# Trace element metabolism in human pregnancy.

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# TRACE ELEMENT METABOLISM IN HUMAN PREGNANCY

BY

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To my Parents and Family

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# Trace Element Metabolism in Human Pregnancy

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The aims of this study were:

i. to investigate the dietary intake of the essential trace elements, zinc and copper during pregnancy,

ii. to assess the possible effects of these intakes on the outcome of pregnancy,

iii. to study the absorption and retention of zinc, copper and manganese in normal primigravidae and those at risk of delivering small,light infants.

Intakes of zinc and copper were calculated from weighed dietary surveys using food composition tables and the validity of these tables to calculate dietary nitrogen, zinc and copper was assessed by comparing analysed and calculated values. To investigate the effect of pregnancy on the absorption and retention of zinc, copper and manganese, metabolic balance studies were conducted on pregnant and non-pregnant women.

Dietary intakes of zinc were less than the recommended level (U.S. Nat. Acad. Sci 1980). Zinc intake correlated with protein intake. Dietary copper intakes were lower than levels previously reported and less than the 'safe and adequate intake' (U.S. Nat. Acad. Sci. 1980). Dietary intakes of zinc and copper were not significantly different between a group of women who delivered small,light infants and those who delivered normal healthy infants.

These recent findings suggest the U.S. Nat. Acad. Sci. recommended dietary intake for zinc during pregnancy maybe over estimated and therefore require reviewal and reassessment.

Zinc and copper were retained in normal primigravidae during the third trimester; this may be related to the concomitant increase in fetal and maternal weight. Zinc and copper balance were negative in the primigravidae at risk of delivering small,light infants. This requires further investigation. Net intestinal losses of manganese was apparent in all groups of pregnant women.

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# Chapter I

# Introduction

During the past thirty years there has been a remarkable advance in our understanding of the physiological and biochemical roles of trace elements in both animal and plant species. With the development of analytical procedures such as atomic absorption spectrophotometry, neutron activation, and X-ray microanalysis, the concentration and distribution of an element in the tissues, cells and organelles can be measured. At present nine trace elements are generally accepted as essential to animals, iron, zinc, copper, manganese, molybdenum, chromium, iodine, cobalt, selenium, and evidence is accumulating that another five, vanadium, nickel, silicon, fluorine and tin may also be essential. At least the first nine are thought to be essential for the optimal health of humans. Essential elements perform functions indispensable to the maintenance of life, growth and reproduction. Consequently, an inadequate intake of an essential trace element may impair cellular and physiological functions and causes illness. Non-essential trace elements are also present in tissues in minute quantities. Some of these, e.g. lead, cadmium, mercury may, in excessive amounts, interfere with normal body functions and are therefore considered toxic trace elements but it should not be forgotten that all metals are potentially toxic.

Examination of the periodic table shows that eight of the fourteen essential trace elements, namely vanadium, chromium, manganese, iron, cobalt, nickel copper and zinc occur in the first transition series of elements. These elements tend to be reactive chemically, readily forming compounds and catalysing reactions; they are used extensively in industry for this purpose. In the body

they function similarly, contributing to enzyme systems either in weak ionic association or as integral components of metalloenzymes. In the latter associations removal of the metal results in inactivity of the enzyme; the protein molecules of the enzyme are associated with a constant amount of metal which normally cannot be replaced in vivo by any other element. If non-essential trace elements present in the body, e.g. lead, cadmium, mercury, reach excessive levels they may interfere with the metabolism of essential trace elements by substitution in, or inhibition of, metalloenzymes. For example, renal dysfunction associated with cadmium toxicity may be due to the adverse effects of cadmium on zinc metalloenzymes necessary for reabsorption of proteins from the kidney (Piscator, 1976). In a state of trace element deficiency, or toxicity the recognition of a specific metalloenzyme may help us attribute physiological or clinical manifestations of the condition to the effect on the enzymes at the cellular level. For example, a dietary copper deficiency in cattle can cause "falling disease" (Bennetts, Beck and Hartley, 1948). A reduced activity of the copper containing enzyme, lysl oxidase is thought to result in myocardial defects which cause sudden death in the deficient cattle. Lysl oxidase catalysis the cross-linking of collagen and elastin in connective tissue formation. Copper deficient animals frequently die suddenly from myocardial and arterial defects (O'Dell et al, 1961; Shields et al, 1962).

