study design it is not certain if a long recovery period alone would have resulted in the full recovery of the parameters examined.

The poor recovery of body mass and length in the deficient rats was similar to that seen in the studies on PEM children and rats (Soliman et al., 1986; Castillo-Duran et al., 1987; Glore and Layman 1987). The studies by Soliman et al. (1986) and Castillo-Duran et al. (1987) included pre and post nutritional rehabilitation weight and height data. In both studies nutritional rehabilitation resulted in an increase in the weight for age and height indices of the children (3-36 months old). After nutritional rehabilitation the children were 72% to 80% of their expected weight for height and 90% to 96% of their expected weight for height. Only the Castillo-Duran (1987) study indicated that there was an increase in linear growth. The study by Glore and Layman (1987) achieved decreased somatic and linear of young rats by limiting the feed intakes of nursing dams and the pups after weaning. After 196 days of nutritional rehabilitation the growth retarded rats recovered their body mass to 75% and their body length to 95% of the well fed control rats. Since, Glore and Layman (1987) made the young rats malnourished by limiting the feed intake of the dams and by limited feed intakes after weaning, one could assume that these rats were both protein and energy malnourished. Unfortunately, the Glore and Layman (1987) study did not confirm the type of malnutrition the underfeeding produced or the degree of recovery achieved through nutritional rehabilitation.

Type of Malnutrition and Body Composition

The type of malnutrition produced by the experimental diets was confirmed by biochemical assays. Changes in gastrocnemius muscle and inguinal fat mass were also employed in the assessment of nutritional status. The gastrocnemius and inguinal fat weights also provided insight into the effect of the experimental diets on body composition.

Zinc status

The Zn status of the rats was assessed via serum and femur concentrations. The serum and femur Zn concentrations of the ZP and Z groups were both significantly less than C group (26% and 22%, respectively). All of the ZP and Z rats exhibited growth retardation and reduced feed

intakes, and some had changes in fur texture. These are classical signs of Zn deficiency (Cousins and Hempe, 1990). It has been recognized that serum Zn concentration is not an effective measure of Zn status as it is readily influenced by numerous pathological conditions and is not sensitive to slight changes in Zn intake (Deeming and Weber, 1977; Solomons, 1979). Nevertheless, in well-controlled experimental conditions where Zn deficient and nutritional complete diets are employed, serum Zn concentration can be used as an indicator of Zn status (Hurley et al., 1982). Precautions were taken throughout the study to prevent Zn contamination of the diets and the rats were not given access to potential Zn containing materials. The rats of this study did not suffer from infection or illness, which can decrease the accuracy of serum Zn measurements. Skeletal femur Zn concentration was also determined as it can be used to indicate Zn deficiency (Demming and Weber, 1977, Giuglaiano and Millward, 1984). In this study and in other studies conducted in this laboratory the feeding of a Zn deficient diet to rats and mice resulted in decreased serum and femur Zn concentrations as well as alterations in feed intake and fur texture. Similar signs of Zn deficiency were observed in a study by Taylor et al. (1988). Taylor et al. (1988) fed 3-week-old male rats a <1.1 ppm Zn deficient diet for 3 weeks. The authors reported that the Zn deficient rats experienced growth retardation, alopecia and skin lesions on paws. The serum Zn concentration of the Zn deficient rats was 19% of the Zn adequate group. The degree of serum Zn concentration reduction observed in the Zn deficient rats of the Taylor et al. (1988) study was similar to what was found in the Z group where the serum Zn concentration was 22% of the C group in the present study.

The P group also exhibited decreased serum Zn concentrations and femur concentrations. A decrease in serum Zn concentration in rodents fed a 2% protein diet has been observed in other studies conducted in this laboratory (Lepage, 1997; Bossuyt, 1998). Filteau and Woodward (1982) reported that 21 day-old mice fed a 1.7% protein diet for 14 days had serum Zn concentrations that were significantly lower than mice fed a 18.5 % protein diet. Zinc supplementation of 165 ug Zn per gram of feed did not improve the low serum Zn concentration associated with the 1.7% protein diet (Filteau and Woodward, 1982). The authors stated that the degree of serum Zn suppression observed in the 1.7% protein mice was similar to that of mice

fed a Zn deficient diet for 2 weeks in previous experiments conducted in their laboratory. In the present study, the degree of serum Zn suppression observed in the P rats was also similar to the Zn deficient rats. Wapnir et al. (1985) reported that young rats (74 g) fed a 4% protein diet for 4 weeks had plasma Zn concentrations that were 48% of the control group fed a 23% protein diet. The authors called this phenomena 'relative hypozincemia'. The diets fed to the two groups only differed in protein content. The objective of the Wapnir et al. (1985) study was to explore if the intestines of PEM rats absorbed Zn in the presence of low molecular weight ligands differently from well-fed rats. The authors indicated that the PEM appeared to reduce the efficiency of Zn absorption compared to the well-fed control group. In future studies it would be interesting to examine if intestinal Zn absorption is reduced by protein deficiency alone and if changes in intestinal absorption could explain the development of relative hypozincemia during protein deficiency.

Interestingly, the double deficient ZP group did not have lower serum or femur Zn concentrations than the P and Z groups (Figures 21 and 22). The Zn status of the Z and P groups was impaired by Zn and protein deficiency, respectively. Instead of having a negative synergetic effect on serum and femur Zn concentrations, the double deficiency appeared to have a positive one. Bettger (1985) fasted young (100 g) rats for 24 hours and fed one group a single meal that was <1 ppm Zn and 20% protein. Sixteen hours later the plasma Zn concentration of this group of rats was significantly less than a group rats fasted and fed a < 1ppm Zn and 5% protein meal. Bettger (1985) stated that higher dietary protein content was the factor that led to the rapid fall in plasma Zn concentrations. Wallwork et al. (1983) observed an inverse relationship between plasma Zn concentration and dietary protein level in rats fed a 6 ppm Zn diet for 30 days. As the protein content of the diet was increased from 6% to 11.3% and 15%, the plasma Zn concentration decreased. The studies by Bettger (1985) and Wallwork et al. (1983) and the present study indicate that the ratio of dietary protein to dietary Zn was a determinate of serum Zn concentration.

The E group had a serum Zn concentration that was significantly greater than the C group (Figure 21). Heard et al. (1977) reported that rats with restricted feed intakes had higher

hemoglobin and plasma concentrations than ad libitum controls. Although water was readily available, the authors suggested that feed restricted rats suffered from some degree of dehydration. In a study by Schneeman et al. (1986) a group of young rats (65-85 g) were pair fed to a Zn deficient group. All groups were fasted overnight before termination as part of the experimental protocol. The pair fed group had a serum Zn concentration that was 1.1 times greater that the Zn adequate control group. In the present study the E group also had an elevated serum albumin concentration (1.1 times greater than control). This raises the question of hydration status of the E group. Future pair feeding studies should include an assessment of hydration status to determine if the elevated serum Zn and albumin concentrations observed in the present study were due to the experimental diet or due to dehydration.

Protein Status

The protein status of the ZP and P groups was confirmed through the assessment of liver lipid and serum albumin concentrations, and inguinal fat pad weight. At the end of the deficiency phase, only the ZP and P groups had significantly depressed serum albumin and elevated liver lipid concentrations compared to the C group (Figures 19 & 20). A comprehensive study by Lunn and Austin (1983) examining the affects of dietary protein and energy on rat plasma albumin concentrations reported that hypoalbuminemia occurred when 0.5% to 6% protein diets were fed ad libitum. The ZP and P rats in this study also exhibited a significant decrease in serum albumin with ad libitum feeding of a 2% protein diet. The protein deficient rats in this study also had significantly elevated liver lipid concentrations (hepatic steatosis). Jacobs et al. (1984) fed 227 g rats a 0.03% protein diet for 37 days and reported hepatic steatosis secondary to dietary protein deficiency. It is also important to note that the ZP and P were able to maintain their inguinal fat pad mass during the deficiency phase (Figure 18). A less severe wasting or maintenance of subcutaneous adipose tissue is a feature of protein malnutrition (McLaren, 1981; Latham, 1990). These changes in liver lipid and serum albumin concentrations and maintenance of fat mass demonstrate that the ZP and P were protein deficient at the end of deficiency phase. By the end of the recovery phase, the serum albumin and liver lipid concentrations of the ZP and P groups were similar to the C group. The inguinal fat pads of the P group fully recovered, but those of the

ZP group were still significantly less than control. The reason for the different degrees of adipose recovery is not clear. In light of the incomplete recovery of inguinal fat pad mass in the Z group, one might suspect that the incomplete recovery of in the ZP group was due to the superimposition of Zn deficiency on protein malnutrition. An assessment of whole body composition via carcass analysis or magnetic image resonance would be helpful in assessing any potential effects of Zn deficiency on whole body adipose stores.

Energy Status

Both the Z and E groups exhibited characteristics of energy malnutrition at the end of the deficiency phase. The same parameters used to identify protein malnutrition were used to define energy deficiency. One might have expected the Z and E groups to exhibit signs of protein deficiency since both groups had low feed intakes (thereby low protein intakes) during the deficiency phase. However, the serum albumin and liver lipid concentrations of the Z and E groups were similar to that of the C group at the end of the deficiency phase (Figures 19 and 20). The E group had a slightly elevated serum albumin concentration, which has been associated with energy restriction (Lunn and Austin, 1983). The most striking sign of energy malnutrition at the end of the deficiency was the changes in inguinal fat pad mass (Figure 18). The inguinal fat mass of the Z group was slightly (though not significantly) lighter than those of the W group and no inguinal fat pads could be excised from the E group. The Lunn and Austin (1983) study found that energy restriction was associated with decreased fat mass as assessed by total body water measurements. Wasting of fat tissue is a primary characteristic of marasmus, a severe form of energy malnutrition (McLaren, 1981 and Latham, 1990). After the recovery phase, the inguinal fat mass of the E group fully recovered, but the Z group inguinal fat mass was significantly less than control. As mentioned before, the mechanism behind the incomplete recovery of the Zn deficient groups' body composition is unclear.

