Severe malnutrition, growth and zinc supplementation

A thesis submitted for the degree of Doctor of Medicine in the University of London by

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The work in this thesis is based on research carried out whilst a research fellow at the Department of Child Life & Health, University of Edinburgh, Scotland.

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Dedication

For my parents

Abstract

Aims

To describe early linear and ponderal growth, collagen turnover, bone osteoblastic activity and growth, and insulin like growth factor (IGF) axis activity during rehabilitation of severely malnourished children and compare the effect of 3 regimens of zinc supplementation.

Methods

141 children were randomised in a double blind trial to either regimen 1: 1.5 mg/kg/day (mg of elemental zinc per kg body weight of zinc sulphate) for 15 days followed by placebo for 15 days, or regimen 2: 6.0mg/kg/day for 15 days followed by placebo for 15 days, or regimen 3: 6.0mg/kg/day for 30 days. Markers of collagen formation (serum pro-collagen type 1 c terminal propeptide (P1CP), and type 3 n terminal propeptide (P3NP)) and degradation (plasma cross-linked telopeptide of type 1 collagen (1CTP)), osteoblastic activity (bone alkaline phosphatase (BAP)), IGF1 and insulin like growth factor binding proteins 2 & 3 (IGFBP2 and IGFBP3) were assessed.

Results

There were no significant differences between zinc regimens in anthropometric or biochemical marker change. Mortality was significantly increased in children who received high dose zinc (6.0mg/kg) initially (Yates corrected chi-square p value of 0.033). Linear growth was significantly associated with initial weight-for-height Z score (WHZ). Plasma IGF-I, IGFBP-3, PICP, P3NP and BAP were low and increased whilst IGFBP-2 and ICTP were increased and fell during refeeding ($P < 0.001$).

IGFBP2 correlated positively with ICTP and negatively with PICP, BAP, IGF1 and IGFBP3 ($p<0.01$). Ponderal growth correlated with increases in IGF-I, IGFBP-3, PICP, P3NP and BAP over 30 days ($P <0.01$). Linear growth over 90 days correlated with increases of IGFBP-3 ($P<0.05$), PICP ($P <0.01$) and P3NP ($P <0.01$).

Conclusions

Alterations in the Growth Hormone /IGF axis in response to diet may control the coupling of bone resorption to formation and collagen deposition. There is no benefit in using higher dose zinc regimens and they might contribute to increased mortality.

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Statement of Originality

I hereby confirm that I was involved in the study design, supervised all field-work and data collection, conducted data analysis (initially with the advice of Dr Rob Elton – Univ of Edinburgh) and wrote this thesis. Rhona Stephens, Jean Wade, Pat Crofton (all of University of Edinburgh) and Hazel Miller (University of Glasgow) carried out laboratory analyses.

Publications based on thesis

Doherty CP, Sarkar MAK, Shakur MS, Ling SC, Elton RA, & Cutting WA (1998): Zinc and rehabilitation from severe protein-energy malnutrition: Higher- dose regimens are associated with increased mortality. American Journal of Clinical Nutrition. Vol 68, 742-749

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Abbreviations

WHZ	Weight for height Z score
WAZ	Weight for age Z score
HAZ	Height for age Z score
LLL	Lower leg length i.e. knemometric length
MUAC	Mid upper arm circumference
SFT	Skin fold thickness
P1CP	Procollagen type 1 C terminal propeptide
P3NP	Procollagen type 3 N terminal propeptide
BAP	Bone alkaline phosphatase
1CTP	Collagen type 1 cross linked telopeptide
PYD	Pyridinoline
DPD	Deoxypyridinoline
IGF1	Insulin like growth factor 1
IGFBP3	Insulin like growth factor binding protein 3
IGFBP2	Insulin like growth factor binding protein 2
IUGR	Intra-uterine growth retardation
GH	Growth hormone
BMI	Body mass index
ICP	Infancy, Childhood and Puberty
NCHS	National Centre for Health Statistics
Zn	Zinc
Cu	Copper
IL	Interleukin
TH	T helper cell

NRU	Nutritional Rehabilitation Unit
WHO	World Health Organisation
RIA	Radioimmunoassay
ELISA	Enzyme linked immunosorbent assay
OP	Out-patient
IP	In-patient
ARI	Acute respiratory infection
CRP	C reactive protein
CI	Confidence Interval

Chapter 1: Introduction

Rehabilitation from severe protein energy malnutrition and optimal catch-up growth require the provision of adequate macro and micro-nutrients. The ideal diet has long been the subject of debate, and there has been much discussion of the importance of micronutrients. Zinc deficiency has been implicated as a limiting factor in recovery (Hambidge 1997). Currently there exists no reliable test for detecting zinc deficiency, particularly in the presence of co-existing protein-energy malnutrition, other than empirically, by detecting a positive response to supplemental zinc.

Linear growth retardation is common in developing countries and has a multi-factorial origin. Zinc deficiency in particular has been increasingly implicated. However zinc supplementation of growth-retarded and presumed zinc-deficient children have had mixed effects on the promotion of linear catch-up growth (Bates 1993; Gibson 1989; Hambidge 1985; Nguyen 1996; Rosado 1997; Walravens 1989). This probably reflects both the diversity of populations in which zinc supplementation was employed in terms of age, degrees of growth retardation, dietary intake, availability of zinc and other growth limiting factors, as well as study design.

Zinc supplementation trials in children recovering from severe protein-energy malnutrition have demonstrated benefits in terms of ponderal growth (Golden 1981) and immunity (Schlesinger 1992). The process of linear growth and its relationship to ponderal growth is not well understood. In one of the few studies that has looked at this in the early phase of rehabilitation from severe malnutrition Walker et al looked at catch-up growth retrospectively in a group of 369 malnourished hospitalised children and assessed influences on linear growth (Walker 1988). Their group as a

whole did not demonstrate linear catch up but a subgroup, who were more stunted initially, did. Two-thirds of the group grew linearly only when they had attained 85% weight for length and they speculated that the diets were limiting in one or more nutrients essential for linear growth such as zinc. Further studies have demonstrated that zinc promotes deposition of lean tissue in this period and could thus influence linear growth (Golden 1992).

To clarify these conflicting studies I designed a randomised, double blind, intervention study of the effect of three regimens of oral zinc supplementation on the recovery of severely malnourished children admitted to an in-patient nutritional rehabilitation unit, focusing on anthropometric and knemometric outcomes and biochemical markers of collagen and bone turnover and growth hormone axis activity.

1.1 Objectives

1. To describe the patterns of and relationship between ponderal and linear catch-up growth in the recovery phase from severe malnutrition

2. To describe bone, soft tissue and collagen turnover during this phase

3. To assess the effects of three different regimens of zinc supplementation on the above processes and thus the optimal dosage range for severely malnourished children. These three regimens have been chosen to reflect the spectrum of current opinion of the zinc requirement of the malnourished child.

1.2 Linear Growth

1.2.1 Assessment

Linear growth is difficult to measure accurately and is assessed either as supine length or standing height. Supine length is best assessed in children less than two years using a baby board with supports for the head and feet. An assistant is required who holds the baby's head in firm contact with the headboard so that line between the center of the auditory meatus and the lower border of the orbit (Frankfurt plane) is vertical. The measurer then straightens the child's legs by gripping the ankles and takes the reading. The standing height of children over two years is measured using a stadiometer. The child should be in his/her bare feet with heels together, buttocks and shoulder blades against the stadiometer, looking straight ahead with a headboard resting at right angles against the highest point of the head. The measurer should ensure that the Frankfurt plane is horizontal and apply gentle pressure to the mastoid process to extend the head checking that the heels have not lifted off the baseboard (Cox 1992). Assessing height accurately in the two to three year age group is difficult and to obtain accurate measurements it is safest to measure supine length even in children up to five years old. Length or height should be reported to the nearest 0.1 cm but it should be remembered that the measurement error is nearly 0.5 cm. The rate of change of height (velocity) has been promoted as a better expression of linear growth than height alone (Brook 1986), but inherent lack of precision in estimating velocity may limit its reliability in assessing growth in short children (Voss 1991).

1.2.2. Indices & reference populations

Growth is measured using weight, height, mid upper arm circumference (MUAC) etc and sex and age. Single anthropometric measurements however are uninterpretable, and indices e.g. weight-for-height, are combinations of measurements that allow growth data to be compared within and between groups, e.g. a single weight is meaningless unless related to a child's height or age. In turn, growth indices must be related to a reference population for meaningful interpretation. The WHO has endorsed the use of that population defined by the US National Center for Health Statistics (NCHS) as a reference (World Health Organisation 1995). However the use of references based on a population of infants and children from one country to assess the growth of children in another e.g. developing country, has proved controversial. Differences in the genetic potential for growth are often quoted as a rationale for having country or region specific growth references. However whilst genetic differences do exist, it is environmental factors that have the larger effect on the potential for growth. Martorell, who looked at the heights of school age children from different socio-economic groups in different countries, demonstrated that variation between height of the least socio-economically deprived groups in both developing and developed countries was less than the variation within countries between socio-economic groups (Martorell 1985).

The WHO has acknowledged that reference data will be used as standards and recommends that care be taken to choose references that resemble standards (World Health Organisation 1986). It further acknowledges that since the mean heights of young children of many affluent populations differs little between ethnic groups it should be possible to construct a standard that reflects the growth potential of all

children throughout the world. The WHO chose the NCHS reference because the population on which it was based lived in a healthy environment, was well nourished and had probably met its full growth potential. As a standard, its limitations must be recognized. The growth curves were originally constructed in 1975 from four sources. The 0 –23 month data of recumbent lengths came from the Fels Research Institute Longitudinal Study of 1923 to 1975. The infants included in this data set were predominately formula-fed and were from a relatively restricted genetic, socio-economic and geographic background. The 2 – 18 y old data of standing heights came from three U.S. surveys from 1960 to 1975. Across most populations there is little difference in mean growth in height or in the distribution around the mean but the inclusion of both healthy and sick, breast as well as formula-fed infants in this reference should be remembered, particularly when comparing individuals or particular groups against the reference e.g. breast-fed infants (see below). With these limitations in mind, NCHS data should perhaps be used as a tool to identify children at risk of malnutrition rather than as a standard to be attained or as a means to label children as malnourished. An expert committee of the WHO has recently recommended the development of a new reference for infants and children, which will be a complex and costly undertaking (De Onis 1996).

Growth, however, is expressed as rate of change in weight, height etc (velocity), and can only be assessed by comparing indices over time against the reference population. In assessing individual children it must be emphasized that plotting a child's weight and height over time allows assessment of the child's own growth curve in relation to a reference population. Stunting is a deficit in height-for-age and signifies slowing of skeletal growth. In general, it reflects a chronic process. The prevalence of nutritional

deficits varies with age and low weight-for-height often peaks in the second year of life, whilst low height-for-age starts earlier and decreases by three years. Interpretation of these indices must take into account age. Thus a low height-for-age among one year olds reflects current health and nutrition, while among six year olds it suggests a past problem, but may also indicate concurrent stunting in the same population among younger children.

1.2.3. Growth – the Infancy, Childhood and Puberty (ICP) model

Linear growth is a complex process occurring in three distinct phases – infancy, childhood and puberty (Karlberg 1989). The infancy phase is a continuation of the high fetal growth rate with a rapid decline to three years of age. The onset of the childhood phase is heralded by an abrupt increase in linear growth rate and thereafter continues with a lower, more slowly decelerating velocity. A third distinct phase occurs at puberty before linear growth ceases and adulthood is reached.

These three phases contribute differently to overall linear growth. Maximal rate of growth occurs during the infancy phase, but it is the slower but longer childhood phase that is responsible for two-thirds of postnatal linear growth. The pubertal phase is associated with a second increase in growth velocity but is relatively short-lived and contributes least to the overall sum of linear growth.

The control of the infancy phase is poorly understood but is primarily a function of the intrauterine environment and postnatal nutrition. The onset of the childhood phase, normally in the second half of the first postnatal year, is influenced by the action of growth hormone (GH), which regulates long bone growth in the legs. Delays in onset

of the childhood phase have attracted particular interest with regards to populations of malnourished children in developing countries and are proposed as a determining factor in attainment of final height (see page 32). GH continues its influence throughout childhood and adolescence but sex steroid secretion during puberty superimposes a further spurt in linear growth on the decelerating childhood phase.

The etiology and reversibility of stunting is best considered with reference to the ICP model. Changes in onset and duration of these phases and the effect of nutritional insults and interventions during them can be best understood within this context. For the purposes of this thesis I shall describe in more detail the infancy and childhood phases.

1.2.4. Stunting – epidemiology

Stunting results from growth failure in childhood, which is commonly nutritional in origin. UNICEF estimates that 40% of the world under five population (226 million) are moderately or severely stunted. As a marker of deprivation stunting also predicts other functional consequences of severe nutritional insults early in life. Cognitive deficits, decreased work ability, increased morbidity and increased obstetric risks have all been associated with stunting. Stunted rural Guatemalan children had lower literacy scores, had completed fewer years at school and scored less well in tests of intellectual function than their normally grown peers (Martorell 1992). Adult height predicted the work capacity of Colombian sugar cane cutters (Spurr 1977), whilst shorter women with smaller pelvic sizes are at a greater risk of obstetric complications (Barnhard 1997; Tsu 1992).

Stunting is a chronic process and it may take many months of sub-optimal growth before it occurs. The degree of stunting is a product of the severity, timing and duration of the nutritional insult. If a normal 12-month-old child stops growing completely then he will take 6 months to fall below –2 Z score for height-for-age i.e. stunted, whereas a 36 month old child will take 13 months to do the same. Equally that same 12-month-old will take 42 months to become stunted if he reduces his growth rate to 70% of normal as opposed to stopping growing completely (Golden 1994). Stunting results from a chronic insult and equally catch-up growth will have to be prolonged to reverse it – the older and the more stunted a child, then the longer that he will have to grow at an accelerated rate before full catch-up growth is achieved. In the environment in which the vast majority of stunted children reside this is usually impossible.

In a study from Pakistan between 75-83% of children were stunted by 24 months of age (Karlberg 1993). The stunting process (defined as height-for-age Z scores) started at 6 months of age and continued to 18 months of age, whereas weight-for-length Z scores increased from a baseline of –1 to 0 at 24 months. Other studies have found that length attained at three years is highly related to adult height but is independent of subsequent linear growth, i.e. that early growth retardation is not reversed later (Martorell 1995).

The requirement for dietary energy is highest in the first year of life when growth velocities are high but stomach volumes are low. Yet commonly used weaning foods in many countries with a high prevalence of stunting are bulky with energy densities too low to support optimal growth. Infections, especially gastrointestinal, are common

in areas of poverty and illiteracy and contribute to malnutrition that makes children more susceptible to further infections. This cycle of poor nutrition and infection in this critical phase of growth leads to stunting.

The relative contribution of diarrhoeal disease and inadequate diet to the commonly observed growth failure of children in developing countries remains uncertain (Briend 1989). At an individual level (Hoare 1996) diarrhoeal episodes cause short term faltering in both ponderal and linear growth, yet whether these children then catch-up and whether their long term growth failure is due to inadequate food intake or recurrent diarrhea is controversial. Malnourished rural Bangladesh children (Briend 1989) grew equally well in the three monthly intervals where a diarrhoeal episode of at least 10 days occurred at the beginning of the interval compared with an interval with no diarrhea. In contrast intervals with at least 10 days of diarrhea occurring in the last 45 days were associated with significantly lower weight gain than those with diarrhea free intervals. These children were free of diarrhea for over 90% of the time but only gained weight to 74% of the NCHS median during diarrhea free periods. The authors concluded that these children were malnourished due to poor food intake rather than diarrhea. A review focusing on evidence of causality concluded that malnutrition was likely to predispose to diarrhea but that there was no conclusive evidence to support the hypothesis that diarrhea is a major cause of permanent growth faltering in whole communities (Briend 1990).

The recognition that diarrhea can be associated with intestinal mucosal injury suggests a mechanism. Recent application of non-invasive tests of mucosal integrity e.g. dual sugar intestinal permeability test, has permitted the study of the relationship between

growth and mucosal injury. 119 rural Gambian infants aged 2 – 15 months had their growth measured and had intestinal permeability assessed monthly until 15 months of age during which time diarrheal morbidity was also recorded (Lunn 1991). All were breast fed until 3-4 months of age during which time their growth approximated to the 50th NCHS centile but by 14 months both height and weight had fallen to the 5th centile. Intestinal permeability was strongly related to mean monthly weight and length growth and predicted 39% and 43% of the observed faltering in weight and length. Growth depressing intestinal permeability values occurred for 76% of the time yet the infants had diarrhea for only 7.3% of time. The intestinal mucosa of these children was abnormal for most of the time and the authors concluded that this was more likely to be due to gastrointestinal infection than malnutrition. Poor intestinal repair after injury was also demonstrated in 20 Gambian infants with persistent diarrhea and malnutrition who had no significant improvement in intestinal permeability one month into rehabilitation (Sullivan 1992).

1.2.5. Prenatal influences

Intrauterine growth has a significant influence on post-natal growth and must be considered in the assessment of growth during infancy. Birth size is a reflection of gestational age, with those born premature, but with an appropriate weight for gestation, usually demonstrating normal postnatal growth. Babies with a low weight-for-gestational age are termed light-for-dates. The relative degree of retardation of linear and ponderal growth can suggest causation. Those infants with length affected less than weight usually have a good postnatal growth prognosis and reflect a short term insult to intrauterine growth in late pregnancy, e.g. placental insufficiency. The baby with length and weight equally affected, however, reflects a more chronic process and the prognosis for postnatal growth is much poorer. Chronic fetal undernutrition, chronic maternal illness or malnutrition, toxin ingestion e.g. alcohol, tobacco, or genetic abnormalities can lead to proportionally small babies.

Growth hormone (GH) secretion is high during fetal life but does not influence linear growth of the fetus. GH deficient children are only 1-2 cm shorter on average than normal infants at birth (Karlberg 1988). GH receptors are present in cartilage but may be immature and whether this small discrepancy in linear growth is a result of this immaturity or a secondary metabolic action of fetal GH remains to be established. Anencephalic and athyroid fetuses do not demonstrate growth retardation indicating that both pituitary GH and thyroid hormone are not vital determinants of intrauterine growth (Vorherr 1982). Placental factors e.g. lactogen and somatomedins, may well influence intrauterine growth and need further study. Placental size and function clearly influence birth weight.

The incidence of low birth weight (<2500g) in 1995 was 15.3% or 21.3 million newborns worldwide of which 20.4 million were born in developing countries (De Onis 1998). IUGR (defined on the basis of birth weight below the 10^{th} percentile of the birth weight for gestational age reference curve - De Onis 1996) in developing countries alone represents 30 million newborn infants per year or 23.8% of births. It is associated with increased mortality, and the strength of the association is greatest in the neonatal period but also extends post-neonatally. Infants weighing 2000-2499g were approximately four and ten times more likely to die in the neonatal period and two and four times more likely to die in the post-neonatal period than those born weighing 2500-2999g and 3000-3499g respectively (Ashworth 1998). Light for dates babies can demonstrate catch-up growth post-natally. Data from developed countries indicates partial catch-up growth can occur by two years of age and thereafter maintain that achieved place in the growth distribution until adulthood. Achieved adult sizes are on average 5 cm shorter and 5 kg lighter than controls (Martorell 1998). In developing countries where the postnatal environment might be less favorable the effect is similar in absolute terms. The influence of body mass index (BMI) at birth for growth retarded children on subsequent catch-up growth is unclear. However birth length and predicted target height (a function of mid-parental heights indicating genetic potential) influences catch-up growth and explained half of the variation in catch-up growth in one study (Luo 1998). The effect of birth length predominated up to two years of age and thereafter target height dominated up to eight years. Pubertal catch-up growth in these children was small and was not influenced by fetal experience. Overall the difference in final height of these children was primarily attributable to the difference in the magnitude of catch-up growth

during the first six months of life, confirming that this is the critical period for catch-up growth.

1.2.6. Infancy phase of the ICP model

Infant birth size is linked to that of mother rather than that of father whose influence on growth becomes more apparent with time. If there is a large disparity between the size of father and mother then the likelihood increases that the baby's growth will cross growth reference centiles either up or down. By the age of two years growth 'converges to the mean' and genetic potential predominates as its principal determinant, and the centile trajectory leading to mid-parental height is achieved.

The infancy phase of growth represents a continuation of the fetal phase with a rapid deceleration of growth until three years of age when the childhood phase of growth predominates. Growth during infancy may continue to be influenced by the same factors that determined growth in utero, with nutrition pre-eminent. Human milk has long been recognized as the optimal food for babies, and complementary feeding is generally recommended as starting at around six months (World Health Organisation 1995). Longitudinal studies of infant growth in developing countries indicate that stunting occurs between the ages of four months and two years (see below), coinciding with the transition from breast feeding to complementary foods. The energy density of weaning foods has therefore been proposed as a factor in the etiology of stunting.

The timing of this transition has recently been questioned (World Health Organisation 1998) and is of considerable importance, particularly in developing countries. The

increased risk of disease associated with the introduction of microbial contaminated solid foods must be balanced against the risk of malnutrition from prolonged exclusive breast-feeding – 'the weanling's dilemma' (Rowland 1978). For a sound recommendation as to the optimal timing of complementary feeding an understanding of the growth of breast-fed babies is required.

The common observation of apparent growth faltering of breast-fed babies beginning at three to four months is based on the use of reference growth curves that were constructed from data from predominately formula-fed infants. Breast-fed babies have a different shaped growth curve, and gain less weight in the first year of life than formula-fed babies. Gaining less weight however does not necessarily imply that breast fed infants are not meeting their energy or nutrient requirements. Studies confirm that breast-fed infants have significantly lower energy intakes than formula-fed (Butte 1984) but this is not due to inadequate maternal milk production. Breast-fed babies regulate their own milk intake (Dewey 1986) and may not consume all the milk in the breast during a feed. Despite lower energy intakes and weight gain babies exclusively breast-fed to four to six months of age have similar motor development and lower rates of infection than those formula-fed (Dewey 1991). Thus NCHS growth curves may reflect the 'overfeeding' of formula-fed babies rather than the 'underfeeding' of breast-fed, and the growth of breast-fed babies in developing countries may be more appropriately compared with that of breast-fed babies of affluent populations.

Weight gain of breast-fed babies in developing countries is similar to that of breast-fed babies from more affluent populations (Hijazi 1989) to six months of age although

attained weight differs due to differences in birth weights. Ponderal growth faltering thereafter occurs at six months in those predominantly but not exclusively breast-fed and at nine months in those exclusively breast-fed to six months (World Health Organisation 1994). Linear growth however is poorer and breast fed babies from developing countries are generally shorter than those from developed countries. Infant linear growth is not exclusively a function of nutrition and when maternal height was controlled for the difference in length between Honduran and U.S. breast-fed babies disappeared (Cohen 1995).