A possible role of copper for normal prenatal development of mammals was suggested by Bennetts and Chapman (1937). They found that enzootic ataxia in lambs could be prevented by giving the ewe

additional copper during pregnancy. Affected lambs had light, poorly developed brittle bones and myocardial defects as well as the neurological defects. As far as is known overt manifestations of neuropathological effects attributable to copper deficiency are confined to lambs and human infants with the genetic, X-linked, defect in copper metabolism, Menkes' disease. Copper deficiency in the young of most species results in skeletal and cardiovascular abnormalities, inhibits melanogenesis and erythropoeises and causes growth failure (Danks, 1980). Copper deficiency in the pregnant rat causes extensive fetal death and resorption even though no external signs of copper deficiency may be evident (Hall and Howell, 1969). The teratogenic effects of copper deficiency in a number of species has been extensively reviewed by Hurley, (1981).

Similarly profound manifestations of zinc deficiency in animal species have been reported. At present, in all species, over 200 zinc metalloenzymes have been described occurring in all the major metabolic pathways. Todd Elvehjem and Hart, (1934) demonstrasted the essentiality of zinc to the normal growth and health of the rat. Thereafter, experimental zinc deficiency was demonstrated in a number of other mammalian species, e.g. dogs, mice, calves. The signs of zinc deficiency in these animals were growth retardation, emaciation, conjunctivitis and skin lesions on the abdomen and extremities,(Underwood, 1977).

Zinc plays an important role in nucleic acid metabolism and it has been suggested that impaired DNA synthesis in zinc deprived embryoes may result in congenital malformations (Dreosti, Grey and Wilkins 1972). Pregnant rats given zinc deficient diets develop

symptoms within a few days and even preimplantation embryoes have been shown to be morphologically abnormal if a zinc deficient diet is given from the time of copulation (Hurley and Shrader, 1975). A group of rats maintained on a zinc deficient diet throughout pregnancy produced fewer young per litter than controls, and the young were less than half the normal weight at term. In this zinc deficient group 54% of the implantation sites had signs of fetal resorption and 45% of the term fetuses had congenital abnormalities, thus 99% of the implantation sites were affected (Hurley and Swenerton 1966).

Even transitory periods of zinc deficiency during gestation produced abnormalities which varied according to the developmental events occurring at that time. A group of dams fed with a zinc deficient diet throughout gestation produced pups with greatly reduced birthweights and congenital malformations of practically all organs. Even in this acute deficiency state of the fetus maternal zinc stores were not mobilized to meet the requirements of the offspring (Hurley and Swenerton 1971). The consequences of zinc deficiency during pregnancy in various animal species has been extensively reviewed by Hurley (1981).

Manganese deficiency in animals results in a number of structural and physiological defects which are similar in several species. Some manganese enzymes have been isolated, namely, pyruvate carboxylase, mitocondrial superoxide dismutase, and avinmangin. (Prasad, 1978). In contrast to the specificity of zinc and copper, manganese is largely involved in non-specific enzyme activation reactions, for example the glycosyl transferases

necessary for normal mucopolysaccharide synthesis. (Leach 1971). Skeletal abnormalities found in chick embryoes from manganese deficient hens were the first indication that manganese may be important in prenatal development (Lyon and Insko 1937). The effects of prenatal manganese deficiency in a number of species has been described (Hurley, 1981). A postural defect, a congenital ataxia, thought to result from a defective development of the otoliths in the inner ear of manganese deficient animals has been observed by several investigators (Hill et al, 1950, Hurley Everson & Greiger 1958, Erway, Hurley and Fraser, 1970).