Body Composition

The literature review highlighted several studies that documented the changes in body composition during PEM and the incomplete recovery of body composition in 'rehabilitated' children and rats. For this study, the gastrocnemius muscle and inguinal fat pad weights were

utilized to represent body composition. At the end of the deficiency phase, the deficient rats had gastrocnemius muscle and inguinal fat pad masses that were significantly less than the C group (Figures 18 and 26). The protein deficient groups had the lowest muscle weights and were able to maintain their inguinal fat pad mass. The preferential wasting of the gastrocnemius muscle reflected the cannibalization of skeletal muscle mass to meet essential protein requirements during protein deficiency. As mentioned before, the wasting of skeletal muscle mass along with the maintenance of adipose stores are clinical features of kwashiorkor, a severe form of protein deficiency (McLaren, 1981). Since the protein deficient groups did not exhibit signs of frank energy deficiency, one could assume that the growth of the ZP and P was limited primarily by the lack of dietary protein. The gastrocnemius muscles of the Z and E groups were significantly less than the C group, but were not as severely wasted as the protein deficient groups. However, the inguinal fat pads of the Z and E groups more were severely catabolized to meet the energy needs of the energy deficient rats. The Z and E rats mobilized their fat stores in order to fuel a level of growth that was greater than protein deficient rats.

By the end of the recovery phase, none of the deficient groups had gastrocnemius muscle masses similar to the C group (Figure 26). The ZP and P groups still had the lowest gastrocnemius muscle mass. The Z group had a gastrocnemius muscle mass that was significantly greater than the E group. After the recovery phase, the P and E groups had experienced full recovery of their inguinal fat mass along with an incomplete recovery of their gastrocnemius muscle mass. Although the P and E rats did not have the excessive fat mass accretion documented in recovered PEM children (Graham et al., 1969; MacLean and Graham, 1980), they still had compensatory growth that favored the synthesis adipose tissue over skeletal muscle. The incomplete recovery of skeletal muscle mass of the P and E groups also extended to the skeletal muscle histology. The ZP and Z groups experienced a full recovery of muscle histology, but had incomplete recovery of inguinal fat pad and gastrocnemius muscle mass. Previous Zn status appeared to have a unique influence on gastrocnemius mass and histology recovery.

In summary, the body composition of the rats reflected the type of malnutrition expected from the feeding of the experimental diets. The macronutrient status of the rats after the deficiency phase, was a more influential factor on the mass of the gastrocnemius and inguinal fat weights than the micronutrient status. Zinc status did not appear to have a significant independent effect of the body composition of the 6-week-old deficient rats. After the recovery phase, only the P and E groups were able to completely recovery their inguinal fat mass and none of the deficient groups were able to completely recovery of their gastrocnemius muscle mass. Previous Zn status appeared to have a unique effect on the recovery of gastrocnemius muscle histology. Gastrocnemius muscle mass and histology will be discussed further in a later section.

Femur Calcium and Phosphate Concentration

In light of the effects of Zn deficient diets on femur Zn concentration (Lepage, 1997; Bossuyt, 1998), it was interesting to examine the effects of the experimental diets on the two major components of skeletal bone Ca and P (Figures 23 & 24). Zn status alone did not appear to be a significant factor influencing the Ca and P concentrations in the femur. As with the linear growth results (Figures 10 & 11), the femur Ca and P concentrations were more adversely effected by the protein deficiency than by energy deficiency. The femur Ca and P results add to the biochemical profile of Zn, protein and energy deficiencies.

Since protein status appeared to be a primary factor of the femur Ca and P concentrations, it is prudent to note that collagen and noncollagenous proteins make up 30% of bone mass (Green, 1994). According to Green (1994), it is the organizational interrelationship between the bone protein matrix and the inorganic minerals (Ca and P) that determine the mechanical integrity of skeletal bone. It would be interesting in future studies to examine if the types of deficiency produced in this study would alter the organizational structure and structural integrity of rat femurs. It would also be interesting to determine femur IGF-1 concentration, as IGF-1 is an important factor in bone development (Spencer et al., 1991).

Insulin-like Growth Factor-1 Status

This study was conducted with the hypothesis that the poor recovery of muscle mass and histology observed in PEM mammals was related to low IGF-1 concentrations secondary to sub-optimal Zn status. IGF-1 status was monitored via serum IGF-1 concentrations. At the end of the deficiency phase, the deficient rats had serum IGF-1 concentrations that were significantly less than the C group (Figure 25). The serum IGF-1 concentrations of the Z and E groups dropped significantly from baseline. The ZP group was able to maintain serum IGF-1 concentrations at baseline. Meanwhile, the serum IGF-1 concentration of the P group increased significantly from baseline. Since the Z and E groups exhibited similar signs of energy malnutrition it would appear that energy deficiency had the most detrimental affect on circulating IGF-1 concentrations. The serum IGF-1 concentrations of the ZP and P groups did not respond in the same dramatic and negative fashion as in the energy deficient groups. Thus, protein deficiency appeared to have a less potent affect on serum IGF-1 concentrations. Prewitt et al. (1982) also found that dietary energy and protein influenced serum IGF-1 concentrations of 6 to 12-week old male rats. The authors stated that dietary protein appeared to be the more influential factor. In the present study, energy deficiency and not protein deficiency appeared to be the more influential variable on serum IGF-1 concentrations.

The different effects of macronutrient deficiency on serum IGF-1 concentrations could be due to the different effects of protein and energy deficiency on the transcription and translation of the IGF-1 gene. As mentioned in the literature review, there are different lengths or species of IGF-1 mRNA. Straus (1994) briefly highlighted how energy and protein deficiency altered the expression of liver IGF-1 mRNA. Straus and Taskemoto (1991) reported that 4 week old rats fed a 20% protein diet at 50% of ad libitum control group intakes for 10 days had a total liver IGF-1 mRNA expression level that was 20% of the control value. The three species of liver IGF-1 mRNA (8.0-kb, 1.0-kb and 1.8-kb) were decreased to a similar degree (19% to 25% of the control group value). Thissen et al. (1991b) reported that 4 week old rats fed a 5% protein diet for 7 days had a liver IGF-1 mRNA expression level that was 47% of the control group fed a 20% protein diet. The expression of the 8.0-kb IGF-1 mRNA was 45% of the control value while that of the 1.0-kb and

1.8-kb species was 63% of the control value. Utilizing a similar study protocol as Thissen et al. (1991b), Thissen and Underwood (1991) examined the translational status of liver IGF-1 mRNA in the protein deficient rat. The authors reported that there was a reduction in the number of mRNAs associated with ribosomes and that this could decrease translation efficiency. The above mentioned studies only describe changes in initial steps of IGF-1 peptide production. How different types of malnutrition can influence IGF-1 production has not been fully elucidated. Since protein and energy deficiency appear to have different effects on the early stages of IGF-1 production in the liver, one could speculate that the protein and energy deficiency could have different effects on the latter steps of IGF-1 production resulting in different serum IGF-1 concentrations. Future studies on how IGF-1 gene expression can be altered during different types of malnutrition would help to elucidate the mechanisms of dietary deficiency induced growth retardation.

As mentioned in the literature review, the majority of IGF-1 in serum and tissues is bound to its binding proteins and these binding proteins can influence the bioavailability of IGF-1 (Elgin et al., 1987, Pimental, 1994). Six different binding proteins have be identified (Straus, 1994; Thissen et al., 1994). Therefore, the detected quantity of IGF-1 does not fully reflect IGF-1 status and anabolic potential. The concentrations of the binding proteins, like IGF-1, are regulated by nutrition (Clemmons and Underwood, 1991; Straus, 1994). For example, IGF binding protein 3 has been found to prolong the half-life and the actions of IGF-1 in circulation (Clemmons and Underwood, 1991). Prolonged protein deficiency has been associated with decreased IGF binding protein 3 concentrations and increased IGF-1 clearance from circulation to the tissues (Thissen et al., 1994). IGF binding proteins 1 and 2 are though to assist in IGF-1 transportation to tissues and are up-regulated during chronic nutritional deficiency (Thissen et al., 1994). The nutritional regulation and function of the four other binding proteins have not been fully elucidated. IGF-1 binding proteins 4 through 6 could potentially inhibit IGF-1 availability (Pimental, 1944; Straus, 1994; Thissen et al., 1994). In short, IGF-1 status is a complex pharmacokinetic interaction between the IGF-1 peptide and its six different binding proteins. Since the function and regulation of the IGF binding proteins has not been fully clarified, the actual peptide concentration

is one of the more readily detectable and understood facets of IGF-1 status. It would be very interesting in future studies to examine how Zn deficiency might influence IGF-1 binding protein production and metabolism.

This study also sought to examine the affects of Zn deficiency on circulating IGF-1 concentration. The superimposition of Zn deficiency on protein deficiency held serum IGF-1 at baseline concentrations. In protein deficiency alone, the serum IGF-1 concentration increased from baseline and was significantly greater than the of ZP group at the end of the deficiency phase. Zinc deficiency alone was almost an independent factor of serum IGF-1 concentration. The least squares means comparison between the Z and E groups had P value of 0.08. However, the serum concentration of IGF-1 appeared to be more strongly influenced by the macronutrient status of the rats than the micronutrient status.

As explained in the literature review, pair feeding in Zn deficiency studies is done in order to account for the marked reduction in feed intake in Zn deficient animals. Dorup et al. (1991) argued that pair feeding results in the semi-starvation of the pair-fed group and disproportionate caloric intake in relation to body mass. Roth and Kirchgeβner (1994) were able to examine the effects of Zn deficiency on serum IGF-1 concentrations without the confounding effects of disproportionate caloric intake by force feeding the Zn deficient and control groups. The 2 groups of young rats (108 g) received the same quantity of feed (11.6 g DM/rat/day) and were fed four times per day for 12 days. The Zn deficient rats received a 1.3 ppm Zn diet and the control group was fed a 25 ppm Zn diet. The Zn deficient rats had significantly lower serum IGF-1 and serum and femur Zn concentrations. The serum IGF-1 concentration of the Zn deficient group was 72% of the control group value. The Roth and Kirchgeβner (1994) study demonstrated that Zn deficiency alone was able to significantly reduce serum IGF-1 concentration. Interestingly, the reduction in serum IGF-1 concentration observed in the Roth and Kirchgeβner (1994) study was not as great as that seen in the present study. The Z group had a serum IGF-1 concentration that was 25% of the C group. This difference maybe due to the secondary energy deficiency induced by the Zn deficient diet. Energy deficiency alone (the E group) resulted in a serum IGF-1 concentration that was 51% of the C group. If the effect of energy deficiency were mathematically

removed from the serum IGF-1 concentration of the Z group, Zn deficiency alone would have resulted in a serum IGF-1 concentration of 76% of the C group. This calculated value is similar to the results reported in Roth and Kirchgeβner (1994). In the present study primary Zn deficiency was accompanied by secondary energy deficiency. In light of the Roth and Kirchgeβner (1994) study, it would appear that the combined effects of Zn and energy deficiency produced the marked reduction in serum IGF-1 concentration observed in the Z group.