It seems therefore that the growth rate of breast-fed infants in developing countries is similar to that of breast-fed infants of more affluent populations. Even if growth faltering occurs complementary feeding may not improve growth. The introduction of hygienic pre-cooked complementary food at four months of age did not improve growth before six months of age in a study of breast fed babies of low-income primiparous mothers in Honduras (Cohen 1994).

The timing of the introduction of complementary feeds however cannot be made on consideration of dietary intakes and growth alone. Infant morbidity, mortality and development as well as maternal considerations must all be included. It may well be that the optimal age of transition varies between populations. In affluent populations the benefit: risk ratio for complementary feeding at a particular age will differ from that of a population in a developed country, owing to the lower risk of contaminated complementary feeds.

Being born short or becoming short in the first two years of life is similar in terms of increased risk of adult shortness and thus it is not necessary to know whether a child was born short or not in order to predict adult height. (Luo 1998)

1.2.7. Childhood phase

The childhood phase describes the slowly declining growth rate throughout childhood into adolescence. Its onset is usually between six and twelve months of age, it is abrupt and probably represents the beginning of the influence of GH on linear growth. In children with isolated GH deficiency, this abrupt onset is lost (Karlberg 1988). Long bone growth is particularly dependant on GH and makes up the majority of growth in the childhood phase compared to the infancy phase when truncal growth accounts for the majority of linear growth (Karlberg 1990).

The trigger for the onset of the childhood phase is not understood but it is influenced by growth rate immediately prior to onset (Karlberg 1987). If a normal child has a small infancy component then onset is early compensating for poor gain. The age of onset will influence attained height subsequently – growth attained between six months and three years of age is negatively related to the age of onset (Karlberg 1990).

Late onset of the childhood phase is common in populations of children with disturbed growth patterns e.g. malnourished children from developing countries or children with a chronic disease such as coeliac disease (Karlberg 1988). This delay causes growth faltering and has been proposed as a determining factor in attainment of final height, particularly in developing countries (Karlberg 1994). The reasons behind this delay in onset remain to be identified but as the incidence of faltering clearly reflects socio-economic conditions it seems that environmental factors are more important than genetic. This view is supported by the observation that the growth rates of well-off members of society in these same developing countries do not

falter or show delayed onset of the childhood phase. This phase and especially the first 6 months is the critical phase for catch-up growth (Luo 1998).

In the second year of life growth reflects the influence of both the infancy and childhood components. Seasonality, for the first time, affects linear growth, which fluctuates more in those populations with a delayed onset of the childhood component (Karlberg 1987). As the effect of the infancy phase disappears in the third year growth trajectory becomes more stable.

1.2.8. Reversibility of Wasting and Stunting

Ponderal catch-up growth is relatively easy to achieve in malnourished children through appropriate dietary rehabilitation and can be spectacular. Rates of 10-20 g/kg/day can be generated – up to 10 times the normal rate of gain in the under two-year old age group (Hoare 1996). The optimal macro and micronutrient content of rehabilitation diets has long been debated. Not only must pre-existing deficiencies be corrected but energy, protein and micronutrient content must match the potential for rapid growth – if any one constituent is limiting growth may falter. Recent WHO recommendations summarize the requirements for energy, protein, potassium, sodium, zinc, copper as well as other minerals and water and fat soluble vitamins (World Health Organisation 1999). The exact requirements of severely malnourished children for many of the micronutrients in particular and when to administer them remains to be clarified.

Linear catch-up growth is much more difficult to achieve. It can occur but its potential is limited, in particular, by the severity and length of the nutritional insult, the age at which it occurs and the age at which the potential for catch-up growth occurs.

In general babies are born with the same mean length between and within populations of diverse socio-economic backgrounds. The process of stunting seems to occur between the ages of 6 and 18 – 24 months and is associated with a delay in the onset of the childhood phase of growth.

If a child in a developing country survives the critical growth period up to two years of age, then locally available foods with adequate energy density, and the development of the child's immunity to local pathogens should allow the child to continue to grow at a normal velocity thereafter. The child will often remain at that baseline level of stunting, however, and demonstrate only marginal catch-up growth. The ability to catch-up linear growth has been demonstrated in studies of children adopted into better socio-economic conditions (Proos 1993). Even this catch-up growth in later childhood however is incomplete, and the effect on pubertal timing and final adult stature is not clear. The vast majority of children in developing countries stunted at two years of age will be left with a degree of stunting until adulthood (Golden 1994).

Nutritional interventions in the critical period (Schroeder 1995) of the first two years of life however can generate catch-up growth and reverse stunting. In Guatemala a study of food supplementation demonstrated a differential effect at different ages with only those under three years of age demonstrating catch-up growth and the linear

growth of those three – seven years not affected. The effect of nutritional interventions on stunted adolescents, their age and duration of menarche, and their final attained height is not known. However isolated reports point to the potential for catch-up growth even at this stage. Historical data on black American slaves of the 19th century demonstrate marked stunting until these slaves reached working age i.e. 15 – 17 years old. Thereafter they were given a food supplement to facilitate work and demonstrated marked catch-up growth (Steckel 1987) with the females attaining final adult heights around the 35th NCHS centile at 25 years of age.

The potential for catch-up growth will depend both upon the environment; e.g. dietary macro and micro nutrient supply, patterns of morbidity and the predetermined height potential for that individual child. Height potential is genetically predetermined and normally reflects the parental heights in well-nourished populations. Comparing height potential for individual populations across the world it is clear that differences are primarily due to environmental factors rather than genetic. However parental height is not a reflection of genetic potential if children are born to stunted parents. Animal experiments demonstrate that the progeny of rats that have been nutritionally restricted are small and even when their offspring have been adequately nourished it will take three generations or more for them to attain their true height potential (Stewart 1980). Thus it will probably take several generations of an optimal nutritional environment for the offspring of stunted parents to attain full genetic height potential. This process of environmental regulation of genetic potential is not clearly understood.

In conclusion, the critical period for linear growth is under two years of age and significant nutritional insults before this age are likely to have profound and long term effects on growth. Ponderal growth is relatively easy to achieve but nutritional interventions designed to promote linear growth must be initiated early and be sustained. An understanding of the ICP model of linear growth and the relative contributions of each phase of growth underlies this. Stunting is not a benign adaptation to chronic nutritional insufficiency but has serious consequences in terms of general and reproductive health, school performance and intelligence and adult work capacity.

1.3. Zinc

Zinc is essential for all forms of life. Its single oxidation state enables it to hydrolyse bonds such as those involving amino and carboxyl groups and enables it to form stable complexes with sulphur and nitrogen atoms. This allows for both the stabilisation of proteins and the facilitation of interactions both between proteins and with steroids or nucleic acids. It has structural and regulatory roles in many enzymes, signal transduction pathways and in gene transcription and is thus essential for metabolism, growth and reproduction. It plays a central role in protein, energy, carbohydrate and lipid metabolism, nucleic acid and haemoglobin synthesis, connective tissue turnover, tissue synthesis and gene transcription.

Up to 2 gm of zinc is present in an adult man but most (95%) is locked away in pools from which it cannot rejoin the circulation and influence plasma levels eg muscle and bone. Small plasma and liver pools are accessible and labile and act as the only reserve available in dietary deficiency. Zinc homeostasis is thus dependent on dietary intake and the average man has an intake of 10mg per day. It is absorbed throughout the small intestine involving specific and non-specific carrier mediated processes. Distal intestinal reabsorption of zinc is very important as there is a large entero-pancreatic circulation and the intestinal secretion of zinc can be at least twice the amount consumed daily. During episodes of deprivation zinc homeostasis depends on the liver and intestine with an increase in intestinal absorption and a decrease pancreatico-biliary secretion. Regulation of intestinal uptake is unclear but a defect at mucosal level is believed to underlie the inherited zinc deficiency disease acrodermatitis enteropathica. Meat is a good dietary source of zinc with plants also potentially good sources. However the presence of phytates in plants in practice often

limits the availability of zinc from vegetable sources. Zinc is absorbed efficiently from breast milk but content (μmol/l) falls with time. Colostrum had a mean content of 71.9 to 176 μmol/l at 7 days, 44.3μmol/l at 1 month, and 7.6μmol/l at 7 months (Casey 1989).

Zinc deficiency is often difficult to recognise as there are no characteristic clinical symptoms or signs associated with it. The classic features of isolated zinc deficiency were first recognised in Acrodermatitis enteropathica - a rare autosomal recessive syndrome of zinc deficiency that usually presents in the neonatal period with growth failure, diarrhoea and a circumorificial, retroauricular rash with acrodermatitis.

Zinc deficiency most commonly results from inadequate dietary intakes of bioavailable zinc. Zinc deficient populations were first described in Egypt and Iran (Prasad 1991). Subsequently deficiency has been reported from numerous developing countries particularly in S.Asia and S.America as well as from certain deprived socio-economic groups in more developed countries such as Canada (Gibson 1989) and the U.S. (Walravens 1989). Populations with high levels of existing malnutrition, prolonged breast feeding and high phytate containing diets should be viewed as at risk of zinc deficiency. Simmer et al (Simmer 1990) measured the daily intake of zinc in breast fed Bangladeshi infants aged 1, 2, 6, 9, and 12 months and found them to be between 10 to 30% of the National Academy of Sciences recommended dietary allowances. Important dietary sources of zinc include red meat, sea food and unprocessed cereals and nuts, but Brown et al (Brown 1982) demonstrated that 50% of Bangladeshi children had never received meat, fish or eggs by 30 months age.

Zinc deficiency is an important public health problem. A meta-analysis of 25 supplementation studies in Latin America & the Caribbean (n=8), N.America & Europe (n=8), Africa(n=3) and Asia (n=5) has shown a highly significant effect of zinc supplementation on both ponderal and linear growth in children less than 13 y old. Linear growth was only improved in children stunted initially and the magnitude of the effect on ponderal growth was inversely related to the plasma zinc concentration at entry. The authors concluded that in those settings with high rates of stunting and/or low plasma zinc concentrations, programs to enhance zinc status should be considered to improve children's growth (Brown 1998).

Zinc deficiency rarely occurs alone and as its clinical features are not pathognomonic it is often not recognised other than as part of a global macro/micronutrient deficiency. However zinc can be a limiting factor particularly in anabolic states. Rehabilitation from severe malnutrition can be associated with spectacular ponderal growth rarely seen in any other physiological situation but linear catch-up growth has been more difficult to demonstrate and a permanent height deficit (stunting) often remains (Khanum 1998). Severity, duration and timing of the nutritional insult determine whether stunting ensues with those insults under 2 years of age causing maximal impairment of height velocity (Costello 1989). The promotion of linear catch-up growth in the stunted malnourished child is poorly understood but requires a prolonged period of ponderal catch-up often not achievable in the environment in which the child is recovering.

The control of linear growth and its relationship to ponderal growth is also not well understood. Walker and Golden (Walker 1988) examined catch-up growth in the early

phase of rehabilitation from severe malnutrition retrospectively in a group of 369 malnourished, hospitalised children to assess influences on linear growth. The subjects of their study did not demonstrate linear catch-up, but a subgroup, who were more stunted initially, did. Two-thirds of the group grew linearly only when they had attained 85% weight-for-length and they speculated that the diets were limiting in one or more nutrients essential for linear growth, such as zinc. They subsequently demonstrated that zinc promotes deposition of lean tissue in this period, which may influence linear growth potential (Golden 1992). Lean tissue synthesis increases when zinc is supplemented to severely malnourished children in addition to their rehabilitation diets. In Jamaica this same group showed that zinc supplementation reduced the energy cost of growth of severely malnourished infants on a soya-based high energy feed by favouring lean tissue deposition (Golden 1981).

The recommended dietary allowance of zinc for a one year old is 5mg/day (National Academy of Sciences 1989). A child recovering from severe malnutrition however is already deficient and in need of a higher dietary intake during the recovery process due to often spectacular rates of weight gain. Both the bioavailability of dietary zinc and the continuing losses in these children (due to the high prevalence of diarrhoea) also contribute to difficulty in estimating requirements in this group.

Supplementation trials carried out on malnourished children under three years have employed a diversity of regimens, and led to a variety of outcomes (table 1). Sempertegui (Sempertegui 1996) et al used 10 mg/d for 60 days and did not report any significant ponderal or linear growth effects. Schlesinger et al (Schlesinger 1992) used 2 mg/kg/day for 105 days and reported significant effects on linear growth only.

Behrens at al (Behrens 1990) used 3- 4 mg/kg /d for 14 days in children recovering from diarrhoea and again reported significant effects on linear growth only. Khanum at al (Khanum 1988) used 10mg/kg/d for 21 days and reported significant effects on ponderal growth and did not report on linear growth.

Zinc deficiency is associated with poor immune function - particularly cellular immunity (Beisel 1982; Chandra 1984; Chandra 1991; Fraker 1982; Pekarek 1979) and zinc supplementation of malnourished children improves immune function (CastilloDuran 1987; Schlesinger 1992; Sempertegui 1996). Lymphoid atrophy, decreased delayed cutaneous hypersensitivity responses, reduction in numbers of T helper (TH) cells and deficient thymic hormone activity have been described in association with zinc deficiency. B cell dysfunction, and specifically impairment of phagocytosis, has also been described. This occurs through a down regulation of basic biological functions at cellular level including DNA synthesis, RNA transcription, cell division and activation. The secretion of cytokines is affected and free radical injury potentially increased as zinc also functions as an anti-oxidant and cell membrane stabiliser. Mild zinc deficiency was induced in a group of male volunteers and resulted in reduced serum thymulin activity and a reduction in the ratio of CD4/CD8

Table 1: Zinc Studies – dosage regimen and outcomes

Investigator	Year	Country	Subjects	Zinc regi
Bates CJ	1993	The Gambia	RDBT 110 children between 0.57 and 2.3 years	70mg twice we 1.25 years
Behrens RH	1990	Bangladesh	RDBT 64 malnourished children between 3 and 24 months with acute diarrhoea	3-4mg/kg/d for
Castillo-Duran C	1987	Chile	RDBT of 32 marasmic infants	2mg/kg/d for 6
Gibson RS	1989	Canada	DB pair matched study of 60 stunted boys aged 5-7y	10mg/d
Golden MHN	1981	Jamaica	16 malnourished children 6-16 months	69 micomoles per litre of feed
Halstead JA	1972	Iran	17 adults between 18 d 20 years with poor linear growth	27mg zinc dail
Hambridge KM	1985	USA	107 children with poor linear growth between 2 and 6 years of age	0.2-0.3 mg/kg/
Hemalatha P	1992	India	RDBT of 33 malnourished children aged 1-5 y	6mg/kg/d
Ronaghy HA	1974	Iran	49 boys prepubertal received 40 mg zinc day and control groups	40mg/d for 18
Simmer K	1988	Bangladesh	25 children with PEM 1-7 y	50mg/d for 2 w
Walravens PA	1989	USA	50 infants RDB pair matched 8-27mths of age with poor linear growth	6mg /d for 6 m
Walravens PA	1983	USA	DB, pair matched study of 40 children between 2 and 6 years	5 mg/d for 1 ye

Cavan KR	1993	Guatemala	DB study of 162 school children mean age 81 months	10mg/d for 25
Nakamura T	1993	Japan	21 prepubertal children with poor linear growth	5mg/d for 6 mc
Shrivastava SP	1993	India	Pair matched controlled study of 30 children with mild PEM aged 8-24 mths	5.6mg/d for 3 r
Schlesinger L	1992	Chile	DBCT of 39 marasmic children mean age 7 mths	2mg/kg/d for 1
Walravens PA	1976	USA	DBCT of 68 full term normal infants	4mg/l of infant
Khanum	1988	Bangladesh	60 children with PEM between 5 and 60 months	10mg/kg/d for
Sempertegui	1996	Ecuador	RCDBT of 50 children with PEM aged 1-6y	10mg/d for 60

RDBT- randomised double blind trial
RCDBT – randomised, controlled double blind trial
DBCT – double blind controlled trial
DB – double blind
NSD – no significant difference
SD – significant difference

lymphocyte ratio (Prasad 1998). IL(interleukin)-2 production was also reduced in the T-helper cells of the zinc deficient volunteers but production of IL-4,5,6,and 10 was unaffected implying an imbalance between TH 1 & 2 functions – this is a potentially important pathway by which zinc deficiency affects cell-mediated immunity. The percentage of children under 3 yrs in India who remain anergic/hypergic to a battery of skin tests after 120 days of zinc supplements decreased from 67 to 47% and was associated with a significant rise in CD3, CD4 and the CD4/CD8 ratio (Sazawal 1997).

Diarrhoea frequently accompanies malnutrition whether as an important cause or result of. Diarrhoea usually persists longer in malnourished children due to associated gut mucosal damage and cell-mediated immune malfunction. Zinc supplementation of 937 children with acute diarrhoea in New Delhi resulted in clinically important reductions in the duration and severity in infants and young children (Sazawal 1995). Supplementation of children in India resulted in a significant reduction in the incidence of persistent diarrhoea in children >1 y old, and those with a low plasma zinc as well as on dysentery in boys (Sazawal 1996).

Zinc has also been recently noted to improve cognitive function in Chinese school children. 720 children aged 6-9 y old were given 20mg zinc supplements daily in a 10 week double blind controlled trial with improvement in cognitive and neuropsychologic testing as well as growth (Sandstead 1998).

The available evidence points to zinc deficiency as an important public health problem in both developing and developed countries (Sandstead 1995).

1.4. Collagen formation, degradation and osteoblastic activity

Bone is an active tissue undergoing continuous remodelling such that 10% of the adult skeleton is replaced each year. Linear growth is dependent on skeletal growth, which occurs when osteoblastic bone formation predominates over osteoclastic bone resorption. Bone is continually remodelled and longitudinal bone growth during childhood is dependent on GH (Nilsson 1994). The study of the biochemical markers of bone and collagen turnover allows for a better understanding of the physiology of growth as well providing a mechanism to describe both the pathological processes which disrupt and therapeutic interventions designed to promote growth.

Collagen comprises 90% of the organic matrix of bone and is produced by mesenchymal-derived osteoblasts. It is unique among proteins in the number of post translational modifications it undergoes. This includes among many others the hydroxylation of proline and lysine residues in the rough endoplasmic reticulum to form hydroxyproline and hydroxylysine. Certain hydroxylysine residues are then glycosylated to form O-linked glycosides. Collagen is synthesised as procollagen which is about 50% larger than the final or fibrillar collagen due to extension polypeptides at each end of the molecule. Association of the three procollagen chains and winding up of the helix is driven by the formation of disulphide bonds within the C terminal propeptide. The helical molecule is then transported to the Golgi apparatus where carbohydrate additions are made to the non-helical sections. The molecule is then secreted actively and the N and C terminal propeptides are removed by specific proteases. The helical molecules then spontaneously associate into fibrils in a quarter staggered array. The final extracellular modification is the oxidative

deamination of specific lysine or hydroxylysine residues which determines the tissue specificity of the resultant collagen molecule – lysine oxidation in skin leads to the formation of histidine based cross-links and hydroxylysine oxidation in cartilage and bone leads to the formation of pyridinium based crosslinks

There are currently about 15 types of collagen described. Collagen type 1 is the main constituent of bone and skin and collagen type 3 is essentially absent from bone but is widely distributed in most soft tissues. The principal product of the mature osteoblast is type 1 collagen that makes up 90% of protein in bone.

The pyridinium crosslinks formed in the final stages of type 1 collagen maturation are good candidates as markers of insoluble collagen degradation as they are quantitatively excreted in urine either free or peptide bound. Pyridinoline (PYD) has a wide distribution other than just in bone e.g. cartilage, ligaments and tendons but since these other pool sizes are relatively small they contribute little to urinary output. Its analogue deoxypyridinoline (DPD) is more specific to bone and shows less variance than PYD in urine. It has been found in other tissues but since their turnover is so slow then the presence of DPD in urine can be regarded as specific for bone (Robins 1994).

Collagen type 1 cross linked C telopeptide (1CTP) is a PYD and DPD containing peptide located at the C-intermolecular site of the collagen fibril. Serum levels correlate with bone resorption (Calvo 1996). Urinary DPD is currently regarded as the best overall test of bone collagen breakdown but as it is difficult to collect timed urine samples from children random urines are often used and excretion of markers is

expressed in relation to creatinine. As this reflects muscle mass a further element of variability is introduced (Robins 1994) and plasma or serum levels of 1CTP are used in an attempt to overcome this.

The carboxy-terminal propeptide of type 1 collagen (P1CP) is one of the propeptides cleaved extracellularly during formation of fibrillar collagen and serum level reflects type 1 collagen synthesis. P1CP is not metabolised but rapidly cleared intact by the liver. Although present in skin the mass and turnover of bone is such that most serum P1CP should come from bone and levels are closely related to histomorphometrically assessed bone matrix formation (Eriksen 1993). Indeed serum P1CP predicted response in prepubertal children treated with growth hormone – the percent increase in P1CP after 3 months was correlated to the increase in height velocity at 12 months (Trivedi 1991). Type 3 collagen is more widely distributed and is not present in bone. The N terminal propeptide of type 3 collagen (P3NP) reflects soft tissue formation and has been used also as a growth marker. P3NP response after 5 weeks predicted 6 month growth response to growth hormone therapy (Tapanainen 1988).

Bone specific alkaline phosphatase (BAP) is a zinc containing enzyme associated with the plasma membrane of the osteoblast. It is needed to hydrolyse pyrophosphate, a potent inhibitor of calcium phosphate deposition. It has been found to correlate with 6 month height velocity in short normal children treated with growth hormone (Crofton 1995). Its response to new bone formation is more specific than P1CP but slower as production of P1CP is an early event taking place during proliferation of osteoblast precursor cells and expression of BAP starts after cessation of cell proliferation (Schonau 1997).

1.5. Insulin like growth factor and binding proteins

Linear growth is dependent on skeletal growth, which is the result of chrondocyte proliferation and subsequent endochrondral ossification in the epiphyseal growth plates of long bones. Growth occurs when osteoblastic bone formation predominates over osteoclastic bone resorption. Bone is continually remodelled and longitudinal bone growth during childhood is dependent on growth hormone (GH) (Nilsson 1994). Control of resorption and formation is complex with evidence pointing to the components of the GH/IGF (insulin like growth factor) axis as providing the complexity required to selectively regulate complementary processes to suit demands. GH and insulin like growth factor 1 (IGF1) have different target cells in the epiphyseal growth-plate. GH stimulates slowly dividing prechrondrocytes in the germinative cell layer while IGF1 promotes the clonal expansion in the proliferative layer of the GH primed cell (Nilsson 1994).

IGF's regulated osteoblast and osteoclast proliferation and collagen synthesis in vitro (Rosen 1994) and recombinant IGF1 stimulated bone formation in women (Johansson 1992). Locally produced IGF's acting as autocrine or paracrine agents have been postulated as mediating in the coupling of bone formation to resorption (Hayden 1995; Chihara 1997).