The effects of zinc, copper and manganese deficiency in animal species have been outlined. An important role of these nutrients in normal fetal development is suggested by the profound effects of a deficiency during gestation described in some animal species. The vital importance of these trace elements is extended to human nutrition and reproduction.

The human adult body contains about 1.5 - 2.5g of zinc (Widdowson, McCance and Spray, 1951). Body zinc associated with bone and muscle maynot be nutritionally available unless it is released, adventitiously, when tissue is catabolized. Of whole blood zinc approximately 75% is contained in the erythocytes as a component of carbonic anhydrase. Leukocytes contain about 3% of whole blood zinc, and plasma zinc, (representing 22% of whole blood zinc) is bound to albumin and, $\alpha_2$ - macroglobulin. Despite the isolation of a multitude of zinc metalloenzymes, as yet, no limiting body pool or functional store of zinc has been identified. The absence of such a biologically available zinc pool may explain why symptoms of

zinc deficiency develop rapidly in experimental animals when net catabolism of tissues is prevented.

Nutritional dwarfism associated with possible zinc deficiency has been described in a number of Middle East countries (Sandstead et al, 1967, Halsted et al, 1972) and aetiological factors in the syndrome are thought to comprise of the following. The unavailability of zinc from diets consisting largely of unleavened wholewheat bread. Zinc is widley distributed in food and therefore dietary zinc intake itself is unlikely to be very low, however, zinc availability may be reduced by the presence of phytate (inositol hexaphosphate) which forms an unabsorbable complex with the zinc (Davies & Olpin 1979). Phytate is found in most cereals, thus zinc from these sources may be less available than from animal protein. Prasad, Halsted and Nadimi (1961) suspected zinc deficiency in eleven Iranian male dwarfs suffering from growth retardation anaemia, hypogonadism, hepatosplenomegaly, rough dry skin and general weakness. The habitual diets of these subjects, and those in a later study, (Halsted et al 1972), consisted largely of unleavened bread made from wholewheat flour. Therefore, despite an apparently adequate dietary zinc intake it may be that the absorption of zinc was impaired. A marked improvement in height, weight and sexual development occurred after provision of a 'western type' diet, high in animal protein, low in phytate. The presence of other nutritional deficiencies was not excluded from this study. It may be the syndrome resulted from a combination of nutritional inadequacies which were alleviated by the provision of a 'better' diet. In addition, geophagia was common practice amongst these subjects; clay may interact with the zinc rendering it unavailable for absorption. (Halsted, 1968).

A trial of zinc supplementation in growth retarded but otherwise outwardly healthy Iranian schoolboys was carried out by Ronaghy et al, (1974). They were all maintained on their customary diet; wholewheat unleavened bread was the main staple; randomly divided into three groups, all groups received a nutritional supplement. In one group the zinc supplement 40mg/day was omitted from the preparation. The control group received a placebo preparation. After 18 months a significantly increased height, weight, and bone age had occurred in the group which received a zinc supplement, little difference was found at 6 months. Considering the rapid response to zinc supplementation in zinc deficient animals and acrodermatitis enteropathica patients (Braun et al, 1976) one may have expected a more rapid response in these deficient boys. Dermal losses of zinc maybe high in villagers working in the sun in these very hot countries. Parasitic infestations, schistosomi asis and hookworm disease result in chronic blood loss. As red blood cells contain approximately 12ug zinc per ml, (Prasad, 1978), diseases associated with chronic blood loss will also result in considerable zinc loss. These infestations are prevalent in tropical, moist areas such as the Nile Delta (Sandstead et al 1967).

That these initial suggestions of a zinc deficiency syndrome in man associates with other nutritional deficiencies cannot be excluded. After all, the symptoms were not the typical manifestations of zinc deficiency seen in Acrodermatitis Enteropathica or protein-energy malnutrition. However, the possibility of a milder zinc deficiency with more subtle symptoms occurring in other populations should be

considered. It is possible that people in underdeveloped nations who do not suffer from malnutrition and/or infection may have varying degrees of zinc deficiency associated with a primarily unrefined cereal diet.