By the end of the recovery phase, the serum IGF-1 concentrations of all the deficient rats recovered to values similar to that of the C group. However, the full recovery of serum IGF-1 concentrations was not accompanied by full recovery of gastrocnemius mass and histology.

Gastrocnemius Muscle Mass and Histology

The gastrocnemius muscle biopsy allowed for the assessment of effects of the experimental diets on gastrocnemius mass and histology. However, the study did not include an assessment of hydration status or a regular exercise program. Both hydration and exercise can influence the size of muscle cells (Keeton and Gould, 1986; Oldfors and Sourander, 1985) and in turn influence skeletal muscle mass and fiber diameter. In general, all rats drank readily from the water bottles. Only two rats (one from the E group and another from the C group) had problems in the first few days of the study adjusting to the sipper-tube arrangement. The E group was more active during the deficiency phase compared to the other groups as they habitually stood up on their hid legs to rattle the cage.

Deficiency phase

The Zn, protein, energy and combined Zn and protein deficiencies had significantly different effects on the weight and histology of the gastrocnemius muscle in young growing rats. At the end of the deficiency phase, the gastrocnemius muscle mass appeared to be more adversely affected by protein deficiency than by energy deficiency. Zinc status did not appear to have significant independent effect on gastrocnemius mass. As with the serum IGF-1 concentrations, macronutrient status produced significant differences in gastrocnemius mass compared to micronutrient status.

The nutritional status of the deficient rats influenced the muscle histology. The T1-MFD in the ZP, P and Z groups failed to increase beyond baseline measurements by the end of the deficiency phase. The E group had a T1-MFD that was significantly greater than that observed in the Z group. Although the E group was more active (cage-rattling) group, the larger T1-MFD is unlikely to be due to exercise induced hypertrophy as it is the T2-MFD that readily reflects changes in physical activity (Samat, 1983). This difference between the Z and E groups could represent a unique affect of Zn deficiency on muscle histology. But this difference can not be explained by the serum IGF-1 concentrations. Both the E and Z groups exhibited signs of energy malnutrition and had similar gastrocnemius weights. The only significant difference between the two groups was their Zn status. The Z group was Zn deficient as assessed by serum and femur Zn concentrations while the E group was not. However, this difference between the Z and E groups does not appear to be explained by serum IGF-1 concentration. As mentioned earlier, the serum IGF-1 concentration was almost a significantly different factor between the Z and E groups ($P=0.08$). McNall et al. (1995) reported that rats fed a Zn deficient diet (0.5 mg Zn/kg diet) for 14 days had a serum IGF-1 concentration that was significantly less than the pair-fed group ($P<0.05$). If significant differences between the Z and E serum IGF-1 concentrations were seen in this study as in the McNall et al. (1995) study, then one could associate low serum IGF-1 concentrations and Zn deficiency with poor T1-MFD development.

In the present study, there were significant differences in serum IGF-1 concentration between the ZP and P group however T1-MFD was similar for these two groups. This could be due to the dominant affect of protein deficiency on muscle physiology as seen in the gastrocnemius muscle weight data. The arrested T1-MFD development of the ZP and P groups can be explained by their protein deficient nutritional status. Olfors et al. (1983) also found that the type 1 muscle fibers of the EDL muscle of 6-week-old rats fed a 1.5 % protein diet for 19 weeks failed to grow. The EDL muscle like the gastrocnemius muscle is predominately made up of type 2 muscle fibers (Glore and Layman, 1983).

The type 2 muscle fibers of the protein deficient groups also failed to grow during the deficiency phase. Type 2 muscle fibers reflect changes in nutrition and physical activity more

readily than type 1 muscle fibers (Samat, 1983). The E group T2-MFD increased significantly from baseline, but was still significantly smaller than the C group measurements. Zinc deficiency alone had an intermediate effect on T2-MFD. The Z group T2-MFD was similar to that of the E and protein deficient groups. As with the gastrocnemius muscle mass data, protein status appeared to have a more detrimental affect on T2-MFD than energy or Zn deficiency alone. As with the T1-MFD data, the serum IGF-1 concentration data did not parallel the T2-MFD data.

As mentioned in the literature review, serum IGF-1 concentrations do not always reflect tissue IGF-1 status due to the localized production and actions of IGF-1 (D'Ecrole et al. 1984; Orlowski and Chernausek, 1988; Daughaday and Rotwein, 1989). The determination of gastrocnemius IGF-1 concentrations would have allowed for better analysis of muscle physiology and histology in relation to nutritional and IGF-1 status. Unfortunately, attempts to determine gastrocnemius IGF-1 concentrations using the serum IGF-1 RIA kit were not successful. Currently, there are no commercially available rat tissue IGF-1 RIA kits. Various tissue preparation and extraction procedures including pulverization in liquid nitrogen and altering the extraction and centrifugation times were tried, but none were successful.

Recovery phase

The 3-week recovery period resulted in significant increases in the serum IGF-1 concentrations, gastrocnemius muscle weights and muscle fiber diameters. The serum IGF-1 concentrations of the deficient rats fully recovered, but there was variable recovery of gastrocnemius muscle fiber diameter and incomplete recovery of gastrocnemius mass. During the recovery phase all rats had adjusted to the sipper-tube arrangement and the cage-rattling of the E group stopped.

The T1-MFD and T2-MFD of the ZP and Z groups completely recovered, but the MFD of the P and E groups did not. The P group T1 and T2-MFD were still significantly less than the C group after the recovery phase. The T1-MFD of the E group was also significantly less than that of the C group after the recovery phase. Muscle fiber recovery from Zn deficiency appeared to be more thorough even when it was combined with protein deficiency. The serial serum samples in the Dorup et al. (1991) study revealed that serum Zn and IGF-1 concentration peaked and

troughed in parallel throughout rehabilitation from Zn deficiency in young rats. Serial measurements of serum Zn and IGF-1 concentrations throughout the recovery phase of this experiment might have shed some light on the divergent recovery patterns of the Zn, protein and energy deficient rats.

As mentioned in the literature review, Montgomery (1962) and Hansen-Smith et al. (1979) both reported that children who had achieved their expected weight for height or age following nutritional rehabilitation from PEM still had significant deficits in muscle fiber cross-sectional area. Glore and Layman (1987) created a model of prolonged malnutrition by limiting the feed intake of nursing dams to 50% of the control dam intakes and by limiting the feed intakes of the weaned pups to 40% of control pup intakes until 120 days of age. At 120 days of age the feed restricted rats had significantly a smaller soleus muscle fiber diameter (81% of the control group value) compared to the well-fed control group. The feed restricted rats were recovered on a 20% protein and 62% carbohydrate nutritional complete diet for 196 days. At 316 days of age, the soleus muscle fiber of the feed restricted rats remained significantly less than the control group (92% of the control group value, $P<0.05$). As mentioned previously, the type of malnutrition the feed restricted rats suffered from in the Glore and Layman (1987) study was not confirmed. If one assumes that the rats were suffering from PEM, the results of the present study support the results of the Glore and Layman (1987) study as the muscle histology of the P and E groups did not fully recover following the recovery period.

In the recovery phase, the deficient rats had unfettered access to a nutritionally complete diet, but none of the deficient rats could make up the muscle tissue deficit accrued during the deficiency phase. The deficient groups still had the significantly smaller gastrocnemius muscle mass after the recovery phase. It is not clear if this deficit was due to the lingering detrimental affects of protein and energy deficiency or due to the significantly lower feed intakes of the deficient groups during the recovery phase. It is possible that the deficient rats were unable to consume enough feed to achieve muscle weights similar to that of the C group. Increasing the length of the recovery period might have resulted in improved recovery of gastrocnemius muscle weight, but increasing length of nutritional rehabilitation in the research, clinical or field setting is

not always feasible or practical. Perhaps the 30 ppm Zn control diet (designed for normal growing rats) was unable to meet the nutritional requirements for full gastrocnemius recovery during compensatory growth. Studies by Simmer et al. (1987) and Morgan et al. (1988) reported that Zn supplementation of 10 mg Zn/ kg body weight for children and a 40 ppm Zn diet for mice during recovery, respectively, resulted in improved rates of weight gain and recovery of lean tissue mass. In future studies, it would be interesting to examine if increasing the Zn content of the recovery diet alone would result in improved muscle mass recovery in the present models of nutritional deficiency and rehabilitation.

Overall Recovery

At first glance the Zn deficient groups would appear to have the poorest degree of overall recovery compared to the P and E groups at the end of the recovery phase. If the recovery of the deficient rats is looked at in terms of cellular levels of organization, the recovery of the ZP and Z can be seen as more organized while that of the P and E groups as disorganized. An individual organism can be broken-down into interrelated groups of specialized systems, organs, tissues, cells and biochemical reactions (Keeton and Gould, 1986; Gylys and Wedding, 1995). The P and E groups recovered biochemically from protein and energy deficiency, but did not complete the histological recovery of the gastrocnemius muscle and had an unbalanced recovery of inguinal fat mass in relation to gastrocnemius muscle mass. The ZP and Z group had full biochemical and muscle histology recovery as well as balanced incomplete recovery of inguinal fat and gastrocnemius muscle tissues. The Zn deficient rats were recovering in an organized and balanced fashion from the biochemical to the cellular (muscle histology) and tissue (muscle and fat mass) levels. The P and E groups had a more disorganized and unbalanced recovery as the inguinal fat tissue recovered while the cellular and tissue recovery of the gastrocnemius muscle was incomplete. None of the deficient rats were able to achieve body weights similar to the C group. Other than achieving expected weight for height, the PEM children studied by Hansen-Smith (1979), MacLean and Graham (1980) and Golden and Golden (1981a) experienced the same incomplete and disorganized recovery of adipose and muscle tissue and muscle histology seen in the P and E rats. Montgomery (1962) and Hansen-Smith (1979) reported that the muscle

fiber cross-sectional area of apparently recovered PEM children (>50 days of nutritional rehabilitation) were still significantly smaller than age and sex matched controls. Brooke and Wheeler (1976) and MacLean and Graham (1980) stated that much of the weight gained by PEM children during compensatory growth was fat. The studies by Golden and Golden (1981b & 1992) found that additional Zn in the recovery diet (5-10 mg Zn/ kg diet) decreased the energy cost of growth, favored lean body mass accretion and improved nitrogen retention. Morgan et al. (1988) reported that malnourished mouse pups rehabilitated on a diet that contained 40 ug Zn/g of diet resulted in body weights and compositions that were similar to well-fed control mice. The present study did not include Zn supplementation of the recovery diet. It would be interesting to investigate if Zn supplementation of the recovery diet would favor a more organized recovery in protein and energy deficient rats.