IGF1 is a 7.6kD polypeptide produced both in the liver and locally in multiple cell types in response to a combination of autocrine, endocrine and paracrine factors to promote cell growth. The major regulator of circulating IGF1 is GH (Hynes 1987). Liver produced IGF1 determines circulating levels but skeletally produced IGF1 also contributes. Whether plasma IGF1 reflects local tissue levels is not clear. IGF1 has

short term metabolic actions similar to insulin and longer term mitogenic actions in the epiphyseal growth plate. IGF1 also promotes type 1 collagen synthesis, increases proteoglycan synthesis in chrondrocytes, and inhibits skeletal specific collagenases (Rosen 1994). Plasma levels of IGF1 have been used primarily for evaluation of GH-secretion but do show some circadian rhythm.

Insulin like growth factor binding proteins (IGFBP's) transport IGF's, control tissue specific availability, regulate clearance, modulate receptor interaction with the IGF's and have a direct effect on cells (Clemmons 1998). There are 6 IGFBP's described currently and they are found in all physiologic fluids. Their affinity to IGF1 is greater than the IGF1 receptor and they thus act to partition the IGF's among 3 sites – interstitial fluids, IGFBP's bound to cell surfaces or extracellular matrix and type 1 receptors. The relative affinity that the IGFBP's have for IGF1 is regulated by whether the IGFBP is bound, and whether they undergo proteolysis or phosphorylation (Clemmons 1998). IGFBP3 is the principal IGF binding moiety and its concentration is regulated by GH. Among the IGFBP's it circulates in the highest concentrations and it has the highest affinity for IGF1. IGFBP3 exists in the circulation as a 150kD complex with an acid labile 85kD glycoprotein. Release of IGF1 from this complex is thought to occur following a lowering of pH and is mediated by IGFBP proteases. As IGFBP3 levels modulate IGF1 availability they might reflect GH secretion better than IGF1 however both are currently used for such. IGFBP2 circulates in concentrations of approximately 1/10 of IGFBP3 and its concentration is inversely regulated by GH (Smith 1993) such that on administration of GH there is an increase in IGF1 and concomitant decrease in IGFBP2.

Nutritional factors are important regulators of the GH-IGF1 axis and malnutrition leads to a state of GH resistance (high GH, low IGF 1 and high IGFBP2 levels) which may represent an adaptive mechanism to promote lipolysis and fatty acid oxidation whilst attenuating the anabolic actions of GH on protein synthesis (Soliman 1986). Animal studies demonstrate that nutrient availability regulates GH action at multiple levels with reduced GH binding capacity, post GH receptor defects, reduced IGF1 gene transcription and translation demonstrated in fasted or protein restricted rats as well as increased IGF1 clearance (Thissen 1991; Thissen 1990; Maes 1988). IGF1 and IGFBP2 decrease rapidly in rats in response to nutritional insults whilst IGFBP3 concentrations decline only in prolonged fasting or protein deficiency (Lewitt 1994; Maes 1983).

IGF1 levels in children declined in response to 6 days of protein or energy restriction with an associated increase in IGFBP2 in protein restriction only (Smith 1995). Levels of IGFBP3, the principal carrier of IGF1, are more consistently reduced in humans by prolonged malnutrition along with IGF1 (Ketelslegers 1996). When malnourished Bangladeshi children recovering from Shigellosis were fed either a normal protein (6% of total dietary energy) or high protein diet (12%), better weight gain, higher IGF1 levels and a larger decrease in IGFBP2 levels were noted in the high protein group (Pucilowska 1993). IGFBP2 is highly dependant on dietary protein intake and may be useful in monitoring the response to refeeding in children who have been ingesting suboptimal amounts of protein (Smith 1995).

1.6. Knemometry

Knemometry is the measurement of the distance between the knee and the heel and was first described by Valk in 1983 (Valk 1983). The technique has been proposed as the technique of choice for short term growth studies as it allows for accurate and repeatable measurements of lower leg length. It is a robust, sensitive and precise technique. The estimated technical error (mean coefficient of variation) for measuring standing height with a stadiometer has been assessed as 2.0-3.0mm (Voss 1991) whilst Valk described a technical error of 0.09-0.16mm with a fixed knemometer. Subsequent to this portable knemometers have been developed with technical errors of 0.27mm (Davies 1996). The lower leg however does not grow smoothly and variations in growth rate are seen in healthy children with a marked day-to-day variation of lower leg length that exceeds the error of the measurement itself (Hermanussen 1988). Lower leg growth is pulsatile and consists of sharp growth spurts alternating with periods of decreased growth velocity (Lampl 1992) (Hermanussen 1988). The sensitivity of this technique has also raised concerns that knemometry also detects changes in soft tissue. Daily fluctuations in weight were weakly associated to fluctuations in knemometric length in children over 2.4y (Hermanussen 1988). In infancy and early childhood however long bone growth is maximal with a height velocity of about 50cm/year in the first few months of life. The accuracy of this technique and this growth rate in infancy allow for accurate assessment of lower leg growth over days and weeks during this period.

Knemometric length does display a diurnal variation (Valk 1983) and physical activity prior to measurement does lead to a significant decrease in lower leg length (Hermanussen 1988). Knemometric growth can occur at a steady rate but also in

spurts and shrinkage has been demonstrated which might be associated with catabolic stress (Wales 1987).

Lower leg growth is dependent on long bone growth and lower body segment growth makes up to 84% of total linear growth in the first few years of life (Karlberg 1990). However knemometers do not predict long-term total linear growth very well (Wales 1987). The correlation coefficient between annual height velocity and a 1 month knemometric increment was 0.07, and with a 3 month increment was 0.437 and with a 6 month increment was 0.73 (Dean 1990). In 12 girls with Turner's syndrome treated with GH a 1 month increase in lower leg length had only a 50% positive predictive value in total linear growth (Wales 1989). Total linear growth is also dependant on the growth of short bones in the spine and different parts of the skeleton appear to grow at different rates and times (Roche 1974). Knemometry is also more technically difficult than height or length assessment.

Knemometry has found a niche in assessing short-term responses to therapeutic interventions. Asthmatic children aged 7-14 ys old given inhaled beclomethasone dipropionate had significant suppression of lower leg growth rate compared to children given inhaled fluticasone (Wolthers 1997). Growth hormone deficient children had significant increases in lower leg length 24hrs after a single dose of human growth hormone (Hermanussen 1985).

Chapter 2: Methods

2.1. Location

The study was part of a collaborative effort between the Bangladesh Institute of Child Health and the University of Edinburgh, Scotland. It took place at the Nutritional Rehabilitation Unit (NRU) of the Dhaka Shishu Children's Hospital in Dhaka, Bangladesh. Bangladesh has an estimated 120 million people, 17 million of whom are under 5 years old. 86% of the population live in absolute poverty i.e. that income level below which a minimum nutritionally adequate diet and essential non-food requirements are not affordable. Extreme and widespread poverty is manifest in the infant mortality rate and national nutrition indicators. 91 per 1000 children die in infancy and 67% of the under 5 population are moderately or severely underweight i.e. (>2 standard deviations from median NCHS weight for age) (UNICEF 1996).

The hospital is the only paediatric hospital in Dhaka – a city of 13 million, and the unit is the biggest of its kind in the city. The hospital is locally run and financed – 40% of its beds are 'pay' beds generating the revenue required to finance the other 60% 'free' beds. These beds are open to anyone who presents unwell to the hospital with only a minimal charge. Due to the overwhelming clinical need however the free beds run at 100% occupancy and financial resources are always stretched. Each unit within the hospital also had a degree of autonomy to raise money that it could spend on itself. As there were rarely any children of the 'better-off' social groups admitted to the Nutritional Rehabilitation Unit this resulted in the NRU having relatively fewer resources than the other hospital units.

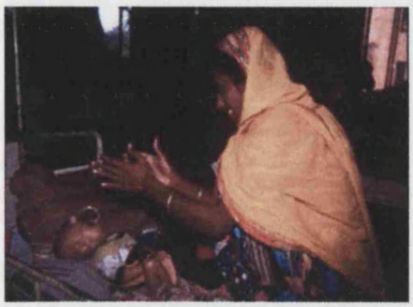
The NRU had 20 beds and was continually full. Severely malnourished children were admitted directly from the 'out-patient' department that effectively operated as an accident and emergency unit or from other hospital units. The unit was run by a senior dietician, with input from 3 hospital physicians and nursing support. The first 4 months of the project involved the development and introduction of an agreed clinical protocol for the management of the severely malnourished child as detailed below to be used by all staff.

The average length of stay on the unit was 11 days and reflected the intense social pressure on the mothers not to remain in hospitals as many were working particularly in the garment industry. This had led to the use on this unit of 'aggressive' refeeding strategies using nasogastric (NG) tubes to increase energy intakes during the initial phase of anorexia associated with severe malnutrition and sepsis.

Nasogastic feeding of anorexic and often septic severely malnourished children

Defined discharge criteria were not developed nor was follow-up arranged or health education promoted. Under the agreed protocol daily health education discussion sessions were introduced, a follow-up clinic was established and discharge guidelines were promoted.

Oxygen, broad-spectrum antibiotics and both oral rehydration and intravenous fluids (the use of which was discouraged) were available on the unit. Stool microscopy and culture, chest Xray, haemoglobin estimation, plasma sodium, potassium, protein and glucose estimation were all available on the unit between the hours of 0900-1200 only.



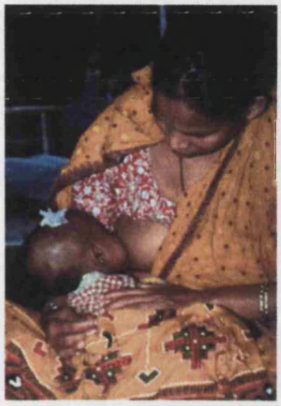
Mothers were integral to the process of nutritional rehabilitation being responsible for basic care, feeding and stimulating the children as well as taking part in the health education sessions.

Mothers were resident with their children sleeping in the same bed as them and were integral to the philosophy of the unit being responsible for basic care and feeding and invited to a health education discussion everyday.

Health education discussion groups

The ethos of nutritional rehabilitation should involve a significant element of health education particularly where children are likely to be discharged quickly back into the same environment in which they became malnourished. This involved a 15-day rolling programme of 45 minute group discussion sessions led by a dietician on a

variety of topics including breast feeding promotion, healthy weaning, cooking, food choices and availability, immunisations, diarrhoea management and the use of oral rehydration solution as well as family planning.



Breast-feeding was actively promoted as part of the health education discussions

Breast-feeding was actively promoted on the unit and health education aimed to encourage the effective nutritional use of local dietary resources available to feed children.

Discharge guidelines included a suggestive minimum of 15 days in hospital , the disappearance of oedema and anorexia, the establishment of catch-up growth without the use of an NG tube, and no evidence of sepsis. Close out-patient follow-up was arranged to ensure continued catch-up growth, to monitor morbidity, to re-enforce health education and ensure completion of the Bangladesh EPI (expanded programme of immunisation) schedule.

2.2. Study children

141 children, who fulfilled the criteria below, were enrolled sequentially from those admitted to the unit over a 13 month period from November 1995 till November 1996. All were aged between 6 and 36 months, with a weight-for-age less than 60% of the NCHS median for age and/or had nutritional oedema. They were clinically stabilised within a week after admission (i.e. acute complications of severe malnutrition being treated and able to commence and tolerate the oral nutritional rehabilitation regimens). Children in whom there was a strong suspicion of underlying tuberculosis (i.e. those children with a tuberculosis contact history and a history of prolonged temperature or cough) were not recruited. All children lived within two hours travelling distance of the hospital, and their carers agreed that their child would remain in hospital for a further 15 days and be followed up for total of 90 days.

Selection criteria

1. Aged 6 to 36 months
2. Weight for age < 60% of NCHS median and/or nutritional oedema
3. Within 1 week of admission to hospital
4. Able to tolerate enteral feeds
5. No clinical suspicion of tuberculosis
6. Live within 2 hrs of the hospital
7. Agreed to follow-up period.
8. Fully informed consent (see below)

2.3. Clinical management protocol

Mortality rates among severely malnourished children vary between units with reports of up to 60% mortality among oedematous malnourished children. A recent review pointed to faulty case management with inappropriate diets high in protein, energy and sodium and low in micronutrients commonplace (Schofield 1996). The WHO has encouraged the use a clinical protocol based approach with the usage of standardised diets and antimicrobials encouraged and as recently produced such a suggested protocol (World Health Organisation 1999).

On recruitment all children were examined and investigated for infection as deemed clinically appropriate. All were treated with broad spectrum antibiotics (usually Ampicillin and Gentamicin) if not already receiving them. If there was a history of invasive diarrhoea either nalidixic acid or mecillinam were prescribed (reflecting the local sensitivity patterns of *Shigella*) and skin sepsis was treated with cloxacillin. They then received liquid dietary regimens with gradually increasing energy densities and protein contents. The volume of feed offered on a particular day depended upon type of malnutrition, the presence of diarrhoea and number of days after recruitment.

The liquid diet used was dried skimmed milk-based (264 kJ, 2.2g protein and 0.3 mg zinc / 100ml) unless diarrhoea was present when a rice-based (259 kJ, 1.1g protein and 0.3mg Zinc / 100ml) was substituted (see appendix 1). These were delivered in two hourly feeds initially at 80 mls/kg/day in oedematous malnutrition or at 120 ml/day in non-oedematous malnutrition with incremental steps of 15mls/kg/day up to 200mls/kg/day over the course of their in-patient stay. These liquid diets were administered by gravity feed through a nasogastric tube initially until appetite

improved enough for children to take the full volume offered by mouth. Breast-feeding was encouraged and solid food was offered *ad libitum*. All subjects on admission were given vitamin A (retinyl palmitate - 200,000 i.u if over 1 year and 100,000 iu if under) and on recruitment were given daily doses of a multivitamin supplement (3000 iu vit A, 30mg vit C, 600 iu vit D, 0.96 mg thiamine, 0.6mg riboflavin, 0.6 mg pyridoxine and 6mg of nicotinamide). Iron supplementation of acutely unwell severely malnourished children has previously be shown to be dangerous (Smith 1989) and was used only in response to an iron deficient blood film taken on day 30 of the trial.

Days 1-15 consisted of in-patient intensive nutritional rehabilitation and health education. The mothers were responsible for primary care of their children and slept with them at night. They collected and dispensed the liquid feed every 2 hours from the nursing staff as well as being responsible for the basic hygiene of the child. The nursing staff administered the ward, dispensed medication and diets and recorded clinical observations. The ward nutritionists were responsible for the feeds and recorded anthropometric and knemometric observations as well as compiling an individual data file on each patient. They also organised and ran a health education programme which consisted of nutritionist led discussion groups for 45 minutes each day on a rotating series of topics e.g. promotion of breast-feeding, healthy weaning, treatment of diarrhoea and use of oral rehydration solution, promotion of vaccinations, family planning and cooking.

Whilst every effort was made to standardise feeding regimens it was recognised that estimated energy intakes were inaccurate. As part of the health education component

of rehabilitation encouraging the mums to use locally available food resources effectively was stressed. Solid foods offered to the children were chosen so that they could both be cooked and resourced locally by the mothers. As a result consistent energy and protein contents of the solid foods in particular were impossible to maintain.

2.4. Randomisation and Zinc supplementation

Stratified randomisation into three zinc supplementation regimens was performed by an independent observer using variable length blocks within six strata generated by age (<13 months and 13-36 months) and type of malnutrition (marasmus, marasmic kwashiorkor and kwashiorkor as defined by the Wellcome classification).

The regimens were chosen to reflect the diversity of current opinion of the zinc requirement of the malnourished child (see introduction – table 1) based on previously published trials

1. Regimen 1: 1.5 mg/kg/day (mg of elemental zinc per kg body weight of zinc sulphate) for 15 days followed by placebo for 15 days,
2. Regimen 2: 6.0mg/kg/day for 15 days followed by placebo for 15 days
3. Regimen 3: 6.0mg/kg/day for 30 days.

A placebo group was not included after extensive consultation in Bangladesh. The majority of malnutrition rehabilitation units had very recently decided that the evidence for benefit was such that randomising malnourished children to placebo was unethical.

The zinc sulphate and placebo suspensions were indistinguishable and both were formulated and provided by Ciba-Geigy, Bangladesh (see appendix 2). All bottles were identical and labelled sequentially 1-300. On recruitment to the study 2 bottle numbers were provided by the independent observer and the corresponding bottles were then selected for that patient and labelled as either bottle A for days 1-15 (either 1.5mg/kg or 6.0mg/kg) and bottle B for days 16-30 (either 6.0mg/kg or placebo). The

mothers were instructed on how to administer the supplement using labelled syringes which they continued to use at home up to day 30.

2.5. Discharge and follow-up

There is tremendous pressure on mothers to take their children out of hospital as soon as possible in Dhaka for economic reasons. Also when the child's acute infection is treated there is often poor recognition of malnutrition as a problem in a society where malnutrition is endemic. Prior to commencement of the study the median length of stay was 11 days. No outpatient follow-up was organised as attendance had been poor and the hospital authorities felt that scare resources could be better spent.

Our subjects were discharged on day 15 on agreed discharge guidelines. Specifically these stipulated that the child should remain an inpatient until at least day 15, the disappearance of oedema and anorexia, the establishment of catch-up growth without the use of an NG tube, and no evidence of sepsis.

A prospective study of 437 severely malnourished children followed up for 1 year in Dhaka demonstrated that morbidity was high, with a mean of seven episodes of diarrhoea during the year (Khanum 1998). Outpatient visits for diarrhoea occurred for 67% of children, and 58% had pneumonia (10% had pneumonia three times). It was concluded that 'the high prevalence of illness highlights the need for continued accessible health care and for interventions to reduce disease acquisition'.

We instigated a follow-up clinic after discharge. To encourage attendance a study nurse would visit homes of discharged children if they had missed an appointment.

Cost of rickshaw journey was re-imbursed to families on attendance. We planned to follow-up all children on days 21, 30, 45, 60, 75, and 90 when anthropometric and knemometric data were recorded and health education re-enforced. All children were examined, infections treated and completion of vaccination schedules promoted. The last blood and urine sample was collected on day 30.

2.6. Ethical approval and informed consent

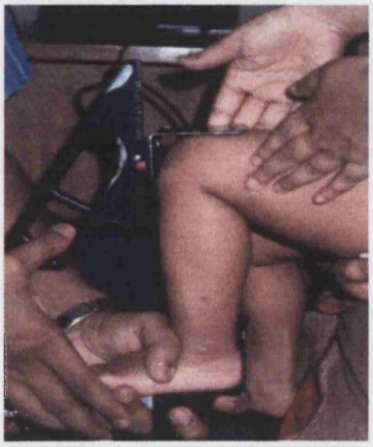
The study was approved by the Paediatrics and Reproductive Medicine Research Ethics Sub-Committee of Lothian Health, Edinburgh, the Ethics Committee of the International Centre for Diarrheal Disease Research, Bangladesh and the Dhaka Shishu Children's Hospital management committee.

Informed consent was obtained from the primary carer of each child. As most carers could not read a standard statement was read out to them in Bengali describing the nature, aims and methods of the study (see appendix 3). It was made clear to the carers that the care of their children would not be affected if they declined to take part in the study. If they agreed to take part then they signed/marked a consent form which was in Bengali and was read out to them. It was also made clear that they could withdraw from the study at any time and that the care of their child would not be affected. Strenuous efforts were made to ensure that the carer understood all of the above.

2.7. Anthropometry

During the in-patient phase body weight was recorded daily, knemometry on alternate days and all other anthropometric variables on days 1, 8, and 15. During follow-up all

nutritional measurements were recorded together on the morning of follow-up. Weight was recorded on an electronic scales (Seca model 835, Todd Scales Ltd, Newmarket, UK) with graduations to 20 grams, and all length measurements were performed supine on a Rollameter (Raven Equipment Ltd, Great Dunmow, Essex, UK) with graduations to 1mm. Skinfold thicknesses were measured with calipers (Holtain Ltd, Crosswell, Dyfed, UK) graduated to 0.2mm and a standard non-stretch tape-measure was employed to measure mid upper arm circumferences with graduations to 1mm. Knemometry is the measurement of the distance between the child's knee and heel measured here with the child sitting and the knee held in flexion of 90 degrees (photo 1). The knemometers were manufactured by the medical physics department of the Royal Infirmary Edinburgh and measured to 0.1mm. Two observers undertook all of the knemometry, skinfold and mid upper arm circumference measurement after an eight week training period. Five knemometric readings were taken at each assessment and the mean was accepted unless the standard deviation was greater than 1mm (chosen to represent a coefficient of variation of 1% of the average knee-heal length in this group). Weight and length were measured by a team of four nurses and four nutritionists who undertook an eight week training course before the study commenced. All staff involved in anthropometric data gathering were subject to regular, unscheduled, formal assessments of measurement technique.



Knemometry

2.8. Biochemical assays

Samples were collected on recruitment, days 15 & 30 and every effort was made to co-ordinate with routine clinical investigations. All blood and urine samples were taken between 0900 and 1100 hours. Plasma (1ml) and serum (1ml) were separated off immediately and stored with urine (3mls) at –20°C. All assays were carried out either in the Department of Biochemistry of the Royal Hospital for Sick Children, Edinburgh or Department of Pathological Biochemistry, University of Glasgow.

CRP was measured by an in-house ELISA assay on Nunc Immuno 1 micrititre plates, using DAKO rabbit anti-human CRP antibodies and calibrated with DAKO Human CRP calibrator. The samples were incubated overnight in plates pre-coated with anti-human CRP. After washing a sandwich was formed by adding anti-human CRP-HRP conjugate followed by a second wash and addition of a chromogenic substrate. After 15 mins incubation the reaction was stopped and the colour read. The precision was 6-8% at values of 0.2-9.9mg/l. Plasma Zn/Cu was determined by an in-house atomic emission method (inductively coupled plasma – atomic emission spectroscopy). The precision ranged from 2-5% at concentration of 4-15 micromol/l.