Since many of the enzymes involved in DNA and protein synthesis are zinc metalloenzymes, clinical and biochemical manifestations associated with zinc deficiency are similar to those seen when protein synthesis is inhibited for some reasons e.g. in essential amino acid deficiency and anti-mitotic drug administration (Golden and Golden, 1981). Hence, one of the first signs of deficiency, skin lesions, occurs in a body tissue with one of the highest rates of cell turnover. Likewise those groups undergoing rapid tissue growth and development, eg. during pregnancy, puberty, post-surgery have the highest zinc requirement and may therefore, be at risk of becoming zinc deficient.

In western countries there is some suggestion that zinc deficiency may occur secondary to other conditions. A reduction of effective absorptive surface due to mucosal disease e.g. in Coeliac disease, Crohn's disease and short bowel syndrome may result in malabsorption and increased faecal zinc, (Crofton et al, 1982). Zinc deficiency should be considered a possible factor in growth retardation associated with malabsorption disorders in childhood Crohn's disease cystic fibrosis and coeliac disease. Aggett et al, 1979 found a reduced absorption and retention of zinc in children with cystic fibrosis and suggested a possible relationship between this and the associated features of the disease.

A major problem in tracing mild zinc deficiency in any population is lack of adequate criterion for diagnosis. Various biochemical parameters have been suggested as indicative of body zinc status, eg plasma, leucocyte, hair and salivary zinc (Meadows et al, 1981, Solomons, 1973). A study in Denver, Colorado (Hambidge et al, 1972) revealed that ten apparently healthy children from upper or middle socioeconomic families had low levels of hair zinc (less than 70ppm), a poor appetite, hypogeusia and low growth percentiles, nine had heights and/or weights on or below the 10th percentile. The commencement of dietary supplementation with zinc resulted in a consistent improvement in taste acuity, appetite, dietary intake, four children showed an increase in growth rate. It was suggested that the poor appetite, growth and hypogeusia in these children maybe attributable to mild zinc deficiency. This study was followed by the supplementation of a commercial infant feeding formula with zinc; male but not female infants grew more rapidly on the supplemented formula (Walravens and Hambidge, 1976).

There is evidence to suggest that the bioavailability of iron may be adversely affected by the presence of zinc at a molar ratio of 1 : 1 (Crofton, Gvozdanovic and Aggett, 1982). Other workers have suggested a similar interaction with pharmacological doses of iron and zinc (Meadows et al, 1982). A recent study by Solomons and Jacobs (1981), showed that non-heme iron effectively depressed zinc absorption when present in the mg ratio of 2 : 1 or 3 : 1 of non-heme iron to zinc, (zinc was present at 25mg $ZnSO_4$), while heme iron had no effect on zinc absorption. They found a similar ratio of inorganic iron to zinc did not have the same effect on zinc

absorption when oysters were the source of zinc ie. 'organic' zinc. This suggests the chemical nature of zinc itself may influence its absorption. High doses of ethylenediamine tetracetic acid (EDTA) reduced the absorption of zinc from aqueous solution in adult subjects (Solomons et al 1979). EDTA is added to various prepared and processed foods e.g. sandwich spreads, sauces, carbonated drinks, beers, salad dressings to prevent oxidative damage by free metals. (Underwood, 1977).