VI. Conclusions

Growth during the deficiency and recovery phases

- The deficiency phase of the study demonstrated that Zn, energy and protein deficiencies result in retarded growth and development on the biochemical, cellular, tissue and whole body levels.
- The 30 ppm Zn control diet produced compensatory growth in the deficient rats, but full recovery did not occur equally on all levels of cellular organization.

Type of malnutrition and Zn status

- The 2% protein diet produced hepatic steatosis and hypoalbuminemia in the ZP and P groups. These are characteristics of protein malnutrition.
- The <1 ppm Zn diets produced hypozincemia and reduced femur Zn concentration in the ZP and Z groups. These are characteristics of Zn deficiency.
- The P group also had decreased serum and femur Zn concentrations at the end of the deficiency phase. Primary protein deficiency was associated with the characteristics of Zn deficiency.
- The Z and E groups had similar liver lipid and serum albumin concentrations and inguinal fat weights. The Z group exhibited characteristics of energy malnutrition.

Body Composition

- The 2% protein diets (ZP and P) impeded gastrocnemius tissue accretion more severely than energy deficiency.
- Energy deficiency in the Z and E groups resulted in a marked wasting of inguinal fat pad mass.
- Recovery from protein and energy deficiencies was associated with full recovery of adipose tissue and incomplete recovery of muscle tissue.
- Recovery from Zn deficiency and combined Zn +protein deficiency was accompanied by incomplete recovery of adipose and muscle tissue.

IGF-1 Status

- Serum IGF-1 concentration was most adversely effected by energy deficiency as seen in the Z and E groups.
- No significant independent effects of Zn status on serum IGF-1 concentrations were identified.

Muscle Mass and Histology

- This study demonstrated that Zn, energy and protein deficiencies have divergent negative effects on muscle mass, muscle fiber diameter and serum IGF-1 concentration.
- Zn, protein and combined Zn + protein deficiency arrested T1 & T2-MFD growth at baseline measurements.
- The ZP and Z groups experienced full recovery of T1 & T2- MFD while the P and E groups did not.
- The recovery data indicated that previous nutritional status and not serum IGF-1 status was the primary factor determining the degree of muscle mass and muscle fiber recovery.

Overall Conclusion - Hypothesis

- The hypothesis of the study stated that the full recovery of muscle fiber diameter would be dependent on the full recovery of serum IGF-1 concentration and Zn status. The results of the study showed that the complete recovery of serum IGF-1 concentration and Zn status was associated with full recovery of muscle fiber diameter in the Z and ZP groups, but not in the P and E groups. Previous nutritional status was the primary factor determining the degree of muscle fiber diameter recovery.

VII. Implications

Several suggestions for future research were mentioned throughout the discussion. The most important implication for future research that this study presents is the need to examine the effects of dietary manipulation (deficiency or supplementation) on all levels of cellular organization. If the examination of the effects of Zn, protein, energy and combined Zn + protein deficiency had stopped at the tissue level, the influence of previous Zn status on the recovery of muscle MFD would have remained unknown.

This study reiterates the need to develop better nutritional rehabilitation protocols that will ensure a full recovery from PEM on all levels of cellular organization. Childhood PEM is a preventable disease. Until the political and social will of the world successfully eradicates this disease, researchers must continue to find ways of maximizing the recovery from PEM. Dietary Zn and Zn status appear to be important factors in the recovery of muscle mass and histology after PEM. The mechanism of this improved recovery is unknown. This mechanism must be identified in order to ensure that every child suffering from PEM is able to fully recover and live full lives unhampered by the effects of childhood PEM.

References

Adams, G.R. & Haddad, F. (1996) The relationships among IGF-1, DNA content and protein accumulation during skeletal muscle hypertrophy. J. Appl. Physiol. 81:2509-2516.

Alnaqeeb, M.A. & Goldspink, G. (1986) Changes in fiber type, number and diameter in developing and aging skeletal muscle. J. Anat. 153:31-45.

Ashworth, A. (1969) Growth rates in children recovering form protein-calorie malnutrition. Br. J.Nutr. 23:835-845.

Baker, J., Lui, J.P., Robertson, E.J. & Efstratiadis, A. (1993) Role of insulin-like growth factors in embryonic and postnatal growth. Cell 75:73-82.

Bates, P.C., Loughna, P{.T., Pell, J.M., Schulster, D. & Millward, D.J. (1993) Interactions between growth hormone and nutrition in hypophysectomized rats: body composition and production of insulin-like growth factor-1. J. Endocrionl. 139:117-126.

Bettger, W.J. (1985) Effect of dietary protein or amino acids on the rapid change in plasma zinc concentration in rats fed zinc deficient diets. Nutr. Res. 5:1153-1159.

Bossuyt, P.J. (1998) Dietary zinc deficiency and PEM decreases in vitro murine T-lymphocyte cell cycle progression. MSc. Thesis, University of Manitoba.

Brooke, O.G. & Wheeler, E.F. (1976) High energy feeding in protein-energy malnutrition. Arch. Dis. Child. 51:968-971.

Castillo-Duran, C., Heresi, G., Fisberg, M. & Uauy, R. (1987) Controlled trail of zinc supplementation during recovery from malnutrition: effects on growth and immune function. Am. J. Clin. Nutr. 45:602-608.

Cheek, D.B., Hill, D.E., Cordano, A. & Graham, G.G. (1970) Malnutrition in infancy: Changes in muscle and adipose tissue before and after rehabilitation. Pediat. Res. 4:135-144.

Chesters, J.K. & Quarterman, J. (1970) Effects of zinc deficiency on food intake and feeding patterns of rats. Br. J. Nutr. 24:1061-1069.

Clegg, M.S., Kenn, C.L., Lonnerdal, B. & Hurley, L.S. (1981) Influences of ashing techniques on the analysis of trace elements in animal tissues. Biol. Trace Elem. Res. 3:107-115.

Clemmons, D.R. & Underwood, L.E. (1991) Nutritional regulation of IGF-1 and IGF binding proteins. Annu. Rev.Nutr. 11:393-412.

Cossack, Z.T. (1988) Effect of zinc level in the refeeding diet in previously starved rats on plasma somatomedin-C levels. J. Pedia Gastro& Nutr. 7:441-445.

Cossack, Z.T. (1986) Somatomedin-C and zinc status in rats as affected by zinc, protein and food intake. Br. J. Nutr. 56:163-169.

Cousins, R.J.& Hempe, J.M. (1990) Zinc. In: Present Knowledge in Nutrition (Brown, M.L. ed.), p.251. Nutrition Foundation, Washington, D.C..

D'Ercole, A.J., Stiles, A.D. & Underwood, L.E. (1984) Tissue concentrations of somatomedin C: Further evidence for multiple sites of synthesis and paracrine or autocrine mechanisms of action. Proc. Natl. Acad. Sci. USA 81:935-939.

Dastur, D., Manghani, D.K., Osuntokun, B.O., Sourander, P. & Kondo, K. (1982) Neuromuscular and related changes in malnutrition: A review. J. Neurol. Sci. 55:207-230.

Daughaday, W.H., & Rotwein, P. (1989) Insulin-like growth factors 1 and 2. Peptide, messenger ribonucleic acid and gene structures, serum and tissue concentrations. Endocr. Rev. 10:68-91.

Daughaday, W.H., Hall, K., Salmon, W.D., Van den Brande, J.L. & Van Wyk, J.J (1987) On the nomenclature of the somatomedins and insulin-like growth factors. Endocrinology 121:1911-1912.

Deeming, S.B. & Weber, C.W. (1977) Evaluation of hair analysis for determination of zinc status using rats. Am. J. Clin. Nutr. 30:2047-2052.

Dorup, I., & Clausen, T. (1991) Effects of magnesium and zinc deficiencies on growth and protein synthesis in skeletal muscle and the heart. Br. J. Nutr. 66:493-504.

Dorup, I., Fylvbjerg, A., Everts, M.E. & Clausen, T. (1991) Role of insulin-like growth factor-1 and growth hormone in growth inhibition induced by magnesium and zinc deficiencies. Br. J. Nutr. 66:505-521.

Doumas, B., Watson, W. & Biggs, H. (1971) Albumin standards and the measurement of serum albumin with bromcresol green. Clin. Chem. Acta. 31:87-96.

Dubowitz, V. & Brooke, M.H. (1973) Muscle Biopsy: A modern approach. W.B. Saunders Company Ltd., Toronto, ON.

Edwall, D., Schalling, M., Jennische, E. & Norstedt, G. (1989) Induction of insulin-like growth factor-1 messenger ribonucleic acid during regeneration of rat skeletal muscle. Endocrinology 124:820-825.

Edwards, R (1985) Basic concepts in immunoassay & Radioimmunoassay. In: Immunoassay-an introduction, pp.12-39. William Heinemann Medical Books, London, England.

Eigenmann, J.E., Patterson, D.F. &Froesch, E.R. (1984) Body size parallels insulin-like growth factor levels but not growth hormone secretory capacity. Acta. Endocrinol. (Copenh) 106:448-453.

Elgin, R.G., Busby,W.H. & Clemmons, D.R. (1987) An insulin-like growth factor (IGF) binding protein enhances the biological response to IGF-1. Proc. Natl. Acad. Sci. USA. 84:3254-3258.

Estes, M.L. & Goss, G.R. (1996) Muscle biopsy: The role of the technologist in optimum freezing, enzyme histochemistry and diagnosis. Presentation at the Canadian Society of Laboratory Technologists Cogress 1996.

Filteau, S.M. & Woodward, B. (1982) The effect of severe protein deficiency on serum zinc concentration of mice fed a requirement level or a very high level of dietary zinc. J. Nutr. 112:1974-1977.

Flanagan, P.R. (1984) A model to produce pure zinc deficiency in rats and its use to demonstrate that dietary phytate increases the excretion of endogenous zinc. J. Nutr. 114:493-502.