IGF1 was measured in plasma using a specific RIA (Mediagnost, Tübingen, Germany). This assay employs an excess of IGF2 to eliminate interferences by IGFBPs (Blum 1994). The between-assay coefficient of variation was <9%. IGFBP3 was measured in plasma using a specific RIA as described (Blum 1990). The between-assay coefficient of variation was <8%. IGFBP2 was measured in plasma using a specific RIA (Elmlinger 1996). Recombinant human IGFBP-2 (a gift from Sandoz, Basel, Switzerland) was used as standard and tracer. The between-assay

coefficient of variation was <11%. PICP, ICTP and P3NP were measured in plasma by RIA (Orion Diagnostica, Espoo, Finland). Before analysis, samples were diluted appropriately in 154 mmol/L sodium chloride to achieve concentrations within the calibration curve; typical dilutions were 1:4 for PICP and 1:2 for ICTP and P3NP. All samples were analysed in duplicate. Samples from each patient were analysed in a single analytical run to minimise analytical variation. Within- and between-run coefficients of variation were <5% and <8% for PICP, <6% and <10% for ICTP, and <6% and <7% for P3NP. Bone ALP was measured in plasma by an enzyme-linked immunosorbent assay (Alkphase-B™; Metra Biosystems Europe, Oxford, UK). This assay utilises a monoclonal anti-BAP antibody coated on a microtitre plate to capture BAP in the samples. Cross-reaction with liver alkaline phosphatase is stated by the manufacturer to be 5%. All samples were analysed in duplicate. Within- and between-run coefficients of variation were <6% and <8% respectively. PYD and DPD were measured in urine by HPLC using a modification of the method of Pratt et al., as described (Pratt 1992), (Crofton 1998). Pyridinium cross-links extracted from demineralised sheep bone (kindly donated by Dr Simon Robins, Rowett Research Institute, Aberdeen, UK) were used as external standards. Results were expressed in relation to creatinine measured on the same urine sample. All samples were analysed in duplicate. Between-run coefficients of variation were 6% for PYD and 9% for DPD.

2.9. Reference data

Reference ranges of normality were limited on many of the biochemical markers of collagen and bone turnover as they were relatively new assays. Reference values for all biochemical data were chosen from that published from well nourished European children of similar age. The 95% reference ranges (2.5-97.5 percentiles) and median for IGF1 and IGFBP3 were 17.6-195.9mcg/l and 57.9mcg/l and 967-3224mcg/l and

1878mcg/l respectively. The 95% reference ranges (2.5-97.5 percentiles) and mean for IGFBP2 were 335-748mcg/l and 522 mcg/l respectively (Blum 1996). The reference range and median for P1CP, P3NP and 1CTP were taken from data on Edinburgh children and were 290-1316 and 488 mcg/l, 5.1-22.3 and 11.6 mcg/l, and 6.9-27.7 and 14.5 mcg/l respectively (Crofton 1997). The reference range and median for BAP were 36-100 and 61 units/l respectively (Crofton 1995). The reference range for PYD and DPD were 200-700 and 30-120 nmol/nmol creatinine respectively (Zanze 1997). The inherent problems in comparing biochemical assays of bone and collagen turnover between malnourished Bangladeshi children and well nourished European children of similar age must be appreciated and these reference data are thus only mentioned in the text of the results for comparison.

2.10. Statistics

Sample sizes were calculated with a requirement for 90% power at the 5% level to show a difference between groups for 11 anthropometric and biochemical outcome variables, and a sample size of 60 was chosen because it was at the upper end of the calculated sample sizes (see Appendix 1). Epi-info V6 (Division of Surveillance and Epidemiology, Epidemiology Program Office, Centre for Disease Control and Prevention, Atlanta, Georgia) was used for data recording and generation of z-scores from NCHS reference data. All anthropometric data were dual entered with a validation performed between the two entry records and against the hard copy of the data at the end of the data gathering period.

Data were then transferred to SPSS v8.0 for analysis (Statistical Product and Service Solutions Inc, Chicago, USA). Differences between groups were compared using t-tests or one-way analysis of variance for quantitative variables with approximately

normal distributions, Mann-Whitney or Kruskal-Wallis tests for ordinal variables, and chi-squared tests for categorized variables, with Yates correction used for 2x2 tables. The three treatment groups were also treated as ordinal for outcomes after discharge, and trends were tested using Pearson or Spearman correlations as appropriate. Analysis of covariance was used to test differences in quantitative outcomes between groups after adjusting for other factors. Univariate regression analysis was used to identify predictors of change in e.g. HAZ score and then construct a regression model to explain change.

Chapter 3: Results

3.1. Initial Anthropometry

141 subjects were recruited and baseline characteristics were similar between treatment groups with no significant differences found (table 2). The average age at recruitment was 15.5 months with 57% of the subjects under 1 year old. The subjects were both severely wasted and very severely stunted with an average initial WHZ of -2.66, WAZ of -4.56 and HAZ of -3.89. Numbers of children with kwashiorkor (10-15%), marasmic kwashiorkor (25-30%) and marasmus (55-60%) were equally distributed.

16 subjects either self-discharged as in-patients (n=6), most commonly due to social pressures on the subject's mother preventing her from completing the in-patient phase, or were lost to follow-up (n=10) (Table 3). A follow-up worker visited each dwelling at least twice after a subject defaulted from follow-up. All defaulters could not be found at their homes and information sought from neighbours suggested that internal migration within Bangladesh, and usually back to ancestral family villages, was responsible.

Table 2: Characteristics of children at entry into trial. Figures shown are mean +/- sd or number of patients.

Zinc Group	*1.5 / Placebo*	*6.0 / Placebo*	*6.0 / 6.0*
Number	49	49	43
Age (months)	15.5+/-8.7	15.0+/-9.0	16.3+/-8.6
Weight for Age Z score	-4.47+/-0.91	-4.56+/-0.98	-4.66+/-0.86
Weight for Height Z score	-2.56+/-0.97	-2.73+/-0.90	-2.71+/-0.93
Height for Age Z score	-3.89+/-1.3	-3.79+/-1.4	-3.98+/-1.45
Malnutrition : M	29	27	26
: M/K	15	14	11
: K	5	7	6
Admission to Recruitment (days)	2.5+/-1.5	3.5+/-2.2	2.7+/-1.8

M is marasmus, M/K is marasmic kwashiorkor and K is kwashiorkor.

Table 3: Outcome and mortality rates in the three zinc supplementation regimens

Outcome					
Regimen	Completed Protocol	In-patient Death	Out-patient Death	Self-Discharge / Lost to follow-up	
1.5 / Plac	43	2	0	4	*49*
6.0 / Plac	38	5	3	3	*49*
6.0 / 6.0	25	6	3	9	*43*
	106	13	6	16	*141*

* A comparison of in-patient and out-patient combined death rate between those children who received 6.0mg/kg/d zinc initially (regimens 2 & 3) and those who received 1.5mg/kg/d initially (regimen 1) demonstrates a Yates corrected chi-squared value of risk of death of 4.52 (p=0.03) (95% confidence interval for relative risk of 1.09 - 18.8).

3.2. Mortality

There was an excess of deaths in the groups receiving 6.0mg/kg/day zinc as in-patients (table 3). This trend was identified in an interim analysis of the first 100 subjects, and enrollment was suspended after 141 recruits. When supplementation regimens 2 and 3 were combined and death rates compared throughout the entire 90 day study period against regimen 1, a Yates corrected chi-squared value of risk of death was significant at 4.52 ($p=0.03$) with exposure to 6.0mg/kg/day as against 1.5mg/kg/day initially, and a 95% confidence interval for relative risk of 1.09 - 18.8.

Cause of death was defined by a clinician's impression (Table 4). Most deaths were sepsis-related and 13 out of the 19 deaths occurred as in-patients.

I next considered those children who died in association with exposure to 6.0mg/kg/day initially and tried to identify factors that could possibly predict for increased risk of death (table 5). Combining regimens 2 and 3 I then compared death rates within subgroups depending on whether a specific possible prognostic factor for death was present or absent. Prognostic factors considered were age, degree of wasting and stunting, severity of initial illness, and type of malnutrition, but none of these factors was found to predict death in association with exposure to the higher initial dose of zinc (Table 5).

Due to the increased infection related mortality associated with high dose zinc supplementation, I will next describe the inflammatory status, plasma zinc and copper status of these children. High dose zinc supplementation can produce a copper deficiency (see discussion) that can be related to an immunodeficiency.

Table 4: Clinical impression of cause of death of individual children.

	Subject	Regimen	Details of Death	Day
1	028	1	Sepsis and diarrhoea	7
2	066	1	Sepsis and ARI symptoms	10
3	008	2	Sepsis and ARI symptoms	8
4	033	2	Sepsis - no localizing symptoms	O.P.
5	039	2	Sepsis - ARI and diarrhoea	O.P.
6	048	2	Sepsis and diarrhoea	22
7	056	2	Sepsis - Disseminated intravascular coagulation	2
8	062	2	Sepsis and diarrhoea	7
9	118	2	? Tubercular meningitis	8
10	134	2	Poor weight gain as OP / ARI - refused re-admission	O.P.
11	024	3	Sepsis - no localizing symptoms	
12	067	3	Sepsis and severe anemia	10
13	068	3	Acute liver failure -? pyrazinimide toxicity	O.P.
14	079	3	Aspirated + cardiac arrest - preceding diarrhoea	20
15	087	3	Sepsis - ARI and severe anemia	3
16	088	3	Failure to progress as O.P, developed oedema and sepsis	O.P.
17	103	3	Sepsis - Diarrhoea and ARI	6
18	113	3	?	O.P.
19	125	3	? Abdominal Tuberculosis - Aspirated and cardiac arrest	8

Note : O.P = Out-patient and ARI = Acute respiratory infection

Table 5: Prognostic factors for death among those exposed to higher dosage zinc regimens initially.

	Death rate when factor	
	Present	*Absent*
Age 12 months or less	8/52	9/40
Weight for Age <45% median	4/24 (17)	13/68 (19)
Weight for Height <70% median	9/43 (21)	8/49 (16)
Height for Age < 85% median	9/42 (21)	8/50 (16)
Admission - Recruitment >3days	5/23 (22)	12/63 (19)
Diarrhoea at Adm : any	9/50 (18)	7/33 (17)
: persistent	5/22 (23)	11/68 (16)
Malnutrition type : Marasmus	9/53 (17)	8/38 (21)
: Marasmic Kwashiorkor	6/25 (24)	11/66 (17)
: Kwashiorkor	2/13 (15)	15/78 (19)
Mother's Education < 2yrs	10/60 (15)	8/32 (25)

Note : Adm - admission

Figures are shown as number of deaths / total number (%) where individual factor is present or absent. The combined denominator figure for individual risk factors should equal 92 (i.e. total number of children randomised to Regimens 2 & 3). In some cases the total figure is below 92 where data collection errors prevented the assignment of individual children to 'present' or 'absent' groups.

3.3. Inflammation and plasma zinc & copper levels

Table 6 shows the mean (and standard deviation) plasma zinc, copper and C-reactive protein (CRP) concentrations for the children in each regimen for days 1, 15 and 30. Table 7 shows the differences in zinc, copper and CRP between regimens. Both mean zinc (normal range 12-18micromol/l) and copper (normal range 10-22 micromol/l) were on the lower limit of normal on day 1 and there were no significant inter-regimen differences.

Mean plasma zinc concentrations of regimens 2 & 3 on day 15 were significantly different from those of regimen 1. Children in regimens 2 & 3 received 6mg/kg zinc until day 15 as opposed those children in regimen 1 who received 1.5 mg/kg. Day 30 zinc concentrations had again increased in regimen 3 and were significantly higher than those in regimens 2 or 1, which had declined to below day 1 concentrations. Regimen 3 children had continued high dose supplementation onto day 30 whilst both regimen 1 and 2 children had ceased supplementation at day 15.

Plasma copper concentrations rose consistently throughout the 30 day period and there were no significant differences between any regimen at any day. Both zinc and copper concentrations are illustrated in fig 1.

Mean CRP concentrations and number of abnormal (defined as above 10 mg/l) measurements decreased over the 30 day period (table 6). There were no significant differences initially between groups (table 7) but combining regimens 2 & 3 and

comparing them against regimen 1 at day 15, there was a significant difference in number of abnormal CRP measurements (table 9) but not in mean CRP.

There were no significant differences in either plasma zinc or copper concentrations between survivors and non-survivors but there were significant differences in initial CRP concentrations (table 8).

There was a significant association between risk of death and elevated CRP at Day 1 (table 9).

Table 6: Mean (standard deviation) plasma zinc (micromol/l), copper (micromol/l) and CRP (mg/l) concentrations and percentage of CRP measurements greater than 10 for each regimen on specified days.

	Day1			Day 15			Day 30		
Regimen	1	2	3	1	2	3	1	2	3
(n)	n=49	n=47	n=43	n=40	n=41	n=34	n=40	n=39	n=30
Zinc	12.28	13.19	12.16	13.7	16.19	18.5	12.02	10.64	19.63
	(7.05)	(4.94)	(4.8)	(5.48)	(6.11)	(8.86)	(5.67)	(3.07)	(10.73)
Copper	16.19	13.71	13.19	18.5	17.12	17.78	21.82	21.47	19.1
	(7.07)	(5.3)	(4.05)	(6.16)	(6.06)	(5.95)	(5.71)	(6.93)	(5.29)
CRP	15.65	12.11	18.63	2.79	9.97	5.03	4.36	6.43	5.5
	(24.7)	(15.99)	(27.5)	(2.99)	(21.29)	(8.07)	(7.06)	(12.02)	(12.36)
CRP>10	34.6	36	37	2.5	19.5	21.4	12.1	12.8	13.3

Figure 1: Zinc and copper plasma concentrations

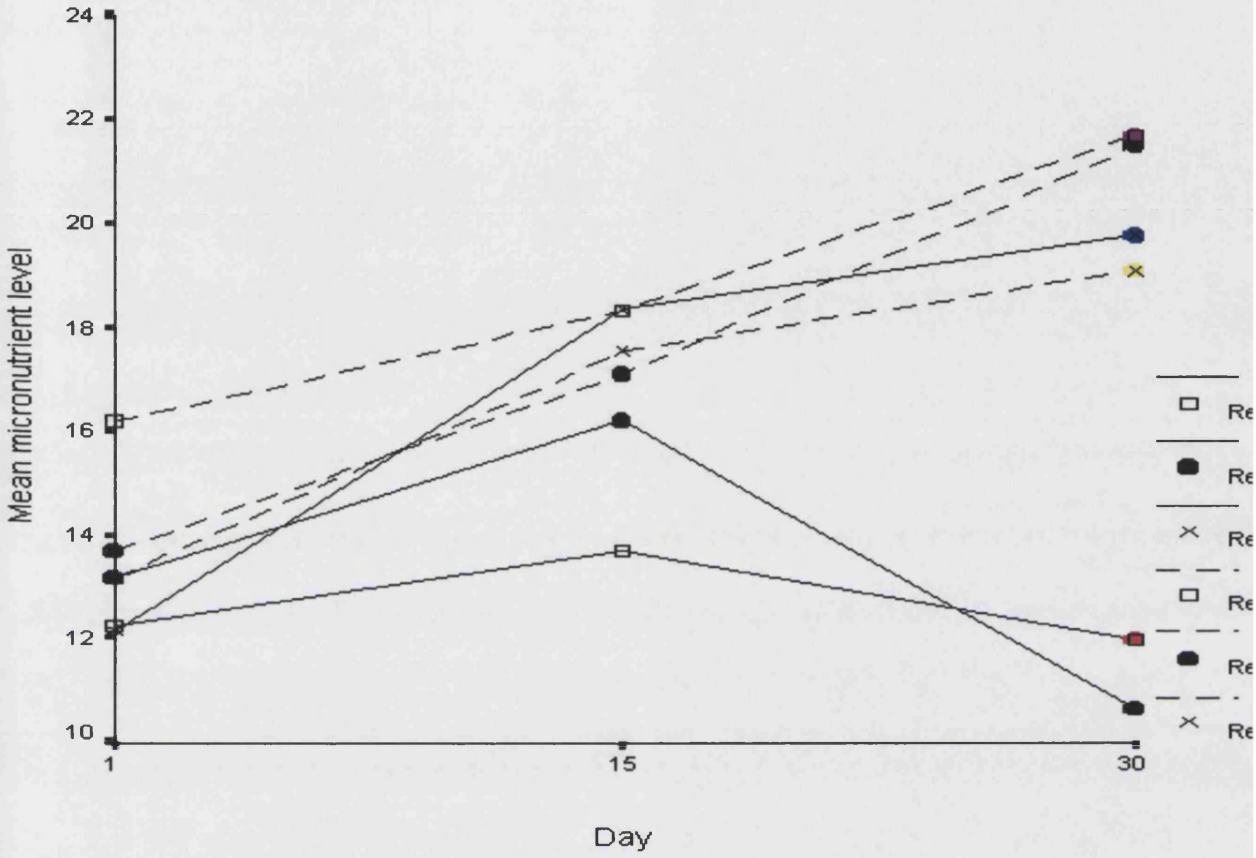


Table 7: Comparison of mean plasma zinc, copper and CRP concentrations between regimens on specified days.

	Day 1*	Day 15**	Day 30**	
		Regimen 1 / 2&3	Regimens 1/ 2	Regimens 2/3
Zn	p=0.23	p=0.002	p=0.22	p=0.000
Copper	p=0.14	p=0.41	p=0.68	p=0.2
CRP	p=0.93	p=0.23	p=0.3	p=0.53

* Kruskal-Wallis 1-Way Anova

** Mann Whitney U

CRP is C reactive protein

Table 8: Initial zinc, copper and CRP concentrations in those children who died compared with survivors.

	Survivors	Deaths	Differences
	n = 122	n = 19	between groups *
Day 1: mean (s.d.) zinc	12.7 (5.77)	10.7 (3.01)	p=0.28
Day 1: mean (s.d.) copper	14.4 (5.99)	14.3 (5.18)	p=0.48
Day 1: mean (s.d.) CRP	13.9 (22.04)	25.1 (28.29)	p=0.008

* Kruskal-Wallis H

Table 9: Chi-square contingency table of days 1 & 15 C reactive protein concentration compared to regimen and occurrence of death.

		Regimen				Death		Chi-Sq	p value
		1	2	3	2&3	No	Yes		
D1 CRP	Normal	32	31	27					
	High	17	17	16				1.95	0.74
D15CRP	Normal	39			61				
	High	1			14			6.01	0.049
D1 CRP	Normal					85	5		
	High					37	13	17.99	0.000

3.4. Ponderal, linear & knemometric growth

There were no significant differences in change of any anthropometric variable between regimens (table 10). Over the 90 days, significant catch-up growth was achieved with an average intragroup improvement of WHZ score of 1.54 to 1.67 and in HAZ score of 0.44 to 0.49 units. Lower leg length grew on average 1.03 to 1.04 cm in the 90 days (table 10).

Neither employing an initially higher dosage (regimen 2) nor prolonging supplementation into the outpatient phase (regimen 3) had any significant effect on promoting further weight gain. Initial WHZ score correlated significantly negatively and inpatient WHZ score change correlated significantly positively with subsequent ponderal growth over both 45 and 90 days (table 11). Over 40% of the children were admitted with nutritional oedema that would have influenced changes in anthropometric indices during the inpatient period. Correlations between changes in inpatient anthropometric indices and subsequent changes over both 45 and 90 days were thus repeated in only those children with marasmus. The correlations noted above in the group as a whole were demonstrable in this smaller subgroup.

Day 1 measurements and change in measurements are compared for 3 age groups in table 12. Those in the 6-12 month age group were less wasted initially and had significantly better linear growth in the second half of the study.

Table 10: Change in anthropometric variables during 90 days of study protocol for each supplementation group.

				95% C.I. for mean difference	
	1.5 / Plac n=43	*6.0 / Plac n=38*	*6.0 / 6.0 n=25*	*6.0/Plac-1.5/Plac*	*6.0/6.0-6.0/Plac*
WAZ	1.35+/-0.69	1.51+/-0.65	1.45+/-0.66	-0.27, +0.52	-0.47, +0.38
WHZ	1.54+/-0.93	1.67+/-0.78	1.62+/-0.86	-0.14, +0.46	-0.39,+0.27
HAZ	0.44+/-0.32	0.48+/-0.38	0.49+/-0.27	-0.11, +0.2	-0.17, +0.18
LLL	1.04+/-0.48	1.03+/-0.49	1.03+/-0.33	-0.23, +0.2	-0.22, +0.22
SFT	3.06+/-1.94	3.63+/-1.87	3.61+/-1.86	-0.29, +1.43	-0.97, +0.94
M.U.A.C	1.66+/-1.4	1.98+/-1.17	1.9+/-1.38	-0.26, +0.89	-0.72, +0.57

Note : WAZ - Weight for Age Z score, WHZ - Weight for Height Z score, HAZ - Height for Age Z score, SFT - Skinfold thickness', M.U.A.C - mid upper arm circumference, C.I.- confidence intervals

Figures shown are mean+/- sd. Knemometry (LLL) and MUAC are in cm whilst SFT is in mm

Table 11: Correlation between initial and inpatient change in anthropometric indices versus total change.

	Linear growth		Knemometric growth		Ponderal Growth	
	D1/45 HAZΔ	D1/90 HAZΔ	D1/45 LLLΔ	D1/90 LLLΔ	D1/45 WHZΔ	D1/90 WHZΔ
D1WHZ	0.33 ***	0.36 ***	0.15	0.2 *	-0.25 *	-0.28 **
D1 HAZ	-0.17	0.03	-0.13	-0.07	-0.18	-0.09
D1 LLL	-0.1	-0.2 *	-0.12	-0.13	-0.01	-0.00
D1/15 WHZΔ	0.1	-0.03	0.47 ***	0.25 *	0.51 ***	0.27 **
MD1/15WHZΔ	0.22	0.16	0.41 **	0.27 *	0.33 **	0.11
D1/15 HAZΔ	0.28 **	0.24 *	-0.06	-0.05	-0.16	-0.12
MD1/15 HAZΔ	0.36 **	0.16	-0.08	-0.002	-0.03	-0.04
D1/15 LLLΔ	0.07	-0.06	0.64 ***	0.25 *	0.21 *	-0.03
MD1/15LLLΔ	0.22	0.12	0.61 ***	0.33 *	0.21	-0.08

Knemometric growth is LLL (lower leg length)Δ.

D'x' 'anthropometric variable' : specified anthropometric reading on specified day

D 'x/y' 'anthropometric variable' Δ : change in specified anthropometric variable between specified days

MD'x/y' 'anthropometric variable' : change in specified anthropometric variable between specified days in those children with marasmus only

$p<0.05$, $**p<0.01$, $***p<0.001$

Note: Day 1/15, Day 1/45 and Day 1/90 are not independent variables.

Table 12: Baseline anthropometry and knemometry (mean (SD and number of subjects)), type of malnutrition and growth (mean change (SD and number of subjects)) in 3 age groups of study subjects.