Forbes, G.B. (1987) Human body composition: Growth, aging, nutrition and activity, pp.125-168. Springer-Verlag, New York, NY.

Froesch, E.R., Schmid, C., Schwander, J. & Zapf, J. (1985) Action of insulin-like growth factors. Ann. Rev. Physiol. 47:443-467.

Furlanetto, R.W. & Cara, J.F. (1986) Somatomedin-C/insulin-like growth factor-1 as a modulator of growth during childhood and adolescence. Horm. Res. 24:177-184.

Gammeltoft, S. (1989) Insulin-like growth factors and insulin: gene expression, receptors and biological actions. In: Peptide hormones as pro-hormones (ed. Martinez, J.). Halsted Press, New York, NY.

Geen, J. (1994) The physiochemical structure of bone: Cellular and noncellular elements. Miner. Electrolyte Metab. 20:7-15.

Ghavami-Maibodi, S.Z., Collipp, P.J., Castro-Magana, M.& Chen, S.Y. (1982) Effect of oral zinc supplements on growth, hormonal levels and zinc in healthy short children. Ann. Nutr. Metab. 27:214-219.

Giugliano, R. & Millward, D.J. (1987) The effects of severe zinc deficiency on protein turnover in muscle and thymus. Br. J. Nutr. 57:139-155.

Giugliano, R. & Millward, D.J. (1984) Growth and zinc homeostasis in the severely Zinc-deficient rat. Br. J. Nutr. 52:545-560.

Glore, S.R. & Layman, D.K. (1983) Cellular development of skeletal muscle during early periods of nutritional restriction and subsequent rehabilitation. Pediatr. Res. 17:602-605.

Glore, S.R. & Layman, D.K. (1987) Cellular development of skeletal muscle of rats during recovery from prolonged undernutrition. J. Nutr. 117:1767-1774.

Glore, S.R., Orth, V.L., Knehans, A.W. & Erdman (jr.), J.W. (1993) Efficacy of dietary zinc supplementation on catch-up growth after protein malnutrition. J. Nutr. Biochem. 4:281-285.

Golden, B.E. (1989) Zinc in cell division and tissue growth: Physiological aspects. In: Zinc in Human Biology (Mills, C.F. ed.), p119. Springer Verlag, New York, NY.

Golden, B.E. & Golden, M.H.N. (1992) Effect of zinc on lean tissue synthesis during recovery form malnutrition. Eur. J. Clin. Nutr. 46:697-706.

Golden, B.E. & Golden, M.H.N. (1981a) Plasma zinc, rate of weight gain, and the energy cost of tissue deposition in children recovering form sever malnutrition on cow's milk or soy protein based diet. Am. J. Clin. Nutr. 34:892-899.

Golden, M.H.N. & Golden, B.E. (1981b) Effect of zinc supplementation on the dietary intake, rate of weight gain, and energy cost of tissue deposition in children recovering from severe malnutrition. Am. J.Clin. Nutr. 34:900-908.

Graham, G.G., Cordano, A., Blizzard, R.M. & Cheek, D.B. (1969) Infantile malnutrition: Changes in body composition during rehabilitation. Pediat. Res. 3:579-589.

Gylys, B.A. & Wedding, M.E. (1995) Medical terminology: A systems approach, 3 ed., pp.43-45. F.A. Davis Company, Philadelphia, PA.

Haltia, M, Berlin, O, Schucht, H. & Sourander, P. (1978) Postnatal differentiation and growth of skeletal muscle fibers in normal and undernourished rats. J. Neurol. Sci. 36:25-39.

Hansen-Smith, F.M., Picou, D. & Golden, M.H. (1979) Growth of muscle fibers during recovery form severe malnutrition in Jamaican infants. Br. J. Nutr. 41:275-281.

Heard, C.R.C., Frangi, S.M. & Wright, P.M. (1977) Biochemical characteristics of different forms of protein-energy malnutrition: an experimental model using young rats. Br. J. Nutr. 37:1-21.

Hintz, R.L., Suskind, R., Amatayakul, K., Thanangkul, O. & Olson, R. (1978) Plasma somatomedin and growth hormone values in children with protein-calorie malnutrition. J. Pediatr. 92:153-156.

Hizuka, N., Takano, K., Shizume, K., Asakawa, K., Miyawa, M., Tanaka, I., & Horikawa, R. (1986) Insulin-like growth factor-1 stimulates growth in normal growing rats. Eur. J. Pharmacol. 125:143-146.

Hohenbrink, K. & Nicol, J.J. (1993) Pediatrics. In: Nutrition support dietetics- Core curriculum, 2ed (Gottschlich, M.M., Matarese, L.E. & Shronts, E.P., eds.). A.S.P.E.N., Sliver Spring, MD.

Howard, C.P., Takahashi, H. & Hayles, A.B. (1981) Children with growth hormone deficiency: Intermittent treatment with somatotropin and oxandrolone. Am. J. Dis. Child. 135:326-328.

Howarth, R.E. (1972) Influence of dietary protein on rat skeletal muscle growth. J. Nutr. 102:37-44.

Hua, K.M., Ord, R., Kirk, S., Li, Q.J., Hodgkinson, S.C., Spencer, S.G., Molan, P.C. & Bass, J.J. (1993) Regulation of plasma and tissue levels of insulin-like growth factor-1 by nutrition and treatment with growth hormone in sheep. J. Endocrinol. 136:217-224.

Hunt, S.M. & Groff, J.L. (1990) Advanced nutrition and human metabolism, pp., 298-306. West Publishing Company, St. Paul, MN.

Hurley, L.S., Gordon, P., Keen, C.L. & Merkhofer, L. (1982) Circadian variation in rat plasma zinc and rapid effect of dietary zinc deficiency. Proc. Soc. Exp. Biol. Med. 170:48-52.

Isely, W.L., Underwood, L.E. & Clemmons, D.R. (1983) Dietary components that regulate serum somatomedin-C concentrations in humans. J. Clin. Invest. 71:175-182.

Isgaard, J., Nisson, A., Vikman, K. & Isaksson, O.G.P. (1989) Growth hormone regulates the level of insulin-like growth factor-1 mRNA in rat skeletal muscle. J. Endocrinol. 120:107-112.

Jackson, A.A., Chir, B., Picou, D. and Reeds, P.J. (1977) The energy cost of repleting tissue deficits during recovery form protein-energy malnutrition. Am. J. Clin. Nutr. 30:1514-1517.

Jacobs, D.O., Trerotola, S.O., Settle, R.G., Rolandelli, R.H., Wolf, G.L. & Rombeau, J.L. (1985) In vitro detection of fatty liver infiltration in protein-depleted rats using proton nuclear magnetic resonance. J. Surg. Res. 39:25-30.

Jennische, E & Hansson, H.-A. (1987) Regenerating skeletal muscle cells express insulin-like growth factor 1. Acta. Physiol. Scand. 130:327-332.

Jennische, E .& Olivecrona, H. (1987) Transient expression of insulin-like growth factor 1 immunoreactivity in skeletal muscle cells during postnatal development in the rat. Acta. Physiol. Scand. 131:619-622.

Jennische, E., Skottner, A. & Hansson, H.-A. (1987) Satellite cells express the trophic factor IGF-1 in regenerating skeletal muscle. Acta. Physiol. Scand. 129:9-15.

Keeton, W.T. & Gould, J.L. (1986) Biological science 4 ed., pp.151-152. W.W. Norton & Company, New York, NY.

Krstic, R.V. (1984) Illustrated Encyclopedia of Human Histology, pp. 376-378. Springer-Verlag. Berlin, NY.

Latham. M.C. (1990) Protein-energy malnutrition. In: Present Knowledge in Nutrition (Brown, M.L. ed.) 6ed. Nutrition Foundation, Washington, D.C..

Lepage, L (1997) Effects of dietary zinc deficiency and malnutrition on the T-lymphocyte zinc-figure protein p56lck in mice. MSc. Thesis, University of Manitoba.

Lui, J.P., Baker, J., Perkins, A.S., Robertson, E.J. & Efstratiadis, A. (1993) Mice carrying null mutations of the genes encoding insulin-like growth fator-1 (IGF-1) and type 1 IGF receptor (IGF1r). Cell 75:59-72.

Lunn, P. & Austin, S. (1983) Dietary manipulation of plasma albumin concentration. J. Nutr. 113: 1791-1802.

MacLean, W.C. & Graham, G.G. (1980) The effect of energy intake on nitrogen content of weight gained by recovering malnourished infants. Am. J. Clin. Nutr. 33:903-909.

McLarne, D.S. (1981) Primary and secondary nutritional disorders. In: Nutrition and its Disorders. Churchill Livinstone, New York, NY.

McNall, A.D., Etherton, T.D. & Fosmire, G.J. (1995) The impaired growth induced by zinc deficiency in rats is associated with decreased expression of the hepatic insulin-like growth factor 1 and growth hormone receptor genes. J. Nutr. 125:874-799.

Merimee, T.J., Zapf, J., & froesch, E.R. (1982) Insulin-like growth factors in the fed and fasted states. J. Clin. Endocrinol. Metab. 55:999-1002.

Merimee, T.J., Zapf, J., & Froesch, E.R. (1981) Dwarfism in the pygmy. N. Engl. J. Med. 305:965-968.

Mitchell, M.K. (1997) Nutrition during infancy. In: Nutrition across the life span. W.B. Saunders company, Toronto, ON.

Mohan, P.S. & Rao, K.S.J. (1979) Plasma somatomedin activity in protein calorie malnutrition. Arch. Dis. Child. 54:62-64.

Montgomery, R.D. (1962) Muscle morphology in infantile protein malnutrition. J. Clin. Path. 15:511-521.

Morgan, P.N., Keeln. C.L., Clavert, C. & Lonnerdal (1988) Effect of varying dietary zinc intake of weanling mouse pups during recovery form early undernutrition on growth, body composition and composition of gain. J. Nutr. 118:690-698.

Nakamura, T., Nishiyama, S., Futagoishi-Suginohara, Y., Matsuda, I. & Higashi, A. (1993) Mild to moderate zinc deficiency in short children: Effect of zinc supplementation on linear growth velocity.. J. Pediatr. 123:65-69.

O'Leary, M.J., McClain, C.J. & Hegarty, P.V.J. (1979) Effect of zinc deficiency on the weight, cellularity and zinc concentration of different skeletal muscles in the post weanling rat. Br. J. Nutr. 42:487-494.