Age Group	6-12 months	13-24 months	25-36 months
Marasmus	47	24	11
MarasmicKwashiorkor	21	12	8
Kwashiorkor	12	5	1
Day 1 WHZ †	-2.3 (0.9, n=80)	-3.0 (0.9, n=41)	-3.1(0.7, n=20)
Day 1 HAZ	-3.6 (1.1, n=80)	-4.1 (1.6, n=41)	-3.9 (1.1, n=20)
Day 1 LLL	15.8 (1.3, n=80)	18.0 (1.6, n=41)	20.7 (1.7, n=20)
Days1/45 WHZ Δ	0.99 (0.8, n=65)	1.51 (0.6, n=29)	1.16 (0.9, n=17)
Days 1/45 HAZ Δ	0.18 (0.2, n=65)	0.12 (0.1, n=29)	0.19 (0.2, n=17)
Days 1/45 LLL Δ	0.46 (0.3, n=65)	0.56 (0.3, n=29)	0.47 (0.3, n=17)
Days 45/90 WHZ Δ	0.53 (0.7, n=61)	0.3 (0.7, n=26)	0.33 (0.5, n=16)
Days 45/90 HAZ Δ ‡	0.38 (0.3, n=61)	0.22 (0.2, n=26)	0.19 (0.2, n=16)
Days 45/90 LLL Δ	0.58 (0.3, n=61)	0.45 (0.4, n=26)	0.53 (0.4, n=16)
Day 90 WHZ §	-0.74(1.1, n=61)	-1.28(1.1, n=26)	-1.63(0.8, n=16)
Day 90 HAZ ¶	-2.99(1.0, n=61)	-3.93(1.8, n=26)	-3.7 (1.0, n=16)
Day 90 LLL ¶	16.9 (1.2, n=61)	18.6 (1.9, n=26)	21.6 (1.5, n=16)

Days 'x'/'y' is day y - day x

† ANOVA between age groups p=0.000

‡ ANOVA between age groups p=0.003

§ Paired samples t test between day 1 and 90 p=0.000

†† Paired samples t test between day 1 and 90 p=0.000

††† Paired samples t test between day 1 and 90 p=0.000

Linear catch up growth was also demonstrated (table 10). Again there were no significant differences between regimens with most catch-up growth occurring during the second half of the study. Knemometric growth (table 10) occurred mostly in the second half of the study and there were no significant differences between regimens.

To examine the relationship between ponderal and linear growth (fig 2) I plotted linear growth over the subsequent 30 days against initial WHZ score for all children at every time point. This demonstrated a strong correlation between WHZ score and subsequent linear growth [$r = 0.3$, $p<0.001$]. Linear growth occurred in the presence of severe wasting and no threshold could be identified. Initial WHZ score correlated significantly with subsequent linear growth over both 45 and 90 days whereas HAZ score did not. Inpatient change in HAZ score but not in WHZ or knemometric length correlated strongly with subsequent change in HAZ score over both 45 and 90 days (table 11) however inpatient change in HAZ score is not independent of total change over 90 days.

Knemometric growth also occurred in the presence of severe wasting but correlated poorly with initial WHZ score (table 11). It correlated well with ponderal indices in the first half of the study and only correlated with linear growth from day 45 onwards (table 13).

Figure 2: Mean change in HAZ score in the subsequent 30 days plotted versus initial WHZ score at that time for data from Days 1, 15, 30, 45, & 60. ($r=0.3$, $p<0.001$)

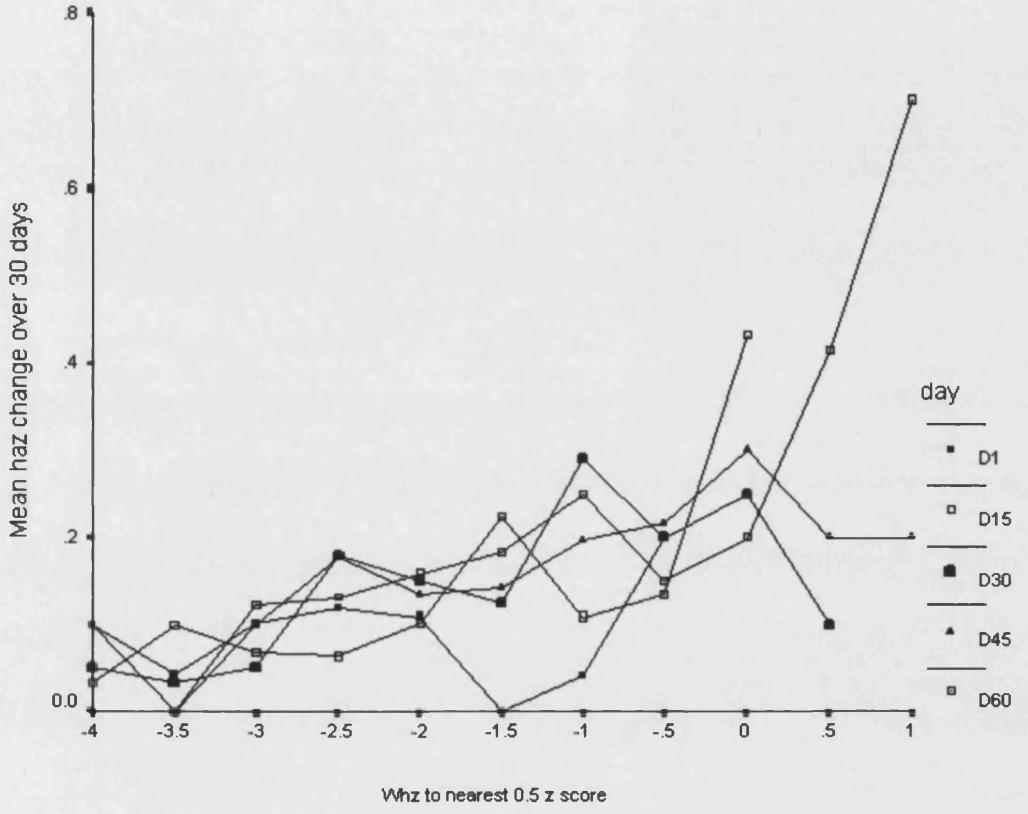


Table 13: Correlation coefficients between changes in knemometry versus changes in other anthropometric indices between Day 1 and subsequent measurement days

Day	T.S.F.T.	M.U.A.C.	WHZ score	HAZ score
15	0.32 ***	0.44***	0.49***	-0.14
30	0.31 **	0.34***	0.51***	0.16
45	0.3 **	0.38***	0.55***	0.19*
60	0.41***	0.43***	0.56***	0.49***
90	0.37***	0.39***	0.48***	0.47***

T.S.F.T = triceps skin fold thickness

M.U.A.C = mid upper arm circumference

* $p<0.05$

** $p<0.01$

*** $p<0.001$

Growth is predominately ponderal initially in severely malnourished children and linear growth occurs when WHZ score approaches the median NCHS reference (fig 2). Univariate regression analysis was used to identify predictors of linear growth and then to construct a linear regression model. Only maternal height and day 1 WHZ score predicted in our model and only accounted for 20% of total variance (Tables 14 & 15).

Figure 3: WHZ and HAZ score increase over 30 days against initial WHZ score (to nearest 0.5 z score) for all time points.

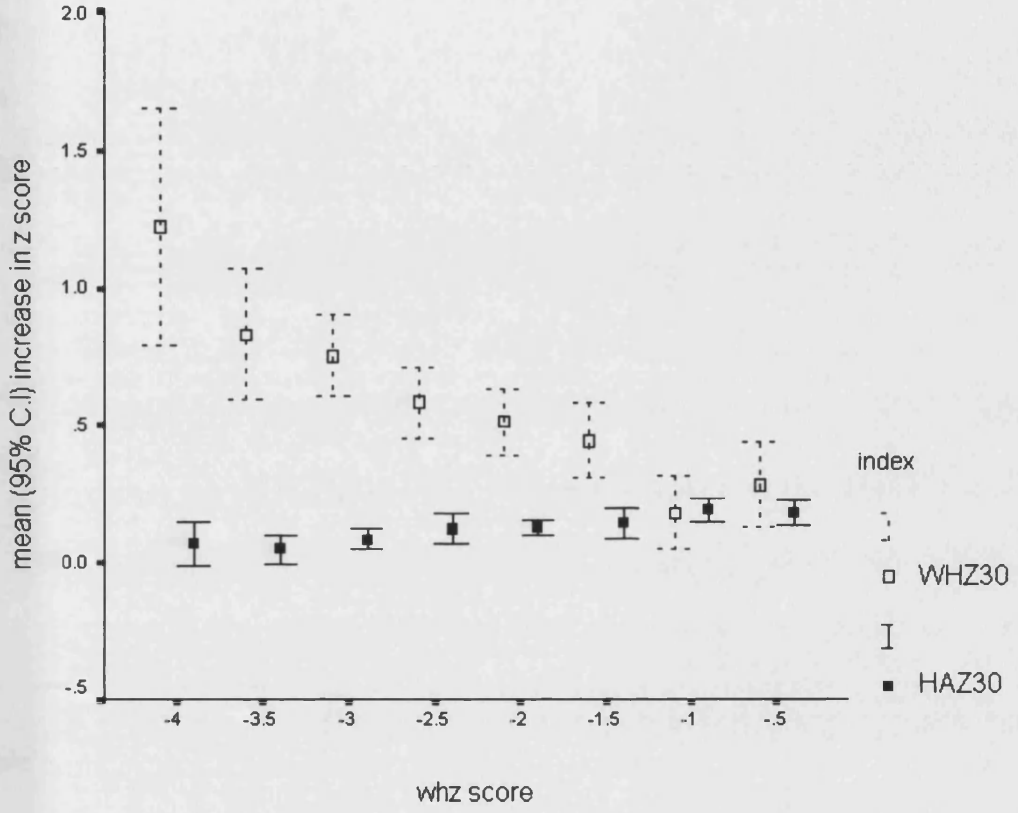


Table 14: Univariate regression analysis for change in HAZ score over 90 days

Sex	y = 0.45 + 0.013 (-0.12, 0.14) x	p = 0.85
Type of malnutrition	y = 0.39 + 0.05 (-0.04, 0.14) x	p = 0.253
Age	y = 0.62 – 0.01 (-0.02, -0.003) x	p = 0.008
WHZ day 1	y = 0.79 + 0.12 (0.06, 0.19) x	p < 0.0001
HAZ day 1	y = 0.5 + 0.01 (-0.04, 0.06) x	p = 0.73
WHZ Δ 1/90 days	y = 0.39 + 0.05 (-0.02, 0.13) x	p = 0.17
Maternal height	y = -1.96 + 1.65 (2.7, 0.01) x	p = 0.009
Maternal B.M.I.	y = 0.11 + 0.02 (-0.01, 0.03) x	p = 0.25

Table 15: Linear regression model of change in HAZ score over 90 days

		Std error	
Constant	-1.49	0.88	
WHZ score day 1	0.122	0.036	p = 0.001
Maternal height	1.54	0.59	p = 0.01

	Sum of squares	df	mean square	
Regression	1.66	4	0.83	p<0.0001
Residual	6.88	70	0.09	
Total	8.5	74		

3.5. Collagen turnover and insulin-like growth factor axis

There were significant changes in all indices of collagen formation and degradation and growth factor activity over the first 15 days (table 16 & fig 4) with inter regimen differences demonstrable only in IGF1 (days 1, 15) and BAP (day 30).

Compared to similarly aged but well nourished European children severely malnourished Bangladeshi children had low insulin like growth factor 1(IGF1) and insulin like growth factor binding protein 3 (IGFBP3) with high insulin like growth factor binding protein 2 (IGFBP2) measurements. They also had low bone alkaline phosphatase (BAP) and variable but high collagen type 1 cross linked telopeptide (1CTP) (fig 4) measurements. On rehabilitation BAP, procollagen type 1 C terminal propeptide (P1CP), procollagen type 3 N terminal propeptide (P3NP), pyridinoline (PYD), deoxypyridinoline (DPD), IGF1 and IGFBP3 measurements all increased associated with a decrease in IGFBP2 and 1CTP measurements (table 16 & fig 4). There were strong positive correlations among BAP, P1CP, IGF1 and IGFBP3 at every time point ($p<0.01$), and between 1CTP and IGFBP2 at every time point ($p<0.01$) (tables 18-20). Conversely IGFBP2 was negatively correlated with P1CP, IGF1 and IGFBP3 at every time point ($p<0.01$) and with BAP on days 15 and 30 ($p<0.05$). 1CTP was negatively correlated with P1CP, IGF1 and IGFBP3 on admission ($p<0.05$). PYD and DPD did not correlate with any other biochemical marker at any time point including 1CTP but did correlate with each other at every time point ($p<0.01$).

Change in P1CP and P3NP correlated significantly with change in IGF1 and IGFBP3 (table 21). Change in BAP correlated significantly with change in IGF1 and P1CP.

Change in PYD correlated significantly with changes in IGF1, IGFBP3 and P3NP. Change in IGFBP2 did not correlate with change in collagen formation, degradation or bone osteoblastic activity (table 21).

Table 16 (a): Collagen synthesis, degradation and osteoblastic activity.

	1.5 / Plac			*6.0 / Plac*			
Day	*1*	*15*	*30*	*1*	*15*	*30*	*1*
P1CP	392.5 (211.3)	805.5 (428.4)	948.1 (437.7)	374.2 (330.9)	739.2 (495.8)	916.2 (557.9)	352.5 (162.1)
P3NP	8.6 (5.5)	20.9 (11.4)	22.8 (12.8)	7.1 (5.0)	18.4 (12.8)	23.0 (21.1)	8.5 (7.3)
BAP	39.8 (20.3)	59.8 (48.6)	78.6 (38.6)	37.5 (17.5)	44.6 (15.1)	59.4 (30.5)	35.6 (17.8)
PYD	484.40 (328.8)	689.58 (466)	777.74 (447.7)	442.29 (282.8)	671.51 (455.6)	711.86 (377.8)	453.67 (289.2)
DPD	86.0 (75.0)	125.6 (97.2)	130.5 (80.7)	75.8 (75.3)	113.5 (94.5)	121.8 (77.4)	80.5 (47.0)

Table 16 (b): Insulin like growth factor axis activity

	1.5 / Plac			*6.0 / Plac*			
Day	*1*	*15*	*30*	*1*	*15*	*30*	*1*
IGF1	9.94 (7.62)	34.27 (25.9)	36.33 (34.29)	6.45 (4.55)	20.24 (16.54)	27.59 (23.61)	7.88 (7.62)
IGFBP2	1712.41 (777.93)	1194.27 (795.9)	1022.77 (442.1)	2157.76 (1378.1)	1434.48 (788.4)	1334.9 (888.6)	1794.57 (1004.8)
IGFBP3	737.9 (461.2)	1749.64 (993.55)	1732.1 (992.9)	657.16 (352.87)	1480.57 (847.1)	1620.8 (959.3)	745.31 (526.97)

Figure 4: Collagen, bone and growth factor markers (mean and 95% C.I.) vs. time. Data are shown separately for the 3 regimens. Reference data for age matched well nourished European children are shown as dashed lines. (a) P1CP, (b) P3NP, (c) BAP, (d) 1CTP, (e) PYD, (f) DPD, (g) IGF1, (h) IGFBP3, (i) IGFBP2.

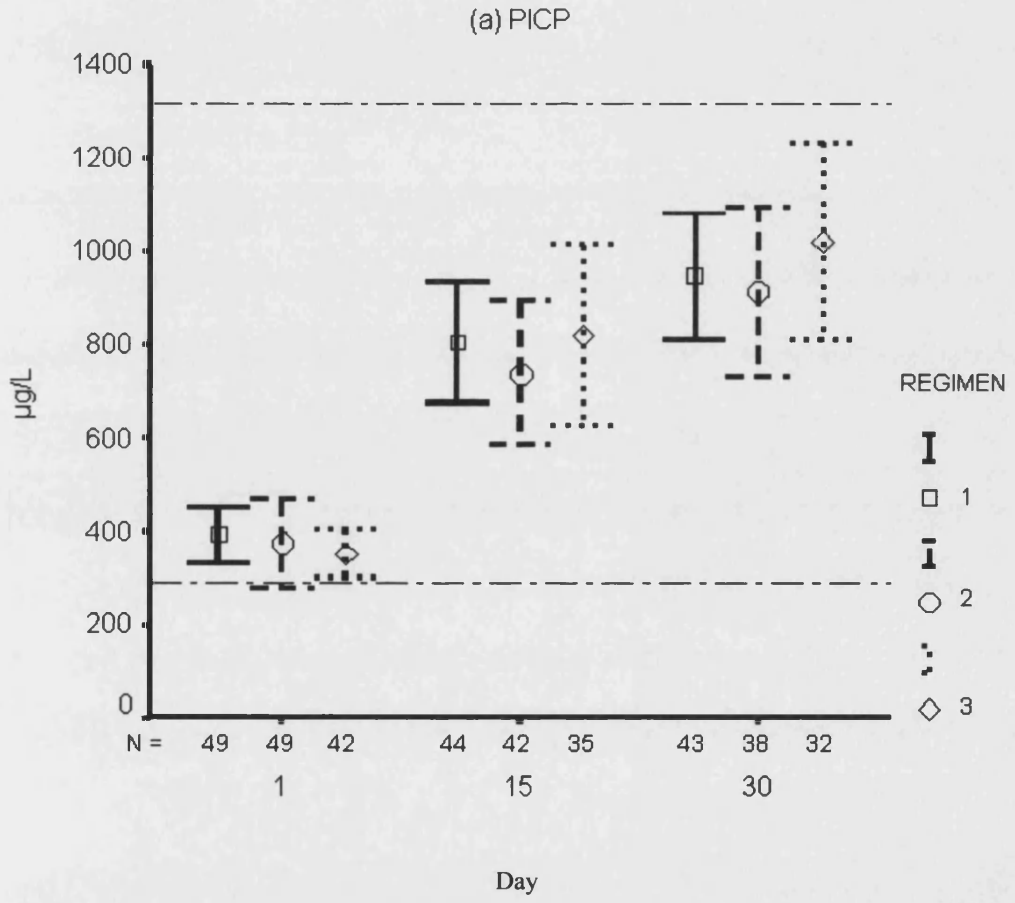


Fig 4b: Collagen, bone and growth factor markers (mean and 95% C.I.) vs. time. Data are shown separately for the 3 regimens. Reference data for age matched well nourished European children are shown as dashed lines

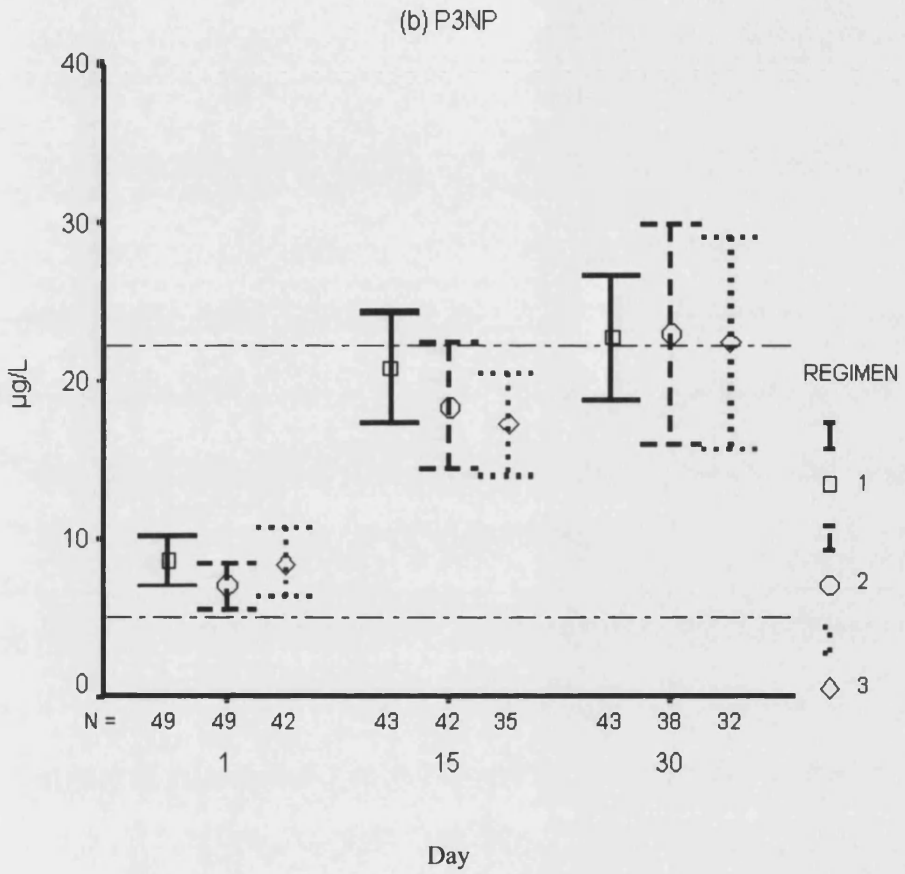


Fig 4c: Collagen, bone and growth factor markers (mean and 95% C.I.) vs. time. Data are shown separately for the 3 regimens. Reference data for age matched well nourished European children are shown as dashed lines

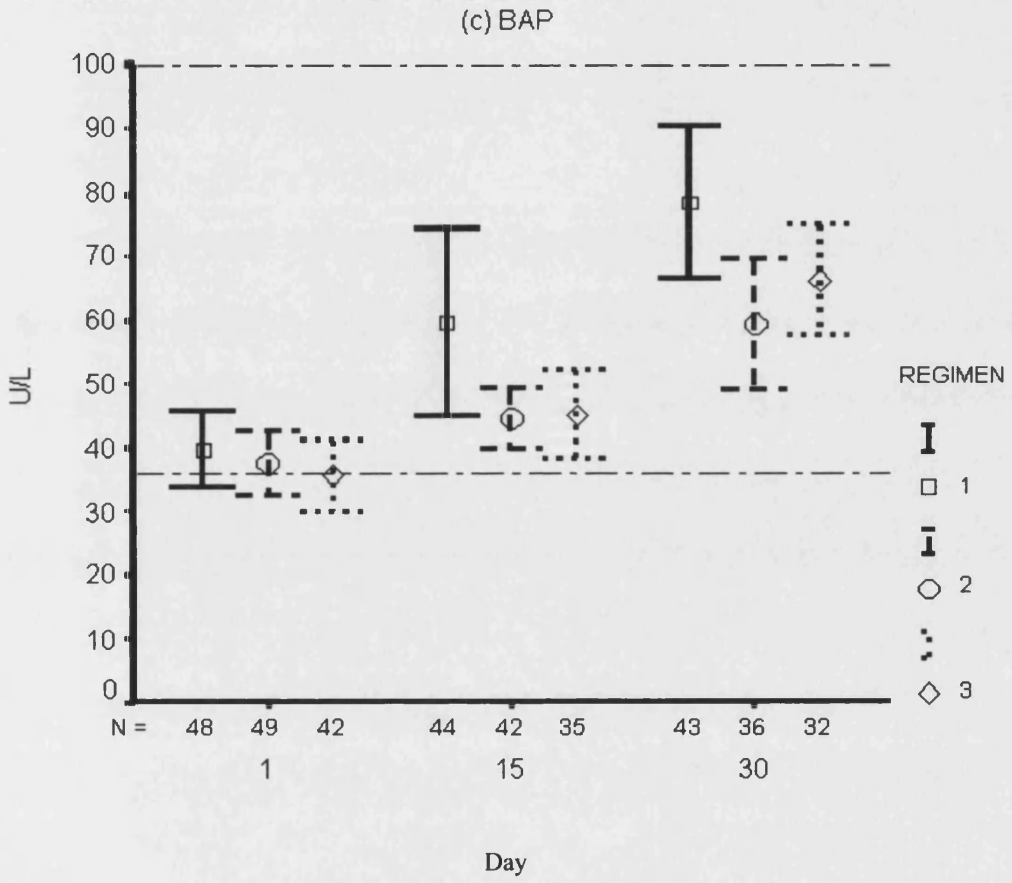


Fig 4d: Collagen, bone and growth factor markers (mean and 95% C.I.) vs. time. Data are shown separately for the 3 regimens. Reference data for age matched well nourished European children are shown as dashed lines

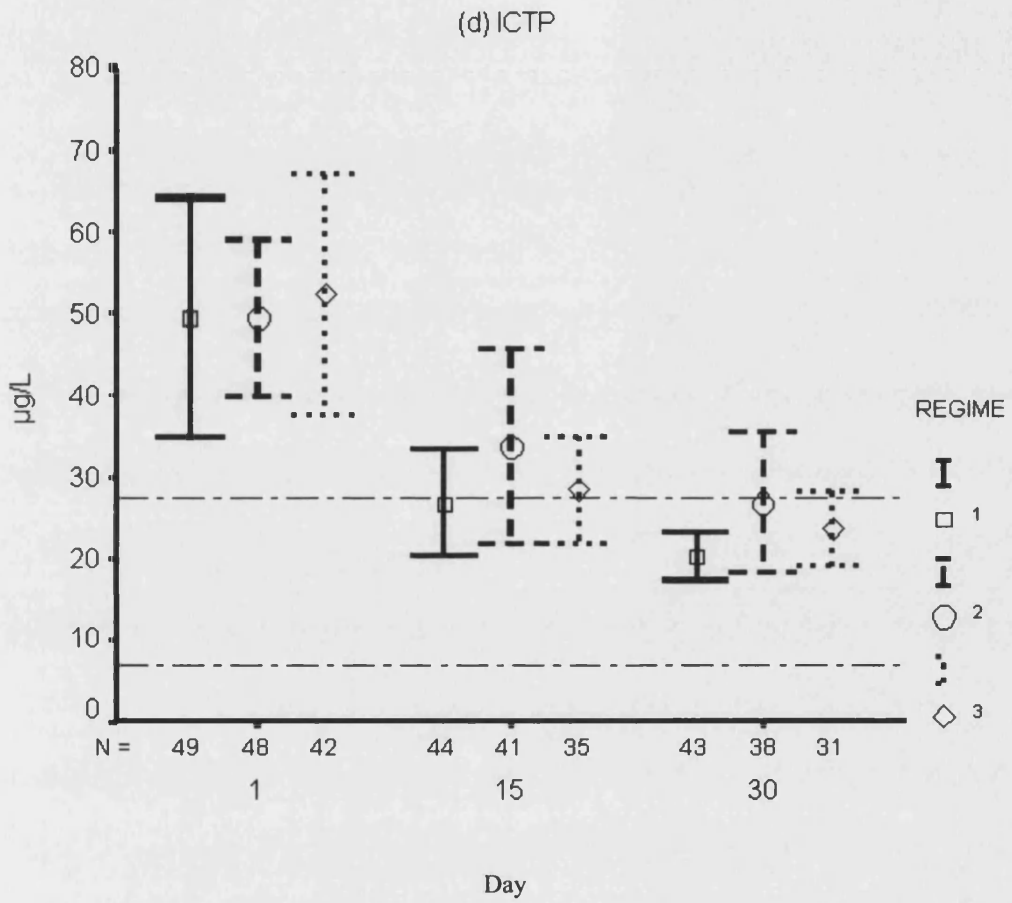


Fig 4e: Collagen, bone and growth factor markers (mean and 95% C.I.) vs. time. Data are shown separately for the 3 regimens. Reference data for age matched well nourished European children are shown as dashed lines

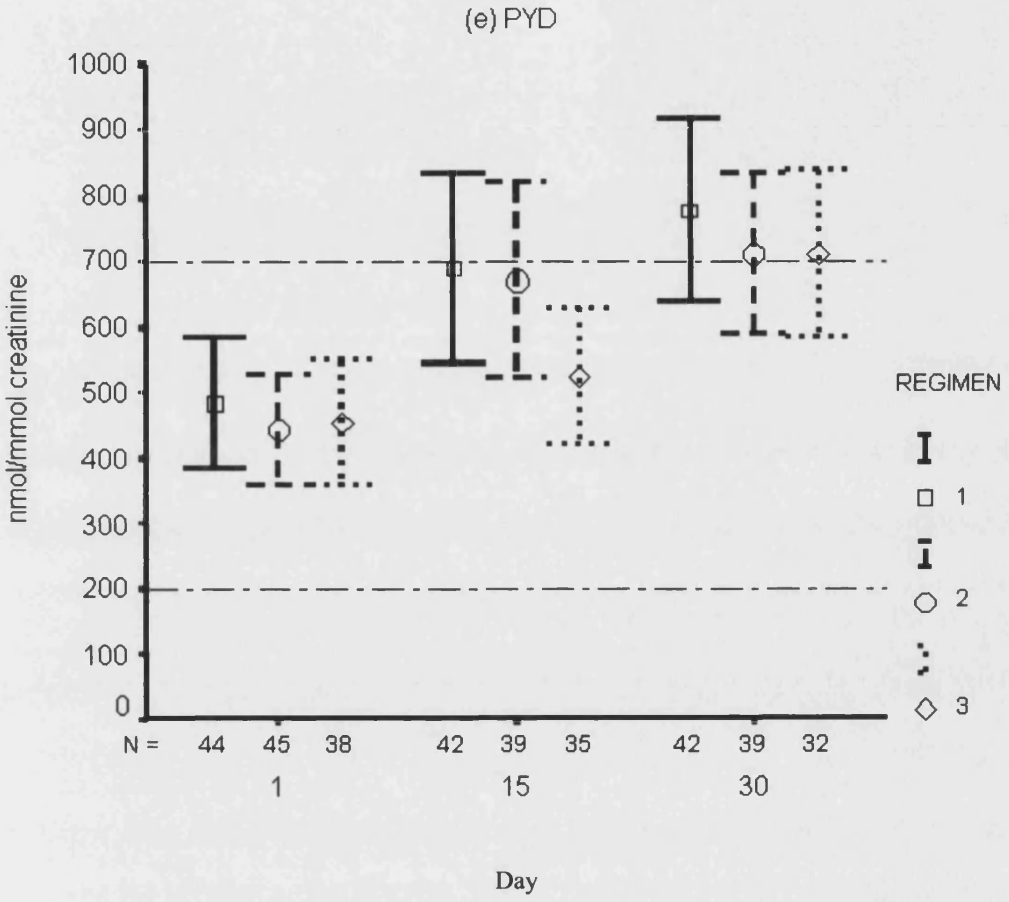


Fig 4f: Collagen, bone and growth factor markers (mean and 95% C.I.) vs. time. Data are shown separately for the 3 regimens. Reference data for age matched well nourished European children are shown as dashed lines

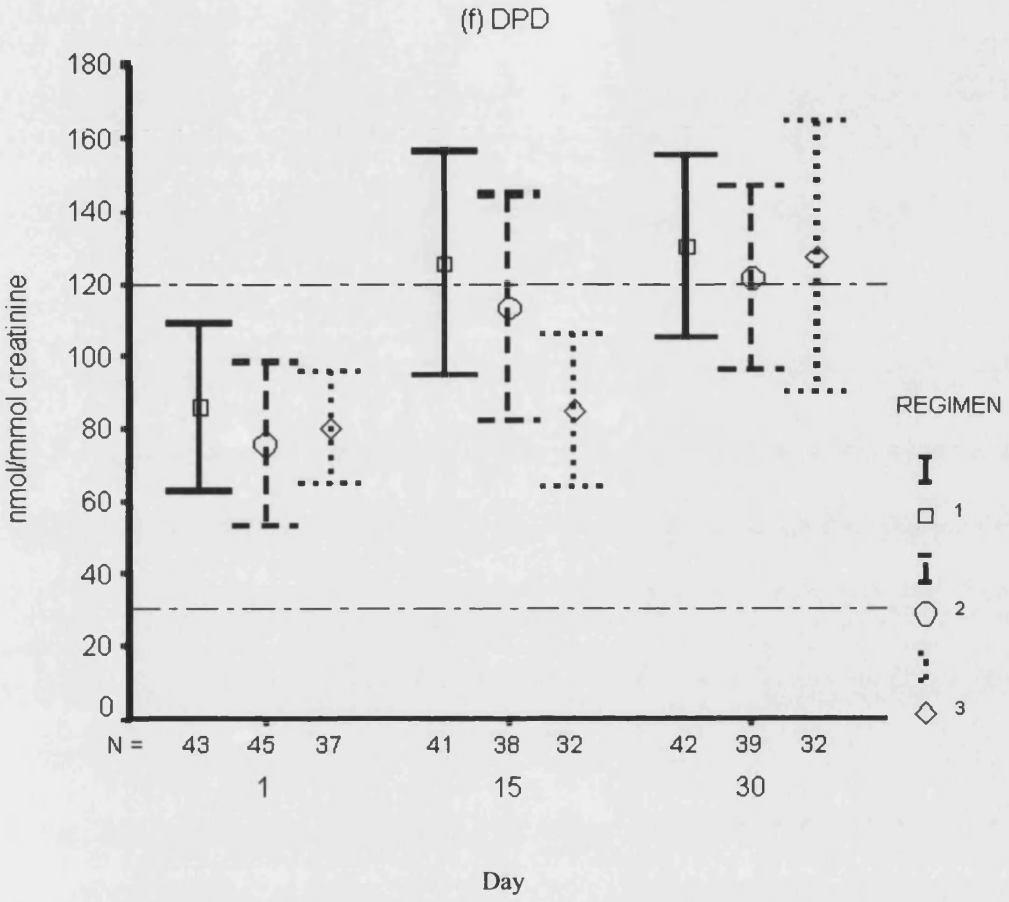


Fig 4g: Collagen, bone and growth factor markers (mean and 95% C.I.) vs. time. Data are shown separately for the 3 regimens. Reference data for age matched well nourished European children are shown as dashed lines. Note that upper limit of IGF1 for European children is 195.9µg/l.

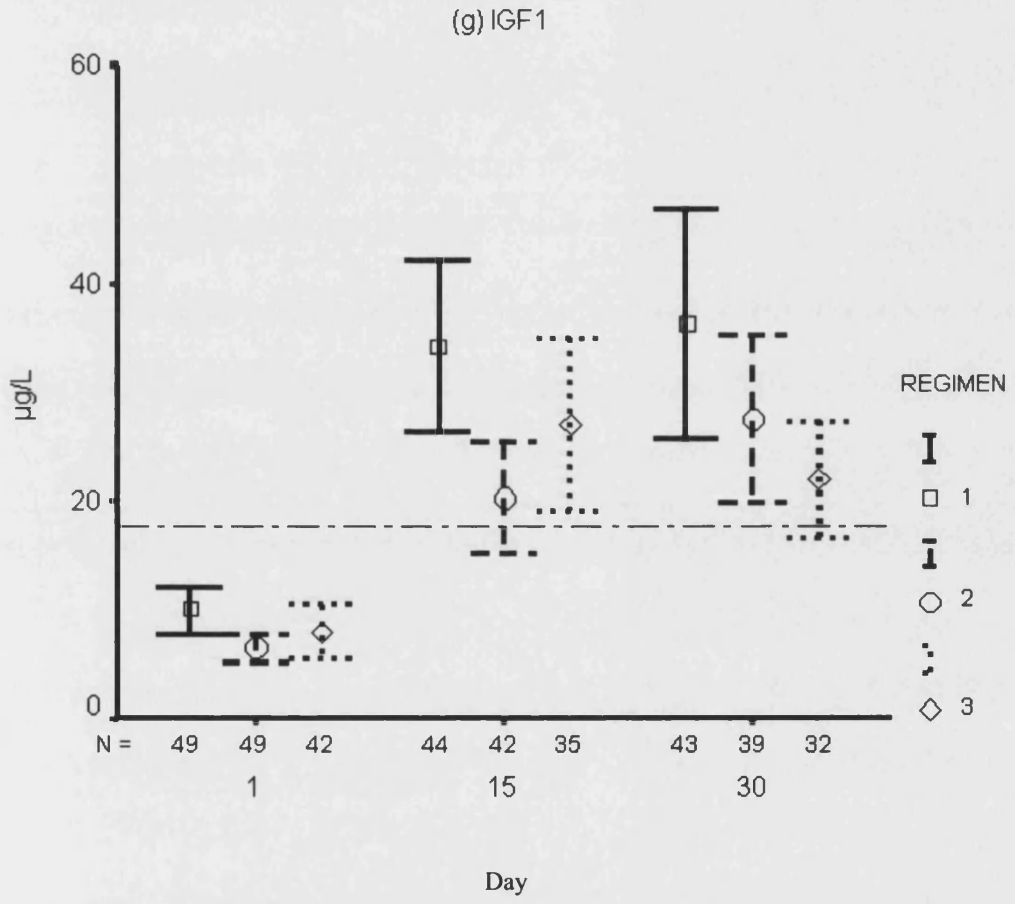


Fig 4h: Collagen, bone and growth factor markers (mean and 95% C.I.) vs. time. Data are shown separately for the 3 regimens. Reference data for age matched well nourished European children are shown as dashed lines

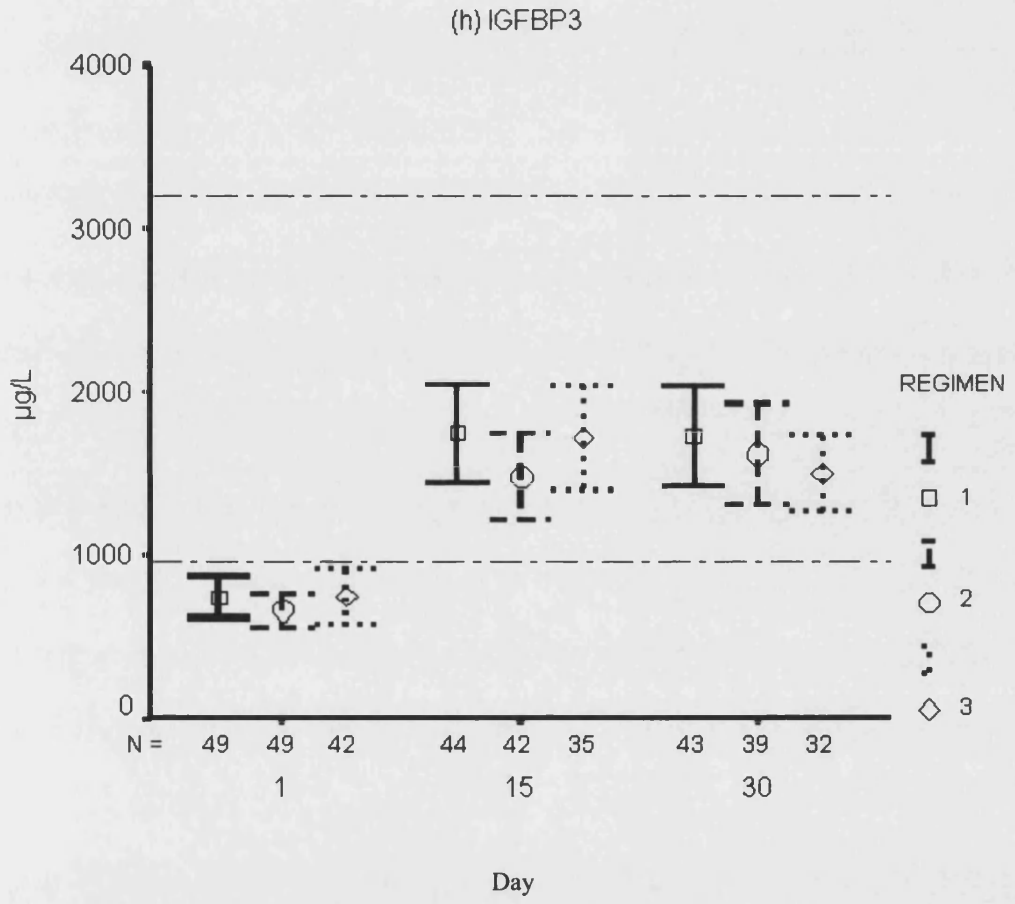


Fig 4i: Collagen, bone and growth factor markers (mean and 95% C.I.) vs. time. Data are shown separately for the 3 regimens. Reference data for age matched well nourished European children are shown as dashed lines

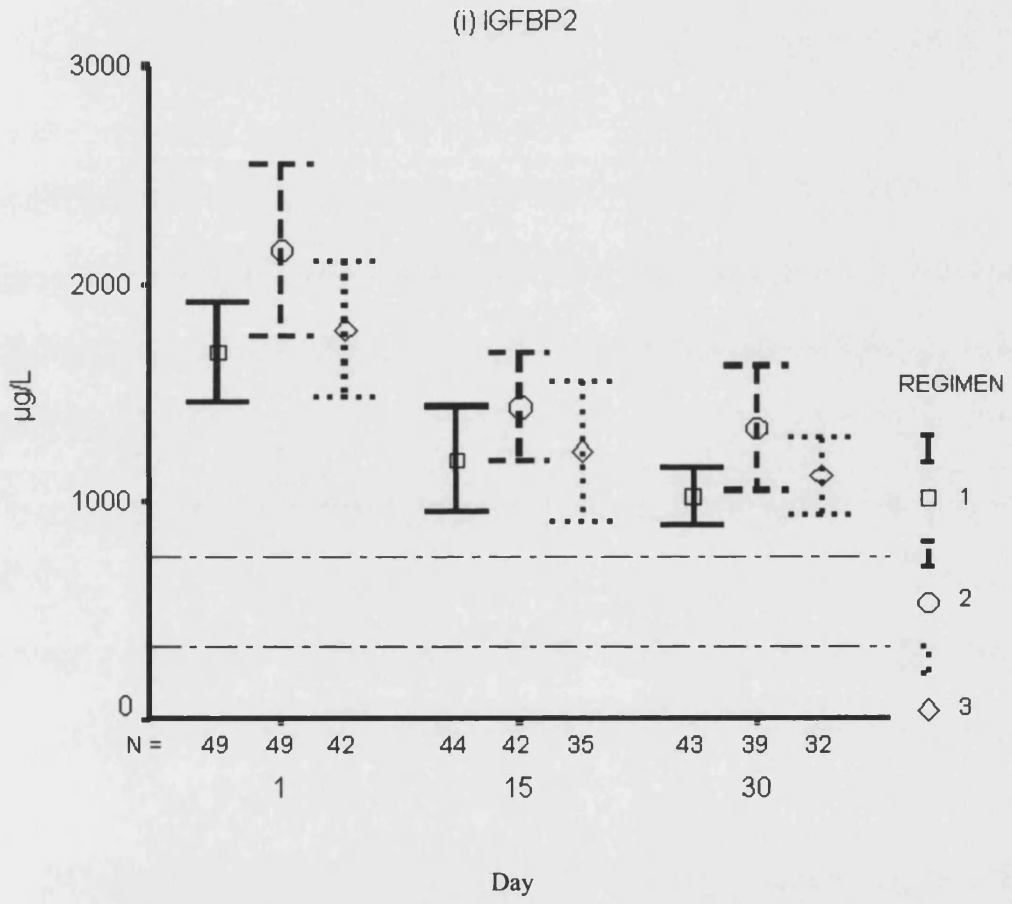


Table 17: p values of differences between inter-regimen means on days 1, 15 & 30 and p values of differences between means on days 1 and 15 and between days 15 and 30

	D1 Regimen	D15 Regimen	D30 Regimen	1/15	15/30
P1CP	0.19	0.52	0.55	0.000	0.002
P3NP	0.18	0.30	0.62	0.000	0.040
BAP	0.78	0.39	0.03	0.000	0.000
PYD	0.76	0.07	0.73	0.000	0.012
DPD	0.15	0.14	0.62	0.000	0.041
IGF1	0.04	0.04	0.13	0.000	0.906
IGFBP2	0.15	0.07	0.34	0.000	0.039
IGFBP3	0.78	0.42	0.89	0.000	0.643

Table 18: Correlation between indices on Day 1

		P1CP	P3NP	ICTP	BAP	IGF1	IGFBP3	IGFBP2
P1CP	Pearson Correlat	1.000	.558**	-.125	.253**	.233**	.265**	-.171*
	Sig. (2-tailed)	.	.000	.143	.003	.006	.002	.043
P3NP	Pearson Correlat	.558**	1.000	.043	.226**	.268**	.190*	-.124
	Sig. (2-tailed)	.000	.	.618	.007	.001	.025	.145
1CTP	Pearson Correlat	-.125	.043	1.000	.107	-.165	-.206*	.204*
	Sig. (2-tailed)	.143	.618	.	.213	.053	.015	.016
BAP	Pearson Correlat	.253**	.226**	.107	1.000	.083	.215*	-.114
	Sig. (2-tailed)	.003	.007	.213	.	.334	.011	.181
IGF1	Pearson Correlat	.233**	.268**	-.165	.083	1.000	.725**	-.260*
	Sig. (2-tailed)	.006	.001	.053	.334	.	.000	.002
IGFBP3	Pearson Correlat	.265**	.190*	-.206*	.215*	.725**	1.000	-.275*
	Sig. (2-tailed)	.002	.025	.015	.011	.000	.	.001
IGFBP2	Pearson Correlat	-.171*	-.124	.204*	-.114	-.260**	-.275**	1.000
	Sig. (2-tailed)	.043	.145	.016	.181	.002	.001	.
PYD	Pearson Correlat	.165	.219*	.134	.280**	.214*	.201*	-.037
	Sig. (2-tailed)	.065	.014	.136	.002	.016	.024	.681
DPD	Pearson Correlat	.308**	.279**	-.012	.109	.262**	.181*	-.003
	Sig. (2-tailed)	.001	.002	.898	.228	.003	.044	.973

**·Correlation is significant at the 0.01 level (2-tailed).

*·Correlation is significant at the 0.05 level (2-tailed).