Oldfords, A., Mair, W.G.P. & Sourander, P. (1983) Muscle changes in protein-deprived young rats. J. Neurol. Sci. 59:291-302.

Oldfors, A., Sourander, P. (1985) Effects of training on skeletal muscle in protein-deprived rats. J. Neurol. Sci., 69:1-8.

Olson, C.L. (1987) Essentials of statistics- making sense of data. Allyn & Bacon Inc, Toronto, ON.

Orlowski, C.G. & Chernausek, S.D. (1988) Discordance of serum and tissue somatomedin levels in growth hormone-stimulated growth in the rat. Endocrinology 122:44-49.

Park, J.H.Y., Grandjean, C.J., Antonson, D.L. & Vanderhoof, J.A. (1986) Effects of isolated zinc deficiency on the composition of skeletal muscle, liver and bone during growth in rats. J. Nutr. 116:610-617.

Payne-Robinson, H.M., Cocks, T, Kerr, D & Picou, D. (1980) Hormonal control of weight gained in infants recovering from protein energy malnutrition. I. The effect of insulin and metabolic rate. Pediat. Res. 14:28-33.

Pell, J.M. & Bates, P.C. (1992) Differential action of growth hormone and insulin-like growth factor-1 on tissue protein metabolism in dwarf mice. Endocrinology 130:1942-1950.

Perwitt, T., D'Ercole, J., Switzer, B & Van Wyk, J . (1982) Relationship of serum immunoreactive somatomedin-C to dietary protein and energy in growing rats. J. Nutr. 112:144-150..

Philipps, A.F., Persson, B., Hall, K., Lake, M., Skottner, A., Sanengen, T. & Sara, V.R. (1988) The effects of biosynthetic insulin-like growth factos-1 supplementation on somatic growth, maturation and erythropoiesis on the neonatal rat. Pediatr. Res. 23:298-305.

Phillips, L.S. & Young, H.S. (1976) Nutrition and somatomedin. 1. Effect of fasting and refeeding on serum somatomedin activity and cartilage growth activity in rats. Endocrinology 99:304-314.

Pimental, E. (1994) Insulin-like growth factors. In: Handbook of growth factors volume 2: Peptide growth factors, pp.55-95. CRC Press, Ann Arbor,MI.

Prasad, A.S. (1984) Discovery and importance of zinc in human nutrition. FASEB 43:2829-2834.

Prentice, A. (1993) Does mild zinc deficiency contribute to poor growth performance?. Nutr. Rev. 51:268-270.

Rechiler, M.M & Nissley, S.P. (1991) Insulin-like growth factors. In: Peptide growth factors and their receptors 1(Sporn, M.B. & Roberts, A.B.). Springer-Verlag, New York, NY.

Rechler, M.M. & Nissley, S.P. (1985) The nature and regulation of the receptors for insulin-like growth factors. Ann. Rev. Physiol. 47:425-442.

Reeds, P.J., Jackson, A.A., Picou, D. & Poulter, N. (1978) Muscle mass and composition in malnourished infants and children and changes seen after recovery. Pediat. Res. 12:613-618.

Reuben, D.B., Greendale, G.A. & Harrison, G.G. (1995) Nutrition Screening in older persons. J. Am. Geriatr. Soc. 43:415-425.

Roth, H.P. & Kirchgebner, M. (1994) Influence of alimentary zinc deficiency on the concentration of growth hormone(GH), insulin-like growth factor 1(IGF-1) and insulin in the serum of force-fed rats. Hom. Metab. Res. 26:404-408.

Salmon, W.D. & Daughaday, W.H. (1957) A hormonally controlled serum factor which stimulates sulfate incorporation by cartilage in vitro. J. Lab. Clin. Med..

Sandstead, H.H., Prasad, A.S., Schulert, A.R., Darby,N.J. (1967) Human zinc deficiency, endocrine manifestations and response to treatment. Am. J. Clin. Nutr. 20:422-442.

Samat, H.B. (1983) Muscle Pathology and Histochemistry, pp.1-33. American Society of Clinical Pathologists Press, Chicago, Ill..

Schneenman, B.O., Lacy, D., Ney, D., Lefevre, M.L., Keen, C.L., Lonnerdal, B. & Hurley, L.S. (1986) Similar effects of zinc deficiency and restricted feeding on plasma and lipoproteins in rats. J. Nutr. 116:1889-1895.

Simmer, K, Khanum, S., Carlsson, L & Thompson, R.P.H. (1988) Nutritional rehabilitation in Bangladesh- the importance of zinc. Am. I. Clin. Nutr. 47:1036-1040.

Smith, H.F., Taiwo, O. & Payne-Robinson, H.M. (1989) Plasma somatomedin-C in Nigerian malnourished children fed a vegetable protein rehabilitation diet. Eur. J. Clin. Nutr. 43:705-713.

Soliman, A.T., Hassan, A.E.H.I., Aref, M.K., Hintz,R.L., Rosenfeld, R.G. & Rogol, A.D. (1986) Serum insulin-like growth factor I and II concentrations and growth hormone and insulin responses to arginine infusion in children with protein-energy malnutrition before and after nutritional rehabilitation. Pediatr. Res. 20:1122-1130.

Solomons, N.W. (1979) On the assessment of zinc and copper nutriture in man. Am. J. Clin. Nutr. 32:856-871.

Spencer, E.M., Liu, C.C., Si, C.C. & Howard, G.A. (1991) In vivo action of insulin-like growth factor-1 (IGF-1) on bone function and resorption in rats. Bone 12:21-26.

Straus, D.S. (1994) Nutritional regulation of hormones and growth factors that control mammalian growth. FASEB J. 8:6-12.

Straus, D.S. & Takemoto, C.D. (1991) Specific decrease in liver insulin-like growth factor-1 and brain insulin-like growth factor-2 gene expression in energy-restricted rats. J. Nutr. 121:1279-1286.

Taylor, C.G., Bettger, W.J., Bray, T.M. (1988) Effect of dietary zinc or copper deficiency on the primary free radical defense system in rats. J. Nutr. 118:613-621.

Thissen, J.P. & Underwood, L.E. (1991) Translation status of the insulin-like growth factor-1 mRNAs in liver of protein-restricted rats. J. Endocrinol. 132:141-147.

Thissen, J.P., Ketelslegers, J.M. & Underwood, L.E. (1994) Nutritional regulation of the insulin-like growth factors. Endocrine Reviews 15:80-101.

Thissen, J.P., Triest, S., Moats-Staats, B.M., Underwood, L.E., Mauerhoff, T., Maiter, D. & Ketelslegers, J.M. (1991b) Evidence that pretranslational and translational defects decrease serum insulin-like growth factor-1 concentrations during dietary protein restriction. Endocrinology 129:429-435.

Thissen, J.P., Underwood, L.E., Maiter, D., Maes, M., Clemmons, D.R., & Ketelslegers, J.M. (1991a) Failure of insulin-like growth factors-1 (IGF-1) infusion to promote growth in protein-restricted rats despite normalization of serum IGF-1 concentrations. Endocrinology 128:885-890.

Tuner, J.D., Rotwein, P., Novakofski, J. & bechtel, P.J. (1988) Induction of mRNA for IGF-1 and 2 during growth hormone-stimulated muscle hypertrophy. Am. J. Physiol. 255:E513-E517.

Vance, M.L., Hartman, M.L. & Thorner, M.O. (1992) Growth hormone and nutrition. Horm. Res. 38(suppl 1):85-88.

VandeHaar, M.J., Moats-Staats, B.M., Davenport, M.L., Walker, J.L., Ketelsleger, J.M., Sharma, B.K., Underwood, L.E. (1990) Reduced serum concentrations of insulin-like growth facto-1 (IGF-1) in protein-restricted growing rats are accompanied by reduced IGF-1 mRNA level in liver and skeletal muscle. J. Endocrinology 130:305-312.

Wallwork, J.C., Johnson, L.K., Milne, D.B. & Sandstead, H.H. (1983) The effect of interactions between dietary egg white protein and zinc on body weight, bone growth and tissue trace metals in the 30-day-old rat. J. Nutr. 113:1307-1320.

Wapnir, R.A., Garcia-Aranda, J.A., Mevorach, E.K. & Lifshitz, F. (1985) Differential absorption of zinc and low molecular-weight ligands in the rat gut in protein-energy malnutrition. J. Nutr. 115:900-908.

Wardlaw, G.M. & Insel, P.M. (1990) Perspectives in nutrition, pp.422-427. Time Mirror/ Mosby College Publishing, St. Louis, MO.

Williams, R.B. & Mills, C.F. (1970) The experimental production of zinc deficiency in the rat. Br. J. Nutr. 24:989-1003.

World Health Organization (1998) The state of the world's children, pp.9-37. Oxford University Press, New York, NY.

Wu, F.Y.H. & Wu, C.W. (1987) Zinc in DNA replication and transcription. Ann. Rev. Nutr. 78:251-272.

Yahya, Z.A.H., Bates, P.C. & Millward, D.J. (1990) Responses to protein deficiency of plasma and tissue insulin-like growth factor-1levles and proteoglycan synthesis rates in rat skeletal muscle and bone. J. Endocrinol. 127:497-503.

Zanconato, S., Moromisato, D.Y., Moromisato, M.Y., Woods, J., Brasel, J.A., Leroith, D., Roberts, C.T. & Cooper, D.M. (1994) Effect of training and growth hormone suppression on insulin-like growth facto-1 mRNA in young rats. J. Appl. Physiol. 76:2204-2209.

APPENDIX A

1. Key to tables
2. Table 1. Weekly weights (in grams) during the deficiency (day 0 – week 3) and recovery (week 4 – week 6) phases.
3. Table 2. Weight gain (loss) during the deficiency phase deficiency (day 0 – week 3) and recovery (week 4 – week 6) phases.
4. Table 3. Weekly feed intakes (in grams) during the deficiency phase deficiency (day 0 – week 3) and recovery (week 4 – week 6) phases.
5. Table 4. Changes in weekly feed intakes (in grams) during the deficiency phase deficiency (week 1 – week 3) and recovery (week 4 – week 6) phases.
6. Table 5. Cumulative feed intake and feed efficiency during the deficiency and recovery phases.
7. Table 6. Body and tail length before and after the deficiency and recovery phases.
8. Table 7. Organ weights (in grams) and organ to body weight ratios at the end of the deficiency phase.
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10. Table 9. Liver lipid concentration before and after the deficiency and recovery phases.
11. Table 10. Serum concentrations of albumin, zinc and IGF-1 at the end of the deficiency and recovery phases.
12. Table 11. Femur concentrations of zinc, calcium and phosphorous and the calcium: phosphorous ratio before and after the deficiency and recovery phases.
13. Table 12. Inguinal fat pad weight, gastrocnemius weight and type 1 & 2 muscle fiber diameters before and after the deficiency and recovery phases.

Key to tables

The values presented in the following tables are the group means +/- SEM. The number of rats per group is equal to 8 unless otherwise indicated in the table footnotes. The weekly values with different lower case letters are significantly different ($P<0.05$) as determined by repeated measures testing. Values before and after the deficiency and recovery phases with different lower case letters are significantly different ($P<0.05$) as determined by Duncan's multiple range test. The lower case letters indicating significance are located under the SEM.

The following codes were used to identify the different experimental groups:

W= Baseline

ZP= Zinc + protein deficiency

P= Protein deficiency

Z= Zinc deficiency

E= Energy deficiency

C= Control

Please note that the baseline data (W) collected on day 0 is presented along side the data collected at the end of the 3-week deficiency phase for easy comparisons.

Table 1. Weekly weights (in grams) during the deficiency (day 0-week 3) and recovery (week 4-week 6) phases

	Group				
	ZP	P	Z	E	C
DAY 0	49.475	48.238	48.413	48.738	49.850
+/- SEM	0.5126	0.509	0.488	0.545	1.135
	a	a	a	a	a
Week 1	46.038	45.475	54.913	57.825	70.000
+/- SEM	0.624	0.446	1.902	2.283	5.380
	c	c	b	b	a
Week 2	46.188	45.775	63.888	75.838	114.550
+/- SEM	0.649	0.617	1.865	1.769	5.686
	d	d	c	b	a
Week 3	46.813	46.113	69.113	77.225	138.700
+/- SEM	0.797	0.686	2.028	1.625	7.024
	c	c	b	b	a
Week 4	97.425	139.263	94.725	143.013	203.163
+/- SEM	0.924	6.052	3.189	6.193	9.041
	c	c	b	b	a
Week 5	149.100	182.550	142.000	185.613	250.663
+/- SEM	2.181	4.520	3.636	6.857	6.363
	c	c	b	b	a
Week 6	192.475	223.013	183.538	216.538	278.613
+/- SEM	3.939	5.198	4.111	7.503	7.971
	c	c	b	b	a

Table 2. Weight gain (loss) during the deficiency (day 0 - week 3) and recovery (week 4 - week 6) phases

	Group				
	ZP	P	Z	E	C
Day 0	0.000	0.000	0.000	0.000	0.000
+/- SEM	0.000	0.000	0.000	0.000	0.000
	a	a	a	a	a
Week 1	-3.075	-2.15	12.075	13.975	30.525
+/- SEM	0.189	0.396	1.208	3.245	5.318
	c	c	b	b	a
Week 2	-0.0375	0.6875	15.3125	18.375	43.3375
+/- SEM	0.238	0.297	3.344	2.845	1.637
	c	c	b	b	a
Week 3	0.05	0.8125	11.225	6.1	38.6375
+/- SEM	0.350	0.402	3.631	4.710	8.080
	b	b	b	b	a
Week 4	51.1875	47.0875	52.6375	56.0125	42.75
+/- SEM	1.074	2.767	1.932	1.695	5.921
	ab	ab	ab	a	b
Week 5	51.675	47.275	43.2875	42.6	47.5
+/- SEM	1.551	3.540	2.832	1.857	3.954
	a	a	a	a	a
Week 6	43.375	41.5375	40.4625	30.925	27.95
+/- SEM	1.965	2.250	3.122	2.260	5.451
	a	a	a	b	b

Table 3. Weekly feed intakes (in grams) during the deficiency (week 1- week 3) and recovery (week 4- week 6)

	Group				
	ZP	P	Z	E	C
Week 1	36.2992	50.7136	37.5991	36.6121	61.1891
+/- SEM	1.701	4.4688	0.9492	0.8827	5.7897
	c	b	c	c	a
Week 2	40.2752	46.6746	49.0623	46.3372	97.9881
+/- SEM	2.3898	3.4265	2.9421	2.0734	3.9767
	b	b	b	b	a
Week 3	32.4625	39.7096	51.5508	51.2127	108.1766
+/- SEM	1.6205	2.4465	4.0719	4.9371	5.2122
	c	c	b	b	a
Week 4	75.663	77.2128	102.5752	97.8621	127.2117
+/- SEM	4.2602	5.9927	4.8202	4.8237	5.439
	c	c	b	b	a
Week 5	95.6382	98.3136	118.111	106.8753	134.6247
+/- SEM	2.5025	3.2249	13.0508	4.3687	4.7411
	bc	bc	ab	bc	a
Week 6	118.4505	113.1627	122.2256	112.2135	139.1593
+/- SEM	5.0981	3.8535	4.0579	3.4111	7.8841
	b	b	b	b	a

Table 4. Changes in weekly feed intakes (in grams) during the deficiency (week1 - week 3) and recovery (week 4 - week 6) phases.

	Group				
	ZP	P	Z	E	C
Week 1	0.000	0.000	0.000	0.000	0.000
+/- SEM	0.000	0.000	0.000	0.000	0.000
	a	a	a	a	a
Week 2	3.9758	-4.0373	11.4624	9.725	36.7999
+/- SEM	2.5336	2.3683	2.7097	2.2404	3.1362
	b	c	b	b	a
Week 3	-7.8129	-6.9665	2.4875	4.8747	10.1873
+/- SEM	1.5125	1.0655	1.6126	3.1022	5.2376
	b	b	a	a	a
Week 4	43.2008	37.5792	51.0248	46.65	19.0375
+/- SEM	4.187	5.1415	2.545	2.7314	4.5874
	ab	b	a	ab	c
Week 5	19.9749	21.1	15.5375	9.0125	7.4125
+/- SEM	3.2088	4.6844	8.7156	1.8251	3.0306
	a	a	a	a	a
Week 6	22.8123	14.85	4.1124	5.3375	4.5341
+/- SEM	3.1249	1.8472	11.4072	1.826	7.1649
	a	a	a	a	a

Table 5. Cumulative feed intake and feed efficiency during the deficiency and recovery phases.

	Group				
	ZP	**P**	**Z**	**E**	**C**
Cumulative feed intake (g)					
Deficiency	109.690	127.050	118.900	116.690	234.850
+/- SEM	4.642	20.292	10.371	9.402	42.935
	a	a	a	a	b
Recovery	408.050	425.140	484.100	448.210	667.650
+/- SEM	14.995	56.333	74.687	52.396	64.572
	c	c	d	cd	e
Feed efficiency					
Deficiency	2.368	2.765	1.770	1.517	1.740
+/- SEM	0.085	0.133	0.035	0.028	0.056
	b	a	d	e	de
Recovery	2.119	2.308	2.170	2.064	2.408
+/- SEM	0.100	0.111	0.081	0.043	0.074
	c	bc	bc	c	b

Table 6. Body and tail length before and after the deficiency and recovery phases

	Group					
	W	ZP	P	Z	E	C
Body length (cm)						
Deficiency	11.975	12.800	12.850	14.100	14.838	18.025
+/- SEM	0.1359	0.2179	0.0845	0.1991	0.0625	0.3331
	a	b	b	c	d	e
Recovery		19.150	19.662	20.037	19.850	21.275
+/- SEM		0.231	0.185	0.063	0.204	0.296
		f	f	g	g	h
Tail length (cm)						
Deficiency	8.945	10.350	10.150	11.888	11.675	14.213
+/- SEM	0.082	0.143	0.169	0.194	0.151	0.315
	a	b	b	c	c	d
Recovery		14.719	14.625	16.288	15.875	18.563
+/- SEM		0.320	0.335	0.146	0.383	0.395
		d	d	e	e	f

Table 7. Organ weights (in grams) and organ to body weight ratios at the end of the deficiency phase

	Group					
	W	ZP	P	Z	E	C
Liver	2.125	2.128	2.133	2.658	2.769	5.600
+/-SEM	0.105	0.092	0.070	0.116	0.095	0.395
	a	a	a	a	a	b
Liver/Body	0.045	0.046	0.047	0.040	0.036	0.041
+/-SEM	0.002	0.001	0.001	0.001	0.001	0.001
	cd	cd	de	ab	a	bc
Kidney	0.550	0.543	0.539	0.819	0.950	1.559
+/-SEM	0.019	0.013	0.015	0.028	0.038	0.062
	a	a	a	b	b	c
Kidney/Body	0.012	0.012	0.012	0.012	0.012	0.012
+/-SEM	0.0004	0.0002	0.0003	0.0004	0.0006	0.0005
	a	a	a	a	a	a
Spleen	0.213	0.104	0.131	0.141	0.156	0.343
+/-SEM	0.013	0.003	0.036	0.010	0.010	0.033
	b	a	a	ab	ab	c
Spleen/Body	0.004	0.002	0.003	0.002	0.002	0.003
+/-SEM	0.0002	0.0001	0.0007	0.0001	0.0001	0.0001
	a	bcd	bcd	cd	d	bcd

Table 8. Oragn weight (in grams) and organ weight to body weight ratios at the end of the recovery phase

	Group				
	ZP	P	Z	E	C
Liver	10.015	8.944	11.353	10.671	12.650
+/-SEM	0.535	0.401	0.580	0.802	0.469
	cd	c	e	de	f
Liver/Body	0.052	0.048	0.051	0.049	0.046
+/-SEM	0.002	0.002	0.002	0.002	0.001
	f	def	ef	def	cd
Kidney	1.948	1.814	2.244	2.124	2.653
+/-SEM	0.102	0.054	0.091	0.100	0.082
	de	d	f	ef	g
Kidney/Body	0.010	0.0098	0.0101	0.0098	0.00960
+/-SEM	0.0004	0.0002	0.0004	0.0003	0.0002
	b	b	b	b	b
Spleen	0.588	0.538	0.504	0.538	0.599
+/-SEM	0.037	0.023	0.020	0.039	0.032
	e	de	d	de	e
Spleen/Body	0.003	0.003	0.002	0.003	0.002
+/-SEM	0.0002	0.0001	0.0001	0.0002	0.0001
	b	bc	bcd	bcd	cd

Table 9. Liver lipid concentration before and after the deficiency and recovery phases [1]

	Group					
	W	ZP	P	Z	E	C
Deficiency	0.033	0.099	0.094	0.033	0.034	0.041
+/- SEM	0.002	0.011	0.020	0.001	0.001	0.002
	b	a	a	b	b	b
Recovery		0.039	0.036	0.038	0.046	0.041
+/- SEM		0.004	0.023	0.002	0.004	0.004
		b	b	b	b	b

[1]The above values represent the group mean +\- SEM with n = 5.