Table 19: Correlations between indices on Day 15

		P1CP	P3NP	ICTP	BAP	IGF1	IGFBP3	IGFB
P1CP	Pearson Correlatio	1.000	.627**	-.066	.350**	.266**	.307**	-.
	Sig. (2-tailed)	.	.000	.471	.000	.003	.001	.
P3NP	Pearson Correlatio	.627**	1.000	-.064	.242**	.426**	.410**	-.
	Sig. (2-tailed)	.000	.	.490	.008	.000	.000	.
ICTP	Pearson Correlatio	-.066	-.064	1.000	-.025	-.173	-.196*	.
	Sig. (2-tailed)	.471	.490	.	.789	.058	.032	.
BAP	Pearson Correlatio	.350**	.242**	-.025	1.000	.268**	.268**	-.
	Sig. (2-tailed)	.000	.008	.789	.	.003	.003	.
IGF1	Pearson Correlatio	.266**	.426**	-.173	.268**	1.000	.807**	-.
	Sig. (2-tailed)	.003	.000	.058	.003	.	.000	.
GFBP3	Pearson Correlatio	.307**	.410**	-.196*	.268**	.807**	1.000	-.
	Sig. (2-tailed)	.001	.000	.032	.003	.000	.	.
IGFBP2	Pearson Correlatio	-.314**	-.318**	.460**	-.224*	-.541**	-.529**	1.
	Sig. (2-tailed)	.000	.000	.000	.013	.000	.000	
PYD	Pearson Correlatio	.214*	.294**	.027	.145	.110	.182	-.
	Sig. (2-tailed)	.021	.001	.776	.120	.242	.051	.
DPD	Pearson Correlatio	.108	.212*	-.038	.115	.100	.073	-.
	Sig. (2-tailed)	.260	.027	.693	.231	.296	.445	.

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 20: Correlations between indices on Day 30

		P1CP	P3NP	ITCP	BAP	IGF1	IGFBP3	IGF
P1CP	Pearson Correlation	1.000	.733**	.116	.356**	.294**	.314**	
	Sig. (2-tailed)	.	.000	.224	.000	.002	.001	
P3NP	Pearson Correlation	.733**	1.000	.146	.256**	.328**	.387**	
	Sig. (2-tailed)	.000	.	.126	.007	.000	.000	
ITCP	Pearson Correlation	.116	.146	1.000	.009	.070	.082	
	Sig. (2-tailed)	.224	.126	.	.925	.466	.388	
BAP	Pearson Correlation	.356**	.256**	.009	1.000	.190*	.156	
	Sig. (2-tailed)	.000	.007	.925	.	.046	.101	
IGF1	Pearson Correlation	.294**	.328**	.070	.190*	1.000	.796**	
	Sig. (2-tailed)	.002	.000	.466	.046	.	.000	
IGFBP3	Pearson Correlation	.314**	.387**	.082	.156	.796**	1.000	
	Sig. (2-tailed)	.001	.000	.388	.101	.000	.	
IGFBP2	Pearson Correlation	-.243**	-.208*	.546**	-.191*	-.423**	-.423**	
	Sig. (2-tailed)	.009	.027	.000	.044	.000	.000	
PYD	Pearson Correlation	.187*	.261**	.056	.126	.438**	.427**	
	Sig. (2-tailed)	.048	.005	.560	.188	.000	.000	
DPD	Pearson Correlation	.132	.192*	-.051	.144	.079	.183	
	Sig. (2-tailed)	.164	.043	.598	.134	.407	.053	

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 21: Correlations between change in biochemical indices over 30 days.

	P1CP						
P3NP	+0.37**	P3NP					
BAP	+0.22**	+0.11	BAP				
PYD	+0.07	+0.18**	+0.12	PYD			
DPD	+0.07	+0.10	+0.11	+0.45**	DPD		
ICTP	-0.03	-0.06	+0.15*	+0.00	-0.05	ICTP	
IGF1	+0.26**	+0.44**	+0.17**	+0.21**	+0.04	-0.02	IGF1
IGFBP3	-0.21**	+0.34**	+0.12	+0.19**	+0.06	-0.14*	+0.58**
IGFBP2	-0.20*	-0.08	-0.00	-0.02	-0.08	+0.11	-0.18**

Change in P1CP and P3NP over 30 days correlated with change in WHZ, HAZ and knemometric length over 30 days and change in WHZ score (less so), change in HAZ score and knemometric length (more so) over 90 days. Change in IGF1 over 30 days correlated with ponderal growth and knemometric growth but not linear growth over 90 days. Change in IGFBP3 levels over 30 days correlated with linear and knemometric growth over 90. Change in BAP levels over 30 days correlated with knemometric growth over 90. Change in collagen degradation markers correlated poorly with growth with only change in 1CTP correlating with knemometric growth over 30 days (table 22).

Kwashiorkor and marasmus were both associated with low IGF1 and IGFBP3 and elevated IGFBP2 levels. IGF1 and IGFBP3 levels increased with rehabilitation and IGFBP2 levels fell with the only differences between marasmic and kwashiorkor children noted in day 15 and 30 IGFBP3 levels ($p<0.05$). There was no difference in change in P1CP, P3NP, BAP, 1CTP, IGF1, IGFBP3 or IGFBP2 between marasmic and kwashiorkor children but there were differences in day 1 P1CP and P3NP. When differences in day 1 HAZ and WHZ were allowed for the differences in days 15 & 30 IGFBP3 and day 1 P1CP respectively were non-significant. Even accounting for differences in initial anthropometry difference in day 1 P3NP remained ($p=0.001$).

Table 22: Correlation between change in anthropometric variables between either days 1 to 30 (1/30) or d biochemical variable between days 1 and 30

	P1CP	P3NP	BAP	PYD	DPD	ICTP	IGF1
1/30WHZ	+0.21**	+0.32**	+0.23**	+0.06	-0.06	+0.10	+0.31**
1/90WHZ	+0.14*	+0.17*	+0.10	+0.07	+0/02	-0.11	+0.15*
1/30HAZ	+0.15*	+0.16*	-0.01	+0.04	+0.04	+0.03	+0.06
1/90HAZ	+0.21**	+0.25**	+0.14	+0.04	-0.01	+0.02	+0.14
1/30LLL	+0.14*	0.25**	+0.14*	+0.01	-0.07	+0.19**	+0.19**
1/90LLL	+0.21**	+0.33**	+0.19**	+0.09	+0.03	+0.08	+0.28**

* significant at 0.05 level

** significant at 0.01 level

Chapter 4: Discussion

The aims of this study were threefold. To describe the patterns of and relationship between ponderal and linear catch-up growth in the recovery phase from severe malnutrition. To describe bone, soft tissue and collagen turnover during this phase and to assess the effects of three different regimens of zinc supplementation on the above processes and thus the optimal dosage range for severely malnourished children.

This study has shown that in severely malnourished Bangladeshi children there were no differences in anthropometric or biochemical marker responses among the 3 zinc regimens. 141 children were recruited making this the largest zinc intervention study to date in this group. Significant catch-up growth was achieved with an average intragroup improvement of WHZ score of 1.54 to 1.67 and in HAZ score of 0.44 to 0.49 units. Mortality differences between the 3 groups of children became evident on analysis and were an unexpected finding. Linear growth was significantly associated with initial WHZ but occurred in the presence of severe wasting. Bone remodelling and soft tissue turnover commenced early and was associated with a fall in IGFBP2 and an increase in IGF1 and IGFBP3.

Despite the evident confusion in the literature on the zinc requirement of the severely malnourished child this study is the only one to date to compare different zinc supplementation regimens in these children. The recommended dietary allowance of zinc for a one-year-old is 5mg/day (National Academy of Sciences 1989). A child recovering from severe protein energy malnutrition however is already deficient and in need of a higher dietary intake during the recovery process due to often spectacular rates of weight gain. Golden et al (Golden 1992; Golden 1981) have demonstrated

that zinc influences rate of weight gain as well as the ratio of lean to adipose tissue deposited by comparing the energy cost of tissue deposition in marasmic children fed low zinc soya based feeds with those fed higher zinc containing milk feeds. Further they demonstrated that zinc supplementation of marasmic children resulted in a greater net absorption of nitrogen and a higher rate of protein turnover, as estimated from urinary ammonia 15N enrichment after oral (15N) glycine. They concluded that additional zinc affected the composition of newly synthesised tissue and intermediary nitrogen metabolism.

Both the bioavailability of dietary zinc and the continuing losses in these children (due to the high prevalence of diarrhoea) also contribute to difficulty in estimating requirements in this group.

There is approximately 30mg zinc/g fat free mass in adult men however only those zinc pools in the liver and plasma (5% of total body zinc) are readily accessible. The much larger zinc pools of muscle and bone are only available in episodes of catabolism. During episodes of deprivation zinc homeostasis depends on the liver and intestine with an increase in intestinal absorption and a decrease pancreatico-biliary secretion.

Simmer et al (Simmer 1990) measured the daily intake of zinc in breast fed Bangladeshi infants aged 1, 2, 6, 9, and 12 months and found them to be between 10 to 30% of the National Academy of Sciences recommended dietary allowances. Important dietary sources of zinc include red meat, sea food and unprocessed cereals and nuts, but Brown et al (Brown 1982) demonstrated that 50% of Bangladeshi

children had never received meat, fish or eggs by 30 months age. In this group all children between 5 and 12 months of age and 85% of children between 24 and 30 months were breast-fed; the average amount of breast milk received by these age groups declined from 632 to 368 g/day. Concurrently, the rate of consumption of cereals increased from 54 to 100% of children, and the amount received increased from 35 to 94 g/day. Breast milk was the major source of all nutrients for younger children.

The zinc content of the rehabilitation diets used in this study was very difficult to measure accurately. The children were initially fed on liquid diets via nasogastric tubes and both the dried skimmed milk and rice based liquid diet had some 3mg zinc per 1000ml of feed. However, as soon as their appetite had returned these children were then encouraged to eat solid food ad libitum. These foodstuffs were of local origin and, as one of the important health education messages to the mothers was that they could provide a healthy diet to their children, it was important that local mixtures of food such as khichuri and curries were included. Whilst every effort was made to standardise the contents of these solid food mixes it was recognised that both the zinc content and the individual estimates of food ingested were inaccurate.

4.1. Anthropometry

This population of severely malnourished children was both severely wasted and very severely stunted with a mean initial WHZ of -2.66 and HAZ of -3.89 respectively. This would indicate both an underlying long-term deficit in growth and nutrition (manifest in HAZ scores) and an acute nutritional insult (manifest in WHZ score). In this study of catch-up growth during recovery from severe protein-energy malnutrition, neither higher dose nor longer duration zinc supplementation regimens had any appreciable anthropometric benefit over the lower dose regimen across the 90 days of the study. On average good ponderal and linear catch-up growth was achieved within all three groups with a wide intragroup variation but no significant intergroup differences. The majority of ponderal growth and practically all of the linear growth occurred during the out-patient phase of the trial when the subjects had returned to the environment in which they had originally become deficient in protein, energy, zinc and presumably other micro-nutrients. Neither the employment of higher zinc dosages for the in-patient period (regimen 2) nor prolonging the period of supplementation to day 30 of the out-patient phase (regimen 3) had a significant effect in promoting further growth. As the confidence limits in table 10 show, any benefit was unlikely to average more than 0.2 - 0.5 units of the three Z scores.

The effect of a single micronutrient supplement to severely malnourished children undergoing refeeding might be expected to be overwhelmed by the beneficial effect of full nutritional rehabilitation. However when sufficiency of that micronutrient limits macronutrient utilisation e.g. zinc, then this experimental design has been effective (Castillo-Duran 1987; Simmer 1988). In the absence of a control group with no zinc supplementation it is difficult to discern whether zinc had no effect in this instance or

rather that that the regimens had no significant difference in effect. On review of the study in Bangladesh it was concluded that there was enough evidence to suggest that severely malnourished children benefited from zinc supplementation and that comparing the effects of different zinc regimens against placebo would be unethical.

During the initial phase of rehabilitation zinc is required for growth, particularly lean tissue deposition, and also for recovery of immune function. The process of replenishing both lean tissue and hepatic pools and allowing for optimal functioning of the immune system provides for an environment in which growth can continue after zinc supplementation has stopped. One of the major tenets of nutritional rehabilitation is the promotion of healthy weaning diets and health education was central to management of these children. The success of this is witnessed by the fact that most of these children continued to thrive after discharge into the same environments in which they initially became malnourished. Dietary variety was a key theme in the advice offered and it may well be that as a result of this that zinc intakes increased. Continuing the higher dosage zinc supplementation into the outpatient phase of rehabilitation did not have any anthropometric benefits.

Ponderal growth was not significantly influenced by supplementation regimen. Severity of initial wasting was significantly negatively correlated with subsequent change in WHZ score and inpatient weight gain was significantly positively correlated with subsequent change over both 45 and 90 days. As individual children approached their median WHZ score rate of weight gain slowed.

Linear growth was not significantly influenced by regimen and largely took place in the second half of the study after weight gain was first achieved. However it occurred even in the presence of severe wasting. Linear growth was significantly positively correlated with initial degree of wasting and inpatient change in height for age z score over both 45 and 90 days but not with inpatient change in weight for height z score even in those children without nutritional oedema. 0.5 z score linear catch up was demonstrated over 90 days 50% of which took place in the last 30 days of the study. Walker et al detailed early linear growth in 369 recovering malnourished children in Jamaica. Only a more stunted subgroup (n=108 and mean HAZ –4.3) demonstrated linear catch up growth and two thirds of these attained at least 85% weight for length before they began to increase in length (Walker 1988). In this study linear growth occurred even in severely wasted children with no threshold identified. With increased wasting growth was predominately ponderal and linear growth only approached ponderal when initial WHZ score was 1 z score below median. Linear and ponderal growth do occur concurrently with initial WHZ score determining whichever predominates. Whether interventions designed to promote linear growth act via improving ponderal growth to that point where linear growth predominates or allow for linear growth at a lower initial WHZ score is not clear. The switch to skeletal growth, lean tissue deposition and linear growth is not understood but as zinc supplementation promotes lean tissue deposition it thus has been postulated to preferentially promote linear growth.

Identifying predictors of linear growth in these children is difficult. If we use in-patient change in HAZ to predict for linear growth over 90 days it must be appreciated that this is not an independent variable. Change in WHZ score preceding

linear growth has previously been described as a predictor (Walker 1996) but did not predict in our model. Initial WHZ score and maternal height did predict but only accounted for 20% of total variance.

Inpatient ponderal growth predicts well for ponderal growth over the subsequent three months and those children who demonstrated most linear catch-up growth were those who were least malnourished initially and those that demonstrated most linear growth as an inpatient.

Knemometry has been used in well nourished children as an index of linear growth, but the relationship between total linear growth and knemometric growth is not clear as different parts of the skeleton appear to grow at different rates and times. In our group knemometric growth did not correlate with linear growth until after day 45 of the study. Across all time periods knemometric growth correlated positively with change in mid-upper arm circumference, triceps skinfold thickness and weight for height z score. The knemometric measurements behaved more as an index of ponderal growth than of linear growth in this group particularly in the first 45 days. As well as measuring changes in the length of the tibia and fibula the knemometer also measures changes in the soft tissues overlying these and the deposition of fat in the supra-patellar and heel fat pads as well as changes in tissue oedema contributed to measurements. Knemometry offers an accurate and reproducible index of lower leg growth and was hoped to be a useful index of linear growth with the potential benefit of allowing shorter follow-up periods to demonstrate differences between groups. However in this group of children this application is limited.

In summary I concluded that there was no anthropometric benefit in employing the higher dosage zinc supplementation regimens nor prolonging supplementation into the outpatient phase. Linear growth may occur even in the presence of severe wasting but is strongly correlated with initial weight for height z score. The knemometer is of limited use as an index of linear growth in this group of children.

4.2. Mortality

The most striking finding of this study was the increase in mortality associated with initial exposure to the higher dosage zinc regimens (6.0mg/kg/day) as opposed to the lower dosage zinc regimen (1.5mg/kg/day). The inpatient death rate among those recruited to the higher dose zinc regimens was 12% (over 3 months 19%) whilst that for the lower dose zinc group was 4% (over 3 months 4%) - this compared with the unit inpatient death rate of 18% just before starting the trial. This could indeed be a chance finding, but the plausibility of this being a true association must be considered.

Most of these deaths were sepsis-related and occurred during the inpatient phase of rehabilitation, and therefore the effect of supplemental zinc on the immune system of the malnourished child must be considered. Malnourished children are commonly zinc deficient (as evidenced by subsequent response to supplemental zinc). The evidence for this in Bangladesh is particularly strong (Khanum 1988; Simmer 1988). Previous supplementation trials in children recovering from severe protein energy malnutrition have demonstrated significant benefits and dietary zinc deficiency is well described here (Simmer 1990). Zinc is now routinely supplemented on many nutritional rehabilitation units in Bangladesh.

Zinc plays a central role in the immune system and deficiency affects immune function at multiple levels in both the innate and specific arms. GI barrier function, polymorphonuclear and natural killer function and complement activity are all affected (Shankar 1998; Allen 1983; Montgomery 1979). Cell mediatedimmunity is profoundly affected in zinc deficiency. Lymphopenia is common as are defects in specific T and B lymphocyte function (Pekarek 1979; Chandra 1980; Fraker 1982;

Beisel 1982). Secretion and function of cytokines (Beck 1997) and the potentiation of apoptosis (Elmes 1977) are also affected by zinc deficiency. Zinc may have a role in prevention of free-radical induced injury mediated through its antioxidant (Bray 1990) and cell membrane stabilising properties. Lymphoid atrophy, decreased delayed cutaeneous hypersensitivity responses, reduction in numbers of T4 helper cells and deficient thymic hormone activity have been described in association with zinc deficiency. B cell dysfunction, and specifically impairment of phagocytosis, has also been described.

Zinc supplementation of malnourished children improves immune function. Initial observations included enlargement of thymic shadows in marasmic children given 10days of zinc supplementation (Golden 1977). Marasmic children (mean age and weight for age were 7 months and –3.1 z scores) given 2mg/kg/d supplemental zinc for 105 days had significantly improved linear growth, significantly increased conversion of delayed hypersensitivity skin reactions and enhanced lymphoproliferative response to phytohaemagglutin (PHA) and increased salivary IgA concentrations compared to non-supplemented infants (Schlesinger 1992). Twenty-five undernourished (mean age and WAZ: 42 mth and –1.4) Ecuadorian children randomised to 10mg/day of zinc for 60 days had significantly larger delayed type hypersensitivity skin reactions than controls. They also had a significantly decreased incidence of fever, cough and upper respiratory tract secretions during supplementation that disappeared on stopping the supplementation in the second half of follow-up (Sempertegui 1996). Sixteen undernourished Chilean infants (mean age and weight for age: 7mths and 67% of median) given 2mg/kg zinc per day for 90 days

demonstrated significantly better weight gain serum IgA and significantly reduced incidence of pyoderma and percentage of anergy (CastilloDuran 1987).

However, zinc supplementation in the presence of ongoing sepsis and an already compromised immune system may not necessarily be beneficial. Zinc, in physiological range, has been associated with impairment of granulocyte phagocytosis (Mustafa 1971; Stankova 1997; Chavpil 1977). In animal experiments supplemental zinc increased serum levels and was associated with decreased mobilisation of polymorphonuclear cells and macrophages into the peritoneal cavity and phagocytosis (Chavpil 1976). 150mg of elemental zinc twice a week for six weeks given to eleven healthy adult men was associated with a reduction in lymphocyte stimulation response to phytohemmagglutin as well as chemotaxis and phagocytosis of bacteria by polymorphonuclear leukocytes (Chandra1984). Marasmic infants fed a zinc fortified liquid diet (15mg zinc/l) demonstrated reduced phagocytic and fungicidal monocytic activity and a significantly increased number and duration of impetigo episodes (Schlesinger 1993).

Serum levels of zinc decrease sharply in inflammation and infection and may reflect a natural protective mechanism, as lower levels of zinc are associated with both optimal phagocytic function and decreased microbial virulence (Sugarman 1983). This decrease is accounted for largely by a redistribution to the liver (Powanda 1973; Pekarek 1972) serving to favor the immune system in the production of acute phase proteins and haemopoesis (Chavpil 1976). Optimal phagocytosis of latex particles correlates with slightly decreased levels of zinc in human serum and polymorphonuclear cells (Lennard 1974). Calprotectin, an acute-phase zinc binding

protein may play a role in reducing extracellular zinc concentrations in inflammation (Clohessy 1995). Zinc is important in microbial virulence with near physiologic concentrations of zinc necessary for toxin production in *Clostridium perfringens* (Murata 1969) and *Pseudomonas aeruginosa* (Jensen 1980) and in the adherence of *Enterobacter* to epithelial surfaces (Sugarman 1980).

Serum iron levels also fall during infection as a result again of redistribution to the liver, reticuloendothelial system and bone marrow. Early iron supplementation of children with severe protein energy malnutrition led to increased mortality (Smith 1989) probably due to increased microbial virulence and free radical generation.

In our study the length of time between admission and recruitment was between 2.5 and 3.5 days and reflected the units policy on early commencement of incremental feeding regimens and micronutrient supplementation. This reflects both the intense social pressures on mothers to leave hospital as quickly as possible and a general move away from in-patient to out-patient based rehabilitation. However since most children presented to the unit with intercurrent infections, it is unlikely that by three days all children were free of infection. In previous trials of zinc supplementation in severely malnourished children, the zinc supplement was administered at a later stage of rehabilitation when it was much less likely that sepsis was ongoing - a situation unlikely to be representative of practice in most nutritional rehabilitation units.

4.3. Zinc and copper

As well as having a possible directly detrimental effect on the immune system during sepsis, high dose zinc supplementation could aggravate deficiencies of other minerals by decreasing their intestinal absorption. Zinc inhibits copper absorption and copper deficiency can co-exist with severe protein-energy malnutrition (Cordano 1964; Hemalatha 1993). Copper deficiency is also associated with an impaired immune response, specifically a reduction in the antibody response to T cell dependent antigens and a decrease in the microbiocidal activity of phagocytes (Vyas 1983; Babu 1989). Metallothionen, an intracellular polypeptide, plays an important regulatory role in the metabolism of ingested zinc and copper. It binds copper more avidly than zinc. Zinc induces the synthesis of metallothionen (Yuzbasiyan-Gurkan 1992) thus preventing serosal transfer to blood of ingested copper as well as endogenously secreted copper in GI, salivary and pancreatic juices. Zinc in large doses can therefore produce chronic negative copper balance particularly in the presence of a pre-existing copper deficiency. Zinc induced copper deficiency has been documented (Botash 1992; Sandstrom 1994; Honkanen 1991; Porter 1977; Prasad 1978) though very high levels of zinc for prolonged periods are usually required to manifest it. Prasad (Prasad 1978) describes how 150 mg of Zn per day for 23 months precipitated severe copper deficiency in patients with sickle cell disease and Porter (Porter 1977) describes how the same level of zinc supplementation for 13 months again precipitated copper deficiency. However an intake of 18.5mg of zinc per day for just two weeks reduced apparent copper retention in a group of healthy well nourished men (Festa 1985). In 23 previous zinc supplementation studies using 1 to 10 mg/kg/day for two weeks to 1.25 years, no side-effects related to copper status were reported

Seven of these studies (2 to 6 mg/kg/day given for 21 days to 1.25 years) examined biochemical markers of copper status and none showed an effect as measured variously by serum/plasma copper and caeruloplasmin levels or superoxide dismutase activity.