Table 10. Serum concentrations of albumin, zinc and IGF-1 at the end of the deficiency and recovery phases.

	Group					
	W	ZP	P	Z	E	C
Albumin (g/dL) [1]						
Deficiency	3.511	2.047	1.911	4.714	5.269	4.715
+/- SEM	0.178	0.077	0.153	0.298	0.400	0.345
	a	b	b	c	c	c
Recovery		4.428	4.353	4.752	4.458	4.987
+/- SEM		0.520	0.203	0.326	0.234	0.305
		d	d	d	d	d
Zinc (ug/ml) [2]						
Deficiency	1.202	0.422	0.586	0.361	1.901	1.626
+/- SEM	0.056	0.043	0.054	0.061	0.179	0.060
	b	a	a	a	d	c
Recovery		1.778	1.678	1.698	1.615	1.646
+/- SEM		0.053	0.082	0.060	0.051	0.060
		cd	cd	c	c	c
IGF-1 (ng/ml) [3]						
Deficiency	476.864	477.438	664.727	119.356	245.088	1129.480
+/- SEM	60.708	60.568	68.585	17.377	53.100	89.362
	b	b	c	a	a	d
Recovery		1319.924	1359.023	1384.401	1348.859	1462.986
+/- SEM		44.866	34.236	33.149	36.903	14.430
		e	e	e	e	e

[1] The above values represent the group mean +/- SEM, n= 8, except for the W group (n= 6) and the ZP group (n= 7) at the end of the deficiency phase.

[2] The above values represent the group mean +/- SEM, n= 8, except for the C group at the end of the recovery phase.

[3] The above values represent the group mean +/- SEM, n= 8, except for the C (n= 7), ZP (n= 6) and E (n= 7) groups after the deficiency phase.

Table 11. Femur concentrations of zinc, calcium and phosphorous and the calcium: phosphorous ratio before and after the deficiency and recovery phases

	Group					
	W	ZP	P	Z	E	C
Femur zinc						
Deficiency	3.408	2.894	2.203	1.672	5.261	4.766
+/- SEM	0.319	0.398	0.446	0.218	0.540	0.319
	cd	bc	ab	a	e	ef
Recovery		4.726	4.976	4.253	4.956	5.036
+/- SEM		0.274	0.157	0.170	0.156	0.260
		ef	ef	ed	ef	ef
Femur calcium						
Deficiency	3204.800	3458.300	3447.700	3809.000	4024.200	4323.000
+/- SEM	167.040	85.754	43.521	32.102	77.483	58.454
	a	b	b	c	d	e
Recovery		4847.400	4762.000	4807.000	4805.600	4985.800
+/- SEM		30.367	47.805	75.840	45.247	41.829
		f	f	f	f	f
Femur phosphorous						
Deficiency	2418.91	2391.73	2482.07	2648.9	2967.96	3219.94
+/- SEM	129.4745	67.1025	30.5142	28.6141	61.0492	43.4177
	a	a	a	b	c	d
Recovery		3346.930	3365.200	3423.740	3489.120	3602.470
+/- SEM		25.0621	36.7791	50.7943	39.6047	39.7496
		de	de	e	ef	f
Calcium: phosphorus ratio						
Deficiency	1.328	1.448	1.389	1.440	1.356	1.343
+/- SEM	0.009	0.010	0.009	0.009	0.006	0.006
	g	a	cd	a	ef	fg
Recovery		1.448	1.415	1.404	1.378	1.385
+/- SEM		0.008	0.014	0.004	0.009	0.006
		a	b	bc	de	cd

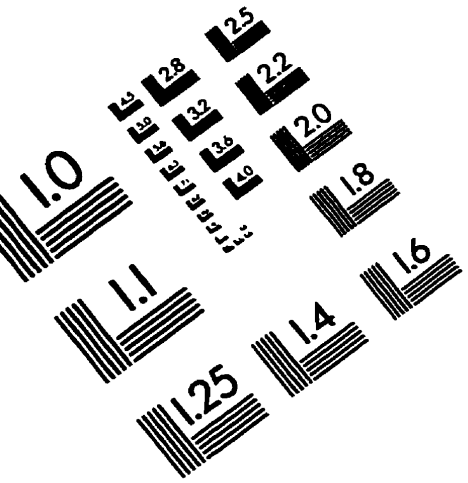
Table 12. Inguinal fat pad weight, gastrocnemius weight and type 1 & 2 muscle fiber diameters before and after the deficiency and recovery phases.

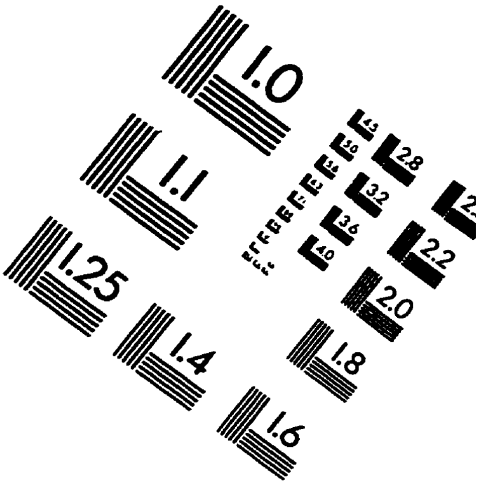
	Group					
	W	ZP	P	Z	E	C
Inguinal fat pad weight (g)						
Deficiency	0.300	0.681	0.710	0.280	0.000	1.744
+/- SEM	0.078	0.054	0.058	0.068	0.000	0.208
	ab	b	b	ab	a	c
Recovery		2.705	3.143	2.530	3.395	3.615
+/- SEM		0.031	0.191	0.295	0.352	0.246
		de	ef	d	f	f
Gastrocnemius weight (g)						
Deficiency	n/a[1]	0.267	0.330	0.468	0.498	0.841
+/- SEM	n/a [1]	0.013	0.043	0.068	0.057	0.052
		a	a	b	b	c
Recovery		1.251	1.129	1.603	1.460	1.848
+/- SEM		0.029	0.044	0.045	0.063	0.055
		d	d	f	e	g
Type 1 MFD (um) [2]						
Deficiency	23.562	25.728	25.368	25.473	31.465	31.535
+/- SEM	0.835	1.405	0.499	0.823	0.856	0.993
	a	a	a	a	bc	bc
Recovery		33.730	31.035	34.458	33.370	36.777
+/- SEM		1.475	1.058	1.987	1.429	0.320
		bcd	b	cd	bc	d
Type 2 MFD (um) [2]						
Deficiency	21.240	22.425	21.363	24.395	26.927	31.163
+/- SEM	1.230	1.247	0.753	1.250	0.985	1.213
	a	a	a	ab	b	c
Recovery		37.833	33.885	38.725	37.657	39.665
+/- SEM		2.030	1.208	1.777	1.462	1.758
		d	c	d	d	d

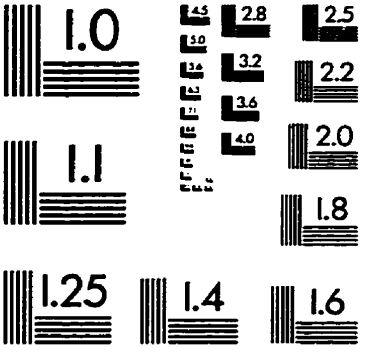
[1] n/a = not assessed

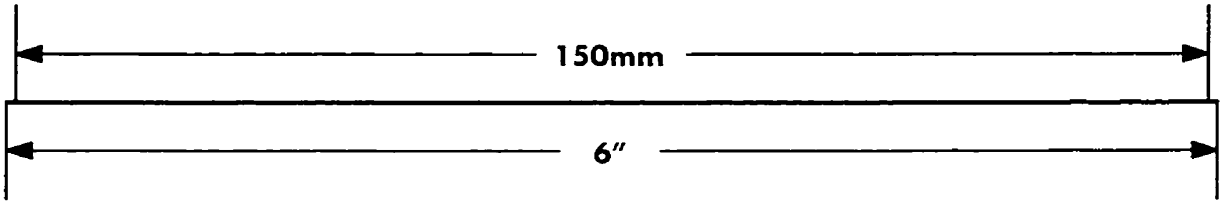
[2] The above values represent the group mean +/- SEM, n=6.

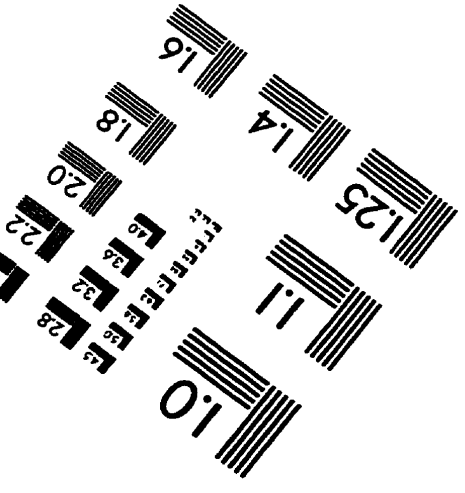
IMAGE EVALUATION
TEST TARGET (QA-3)

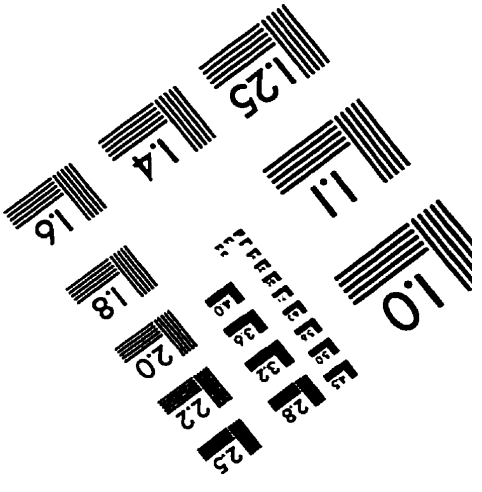












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