Table 23: Zinc studies – effects on copper status

Investigator	Year	Copper status assessment and effect
Bates CJ	1993	Y (hair copper and superoxide dismutase levels) – no effect
Behrens RH	1990	N
Castillo-Duran C	1987	Y (plasma copper) - no effect
Gibson RS	1989	Y (plasma & hair copper, and caeruloplasmin)– no effect
Golden BE	1981	N
Golden MHN	1981	N
Halstead JA	1972	N
Hambridge KM	1985	N
Hemalatha P	1992	Y (plasma copper) – no effect
Krebs NF	1994	N
Ronaghy HA	1974	N
Sachdev HPS	1988	N
Sella GE	1991	N
Simmer K	1988	N
Walravens PA	1989	N
Walravens PA	1983	Y (plasma copper) – no effect
Cavan KR	1993	N
Nakamura T	1993	N
Shrivastava SP	1993	N
Schlesinger L	1992	Y (plasma copper) – no effect
Walravens PA	1976	Y (plasma copper) – no effect
Khanum	1988	N
Sempertegui	1996	N

Severely malnourished children are immunocompromised and frequently present to hospital with intercurrent infections. Infection in these children is often difficult to recognise, tends to be aggressive and disseminates easily. Broad-spectrum anti-microbials are now advocated for all children that present with severe malnutrition

(Schofield 1996). C reactive protein (CRP) concentrations increase with the acute inflammatory response even in the presence of severe malnutrition (Manary 1998; Sauerwein 1997) and are a useful clinical tool in detecting sepsis. Day 1 mean regimen plasma CRP concentrations were between 12 and 19mg/l and between 34 and 37% of values were abnormal with no significant inter-regimen differences. At the end of the inpatient period (day 15) the mean regimen CRP concentration had dropped to between 2 and 10mg/l with no difference between regimens but with a significant increase in numbers of children with an abnormal CRP concentration in the high (regimens 2&3) as compared to the low dosage (1) regimen. Thus the prevalence of inflammation was equally distributed on admission but by day 15 those children that had received the higher dosage zinc supplement had an increased prevalence. At day 30 there were no significant inter-regimen differences in mean CRP concentrations or absolute numbers of children with abnormal CRP concentrations. Rehabilitation was established, children had gone home and most deaths had occurred making analysis of the survivor CRP concentrations difficult to interpret. However it might suggest that this proposed immunosuppressive effect was maximal on admission and thus have implications for the timing of zinc supplementation in these children. Abnormal CRP's were found in 12% of children at day 30 re-enforcing the need for close follow-up as the recovery period for immune function of these children has not been defined.

Those children who died had significantly elevated day 1 mean CRP concentrations compared to survivors and there is a significant association between death and an abnormal CRP. This supported the clinical impression of sepsis as cause of mortality.

Plasma zinc and copper concentrations were measured in these children on days 1, 15, and 30. Plasma zinc concentration increased with supplementation and was significantly higher in the high dosage regimens compared with the lower. Day 30 plasma zinc was significantly higher in those that received supplementation onto day 30. Plasma copper concentration increased throughout the study period and did not differ between regimens. Though inflammation affects plasma copper concentrations a regression analysis of day 15 copper, zinc and CRP concentrations demonstrated that inflammation did not confound the relationship between regimen and plasma copper concentration. There is therefore no evidence that a copper deficiency developed in those children who received the higher dose zinc supplement

In conclusion, neither the use of a higher dosage of zinc initially nor prolonging the period of supplementation resulted in a significant anthropometric benefit among severely malnourished children, and indeed, using the higher dosage was associated with increased mortality. It is recommended therefore that caution should be employed in its use. Further studies are needed to define the relationship between zinc and the immune response, how best to assess zinc status, what is the optimal level of zinc supplementation and when is it best to administer it.

4.4. Collagen turnover and IGF axis activity

In this study plasma IGF-I, IGFBP-3, PICP and P3NP (markers of bone and soft tissue collagen synthesis respectively), and BAP were low and increased within 15 days ($P < 0.001$). IGFBP-2 and ICTP (a marker of type I collagen degradation) were increased and fell during refeeding ($P < 0.001$). There were no differences in biochemical marker responses among the zinc regimens. IGFBP2 correlated positively with ICTP and negatively with PICP, BAP, IGF1 and IGFBP3 ($p<0.01$). Ponderal growth correlated with increases in IGF-I, IGFBP-3, PICP, P3NP and BAP over 30 days ($P < 0.01$). Linear growth over 90 days correlated with increases of IGFBP-3 ($P<0.05$), PICP ($P < 0.01$) and P3NP ($P < 0.01$) over 30 days.

Linear growth is dependent on skeletal growth, which occurs when osteoblastic bone formation predominates over osteoclastic bone resorption. Bone is continually remodelled and longitudinal bone growth during childhood is dependent on GH (Nilsson 1994). Control of resorption and formation is complex with evidence pointing to the components of the GH/IGF axis as providing the complexity required to selectively regulate complementary processes to suit demands. IGF's regulated osteoblast and osteoclast proliferation and collagen synthesis in vitro (Rosen 1994) and recombinant IGF1 stimulated bone formation in women (Johansson 1992). Locally produced IGF's acting as autocrine or paracrine agents have been postulated as mediating in the coupling of bone formation to resorption (Hayden 1995; Chihara 1997).

IGF1 is produced both in the liver and locally in multiple cell types in response to GH to promote cell growth. IGFBP's transport IGF's, control tissue specific availability,

regulate clearance, modulate receptor interaction with the IGF's and have a direct effect on cells (Clemmons 1998). Malnutrition is associated with GH resistance (high GH and low IGF 1 levels) which may represent an adaptive mechanism to promote lipolysis and fatty acid oxidation whilst attenuating the anabolic actions of GH on protein synthesis (Soliman 1986). Animal studies demonstrated reduced GH binding capacity, post GH receptor defects, reduced IGF1 gene transcription and translation in fasting or protein restriction as well as increased IGF1 clearance (Thissen 1991; Thissen 1990; Maes 1988). IGF1 levels in children declined in response to 6 days of protein or energy restriction with an associated increase in IGFBP2 in protein restriction only (Smith 1995). Levels of IGFBP3, the principal carrier of IGF1, are more consistently reduced by prolonged malnutrition along with IGF1. (Ketelslegers 1996). A 3 day fast in obese and non-obese subjects with non-insulin dependant diabetes did not alter IGFBP3 (Bang 1994) whereas in anorexia nervosa it is significantly decreased together with IGF1 and IGFBP2 was markedly elevated (Counts 1992). Increase in IGF1 levels correlated temporally with entry into positive nitrogen balance in 6 malnourished adults who were receiving nutritional support and proved a more sensitive index of acute directional changes in nutritional status than other plasma proteins (Clemmons 1985). When malnourished Bangladeshi children recovering from Shigellosis were fed either a normal protein (6% of total dietary energy) or high protein diet (12%), better weight gain, higher IGF1 levels and a larger decrease in IGFBP2 levels were noted in the high protein group (Pucilowska 1993).

Micro as well as macronutrients influence catch-up growth in malnourished children.10mg of zinc per day for 5 months produced significant increases in weight and height and significant reductions in infectious morbidity in stunted Vietnamese

children associated with a significant increase in IGF1 levels. Between 1 and 5 months post supplementation IGF1 concentration increased from a mean of 2.8 to 3.4 nmol/l with no increase in the placebo group suggesting that zinc deficiency was limiting growth in these children and that zinc stimulated growth through changes in circulating IGF1(Nguyen 1996). 220 prepubertal stunted Japanese children were randomised to receive either 2mg/kg zinc per day for 6 months or placebo. The zinc supplemented group had significantly increased energy intakes, growth velocity and insulin like growth factor 1 levels but urinary excretion of growth hormone was unchanged as were plasma levels of other pituitary hormones. This might indicate that zinc has a role in binding GH to its receptor in hepatic cells (Nakamura 1993). Zinc depletion in rats resulted in reduced body weight gain, serum IGF1 concentration, hepatic growth hormone receptors and serum growth hormone binding protein levels (GHBP). Liver IGF1 mRNA levels were reduced by zinc deficiency suggesting that the decline in IGF1 levels was mediated through decreased hepatic GH receptors and/or GHBP levels (Ninh 1995). Exogenous GH failed to stimulate growth and raise IGF1 in zinc deficient rats suggesting GH resistance as a result of a decrease in liver GH binding sites and/or a decrease in GHBP (Prasad 1969).

I demonstrated both initial low levels of IGF1 and IGFBP3 which increase significantly with rehabilitation and high initial levels of IGFBP2 which subsequently fall. Metabolic adaptation to malnutrition requires a reduction in protein synthesis whilst rehabilitation and catch-up growth demand both lean tissue deposition and bone growth. Changes in both local and circulating levels of IGF's and their binding proteins may regulate this switch. The positive correlation between IGFBP2 and 1CTP and negative correlations with P1CP, IGF1 and IGFBP3 support a role for

dietary protein sensitive IGFBP2 regulating bone formation/resorption coupling directly or through availability of IGF's.

There were no differences in growth between the 3 zinc regimens and no differences in change of IGF1, the binding proteins or any marker of collagen or bone turnover (except for D30 BAP). In the absence of a control group with no zinc supplementation (deemed unethical) it is impossible to further comment on the role of zinc in the catch-up growth of these children. Both type 1 and type 3 collagen formation and bone osteoblastic activity increased significantly early in rehabilitation and change in these markers was significantly positively correlated with change in IGF1 and IGFBP3 over the same period. Type 3 collagen formation increases more relative to European children in the first 30 days of rehabilitation than type 1. Growth is predominately ponderal in this early phase of rehabilitation reflected in early improvement in WHZ but not HAZ scores. Change in P1CP, P3NP, BAP, IGF1 and IGFBP3 all correlated with weight gain over the same period. Change in collagen formation markers and IGF1 levels over 30 days also correlated with ponderal growth over 90 days but less well.

The most significant predictor of linear growth in these children that we were able to identify was their weight for height z scores at presentation. Most linear growth occurred in the later stages of the 3 month follow-up associated with significant ponderal growth. Knemometry behaved as a ponderal index acutely in this group of children reflecting soft tissue deposition as well as bone growth and only after 45 days correlated with linear growth. Change in collagen formation correlated with the small amount of linear growth over the first 30 days and this correlation improved with

linear growth over 90 days. Change in lower leg length over the first 30 days correlated significantly with change in both type 1 bone predominate collagen, type 3 soft tissue collagen, BAP and IGF1 as did change in WHZ score. Improved correlations with change in P1CP and P3NP were associated with lower leg growth over 90 days that correlated in addition with change in IGFBP3 (as did change in HAZ) and change in BAP.

Collagen degradation was assessed both by urinary levels of pyridinium crosslinks and plasma levels of cross-linked telopeptide of type 1 collagen. 1CTP fell with rehabilitation and correlated negatively with type 1 collagen formation (P1CP), IGF1 and IGFBP3 and positively with IGFBP2 levels. Conversely PYD and DPD levels increased and did not correlate with type 1 collagen formation markers or growth factor activity. PYD and DPD were measured in spot urine samples and expressed them as a ratio to creatinine excretion which may well change dramatically in the malnourished catabolic child who becomes anabolic (Branca 1992). 1CTP is a plasma marker and not subject to changes in creatinine production and excretion.

Marasmus and kwashiorkor occupy opposite ends of the clinical spectrum of severe malnutrition and may represent both different types of and responses to nutritional insults. The children with kwashiorkor in our study had significantly better initial HAZ scores than the marasmic children indicating that the chronic nutritional insult underlying their acute nutritional deficit (manifest in WHZ score) was less severe in nature. Similarly aged marasmic Gabonese children demonstrated low initial IGF1 and IGFBP3 levels with increased IGFBP3 proteolytic activity which normalised after 4 weeks of rehabilitation. Gabonese children with kwashiorkor however initially had

low IGF1 and normal IGFBP3 levels with low IGFBP3 proteolytic activity and after rehabilitation IGFBP3 levels fell and proteolytic activity increased (Zamboni 1996). This was proposed as an aflatoxin mediated effect however when comparing Bangladeshi and Gabonese children with kwashiorkor the difference in initial HAZ scores and thus the chronicity of the nutritional insult was impressive (-2.9 vs –0.34). Severe prolonged nutritional insults cause greater deficits in HAZ scores as well as IGFBP3 levels and difference in IGFBP3 levels between children with marasmus and kwashiorkor disappeared when differences in initial HAZ score were controlled for. Linear catch-up growth also correlated significantly and positively with change in IGFBP3 levels.

In conclusion, bone remodelling and soft tissue turnover commence early in the nutritional rehabilitation of severely malnourished children and did not differ between the 3 zinc regimens. This switch to collagen deposition and bone growth is associated with a fall in IGFBP2 and an increase in IGF1 and IGFBP3. Alterations in GH/IGF axis local mediators in response to diet may control the coupling of bone resorption to formation and collagen deposition in these children.

Bone remodelling and soft tissue turnover is complex and the measurement of a number of biochemical variables representative of different processes is required to understand this complexity. The combination of assays employed here to document collagen deposition, degradation, and IGF axis changes offer a comprehensive toolbox to allow the identification and assessment of individual processes that combine to produce growth. Nutritional interventions to produce growth (usually combining micronutrients and macronutrients) act at many levels and it is often

unclear to what extent ponderal and linear components contribute overall, to what extent individual processes contribute either to ponderal or linear growth and what micronutrient/macronutrient components of an intervention specifically do. Measurement of the individual components of growth and the use of a toolbox of assays of collagen turnover, bone growth and IGF axis activity may allow a more detailed understanding of the effects of interventions and the optimisation of such interventions to produce optimal patterns of growth. Complex toolboxes produce complex results however and further studies are needed to test the utility of such an approach.

Clinical Implications

The correct micronutrient/macronutrient mix to enable severely malnourished children to realise their full growth potential remains unclear. Much progress has been made in optimising regimens and the current WHO guidelines represent best practice as currently understood. Zinc supplementation is known to be an essential component of this. This study demonstrates that the use of high dose or prolonged zinc supplementation did not produce significant additional benefit and that the use of high dose supplementation was associated with increased mortality.

Zinc supplementation of deficient children improves immune function and there is no evidence that zinc supplements cause harm when given to non-septic immunocompetent children. In the septic severely malnourished child however the immune response to supplementation is less predictable and competing microbes may benefit before the severely compromised immune system can use the supplement to recover. The dose of supplement employed here may well have tipped the balance of benefit in favour of the pathogen and away from the malnourished child.

It must be emphasised that all the organs/organ systems of severely malnourished children are compromised and that the immune system is as damaged as, for example, the gastrointestinal system. This accounts for the largest proportion of early mortality during rehabilitation of these children and underlies the WHO's recommendations that all such children should receive empiric broad-spectrum antibiotic coverage on admission. Nutritional interventions in this group of children must recognise this and the macronutrient/micronutrient mix that is most beneficial for well malnourished

children is not necessarily what is best for septic malnourished children – this has already been demonstrated with iron supplementation of these children.

Until the optimal macronutrient/micronutrient combination is better defined high dose zinc supplementation of severely malnourished children should be avoided.

Future Research

The research documented in this thesis has raised more questions than it has answered. Particular areas and questions have been highlighted as requiring further research and illustrate the need for more 'mechanistic' studies on the effects of supplementation.

Zinc

- a biochemical index of zinc sufficiency unaffected by the presence of inflammation is required to define the zinc status of individuals on entry into supplementation trials.
- a better understanding of the mechanisms and control of zinc flux and storage, and the contribution of host genetic heterogeneity is required to predict results of supplementation
- the effects of inflammation and ongoing sepsis on the response to micronutrient supplementation and whether optimal micronutrient/macronutrient combinations depend on the presence/absence of ongoing sepsis

Growth of severely malnourished children

- The determinants of linear catch-up growth of severely malnourished children remain to be determined – we could only identify factors that accounted for 20% of variance in this study. Whether these determinants are amenable to intervention also needs to be answered.
- The effects of micronutrient/macronutrient supplementation and interaction on the composition of newly laid down tissue, bone growth, GH-IGF axis activity and the promotion of linear as opposed to ponderal growth requires further research.
- We need to understand better what represents optimal ponderal and linear growth of severely malnourished children and to know how aggressive should we be in promoting it.

Appendix 1: Sample size calculation

With 90% power at the 5% level, the following approximate sample sizes would be required (formula $n = 10.5 (SD_1^2 + SD_2^2) / D^2$ where SD_1 and SD_2 are the standard deviations and D is the difference in the outcome measure.

i) Bisgaard *et al* showed the SD of normal lower leg length in this age group to be 153 μm / day, and the daily leg length growth using weekly measurements to be 92 μm / day. Using these figures in the above formula, to show a difference between the groups of 92 μm / day increase in lower leg length, **n = 29**. The accuracy of measurements with the portable knemometer is approximately 50 μm / day.

ii) Kabir *et al* showed a difference in change of height for age Z-score of 0.09 between groups fed normal protein or high protein diet in malnutrition refeeding. Standard deviations of the two groups were 0.04 and 0.12. Using these figures, in order to show a difference of 0.09 in change of height for age Z-score between the two intervention groups, **n = 21**.

iii) Kabir *et al* showed a difference in change of weight for age Z-score over 21 days of 0.23 between groups fed normal protein or high protein diet in malnutrition refeeding. Standard deviations of the two groups were 0.27 and 0.38. Using these figures, in order to show a difference of 0.23 in change of weight for age Z-score between groups, **n = 43**.

iv) Kabir *et al* showed a difference in change of weight for height Z-score over 21 days of 0.25 between groups fed normal protein or high protein diet in malnutrition refeeding. Standard deviations of the two groups were 0.3 and 0.4. Using these

figures, in order to show a difference in weight for height Z-score of 0.2 between groups, **n = 50**.

v) Skin fold thickness and mid upper arm circumference - data for calculation of sample size not available.

vi) Kabir *et al* showed a difference of 23.4 nmol / l in change of IGF 1 following refeeding malnourished children with normal protein *vs* high protein diet. Standard deviations in the two groups were 16.2 nmol / l and 9.2 nmol / l. Using these figures, in order to show a difference of 23.4 nmol / l in change of IGF1 between groups, **n = 7**.

vii) IGFBP3 - no data available for sample size calculations.

viii) Trivedi *et al* showed a difference in P1CP levels of 130 μg / l between children with average height velocity of 2.18 cm / y and 6.02 cm / y. Standard deviations in the two groups were approximately 250 μg / l. Using these data, in order to show a difference of 130 μg / l in change of P1CP between groups, **n = 39**.

ix) Trivedi *et al* showed a difference in P3NP level of 2.1 μg / l between children with average height velocity of 2.18 cm / y and 6.02 cm / y. Standard deviations in the two groups were approximately 4.125 μg / l. Using these data, in order to show a difference of 2.1 μg / l in change of P3NP between groups, **n = 41**.

x) Branca *et al* showed a difference in PYD of 21 nmol / h / m^2 between malnourished and recovered children. Standard deviations of the two groups were 4.6 nmol / h / m^2 and 10.8 nmol / h / m^2. Using these data, in order to show a difference of 5 nmol / h / m^2 in change of PYD between groups, **n = 58**.

xi) Branca *et al* showed a difference of 4.9 nmol / h / m^2 in DPD between malnourished and recovered children. Standard deviations of the two groups were 1.3

nmol / h / m^2 and 3.0 nmol / h / m^2. Using these figures, in order to show a difference of 2.5 nmol / h / m^2in change of DPD, **n = 18.**

Taking the largest anthropometric outcome of these sample size calculations, the total sample size would need to be 150. Allowing for 20% drop out, if we recruit 180 subjects complete data should be available on 150 subjects. Stratified randomisation should ensure that other confounding variables are equally represented although collection of data on possible confounders should enable these variables to be controlled for in a multiple regression analysis if necessary.

Appendix 2: liquid diets

1. **Rice Powder formula**

60gm of rice powder

40ml of soya oil

75gm of egg albumin

Water up to 1000ml

- 2590 kJ energy
- 11 gm protein
- 3 mg zinc

2. **Dried Skimmed Milk Formula**

80gm of dried skimmed milk

65gm of sugar

49ml of oil

Water up to 1000ml

- 2640 kJ energy
- 22 gm protein
- 3 mg zinc

Appendix 3: Zinc and placebo formulations

Zinc as zinc sulphate ($ZnSO_4$) in 200mg tablets.

200mg $ZnSo_4$ tablet equivalent to 81.46 mg zinc.

	Formula 1	**Formula 2**	**Formula 3**
$ZnSO_4$	44gm	352gm	/
Sugar	5.81kg	11.63kg	5.81kg
Citric acid	2.91gm	5.81gm	2.91gm
Na Benzoate	11.63g	23.26gm	11.63gm
Aroma orange	11.63gm	23.26gm	11.63gm
Distilled water to	11.63 litres	23.26 litres	11.63litres
Elemental Zn/ml	**3.75**	**15**	/

Appendix 4: Consent form

Consent form **Name of Patient**_____

Your child has been admitted to this unit because he/she is malnourished. He/she needs to be carefully fed for two weeks or more to make him/her better. We are doing a study to help to find out what is best for a child to be fed at this important time, and how this might make him/her grow.

In the study, we will give some children a multivitamin syrup containing a certain dose of zinc, and others a multivitamin syrup with a smaller dose of zinc. Which children get what dose of zinc will be decided by chance. We will then see you during the admission here, and afterwards in the clinic every 2 weeks for 3 months, to measure your child. Some people have shown that zinc may improve the way a child grows, while others have not shown this. Zinc does not harm your child. By comparing the growth of children in the two groups, we hope to show whether or not zinc helps.

We also want to look at how children grow after being sick. To do this we need to do three blood tests over a 1 month period. These blood tests involve one prick with a needle each, and a small amount of blood being taken for some tests to be done in the laboratory. The blood is taken from a vein, and each time we will take about 1 teaspoon-full (5ml). The tests will give us important information about how the zinc might work. We can give you more information about these tests if you want.

The study involves no risk to your child. There will be some minor, temporary discomfort due to the blood tests.

If you agree, we will include your child in the study. If you do not agree, then your child will still get all the normal treatment on the unit. If you agree now, but later change your mind, then you can remove your child from the study immediately without affecting his/her further treatment in this unit.

If you are happy for your child to be included in the study, please give your consent by signing this form. If you have any questions about the study please ask.

Parent's / guardian's signature / left thumb print.....

Prof Akbar's signature Dr Conor Doherty's signature

Dr Kaseem Sarkar's signature Dr Saleem Shakur's signature

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