The most clear-cut human zinc deficiency disorder is acrodermatitis enteropathica (AE), a rare congenital defect of zinc absorption. Moynahan (1974) reported that AE patients responded clinically and biochemically to pharmacological doses of zinc. Prior to this AE patients were treated with the drug 5, 7-diodo-8-hydroxy quinoline (DIH), (Dillaha Lorinez and Aavik, 1953), but suffered spontaneous remissions and exacerbations. Until the advent of DIH therapy, and subsequently zinc therapy few patients lived beyond childhood. Consequently only during the past decade have reports of AE in human pregnancy appeared. The main characteristic clinical features of AE are skin lesions, particularly around the orifices, diarrhoea, growth retardation and failure to thrive. Clinical signs of the disease first become manifest in infancy but are likely to be delayed until weaning if the infant is breastfed. If untreated, patients with severe AE have a fluctuating, but ultimately relentless downhill course with anorexia, severe failure to thrive and bacterial infections leading to a fatal outcome in early childhood. It is now accepted that the clinical picture of AE is one of profound zinc deficiency, the

pathogensis of which is probably an intestinal malabsorption of zinc. Intestinal absorption of zinc was reduced in young patients with AE (Weismann et al 1979). Oral supplementation with therapeutic doses of zinc salts produces complete remission of symptoms.

A relationship between human congenital anomalies and zinc deficiency has been suggested in pregnancies in Acrodermatitis Enteropathica (AE) patients. (Hambidge, Nelder and Walravens 1975). They suspected that pregnancy in an untreated or partially treated woman with AE could expose the fetus to intense intrauterine zinc deficiency. There have been eleven reported pregnancies in AE patients untreated with zinc. One woman who received no treatment at all had two normal pregnancies followed by two pregnancies during which severe exacerbation of her disease required therapuetic abortion. (Olholm-Larsen, 1978). Another woman who received DIH treatment during the last two trimesters of her pregnancy gave birth to an achondroplastic dwarf; she was treated with DIH throughout her following three successful pregnancies. (Epstein and Vedder, 1960). Of two other pregnancies in AE, treated with DIH throughout pregnancy, there was one spontaneous abortion and an anencephalic stillbirth (Nelder and Hambidge, 1975). Two successful pregnancies in an AE patient treated with zinc sulphate have been reported (Brenton, Jackson and Young 1981).

A relationship between maternal zinc deficiency and congenital malformations of the central nervous system has been suggested in two regions in the world, namely, in Iran and Egypt, where zinc deficiency in human beings has been observed, along with the highest incidence of spina bifida and anencephaly (Sever and Emmanual 1973,

Halsted, 1973). A group studying in Turkey found maternal serum zinc levels were significantly lower in ten women who delivered anencephalic stillbirths compared to ninety who delivered normal infants (Cavdar et al, 1980).

Alcoholism maybe associated with poor zinc status: A distinct syndrome of abnormalities in infants born to chronic alcoholic mothers has been described (Jones et al, 1973). Microcephaly was present in seven of the eight infants. The cause of the fetal abnormalities in these alcoholic women was unknown but poor dietary intake is common among alcoholics and there is some suggestion chronic alcoholism is associated with a low zinc status. (Helwig et al, 1966, Sullivian and Lankford, 1962). A high incidence of malformations and low plasma zinc has been described in infants of alcoholic women, (Flynn et al, 1981).

Low plasma zinc concentrations were found in women who delivered infants with congenital abnormalities. (Soltan and Jenkins 1982). Jameson (1976) measured serum zinc in early pregnancy and found a notably high incidence of complications, especially inefficient labour and atonic bleeding, in women with low serum concentrations. In a group of 234 gravidae eight delivered infants with congenital abormalities. Of these eight, five had the lowest recorded serum zinc measured at 14 weeks gestation. In addition women who gave birth to premature, or growth retarded infants, showed lower serum zinc during early pregnancy than women with normal deliveries and normal infants. From these results and assuming serum zinc at 14 weeks gestation reflected zinc levels throughout pregnancy he postulated that low serum zinc

concentrations during human pregnancy may be a sign of zinc deficiency, implying risks to mother and infant. It is well documented that during pregnancy there is a decrease in plasma zinc (Hambidge and Droegmeuller 1974, Widdowson, 1974, Jameson, 1976, Tuttle, 1983). To what extent, if any, the fall indicates an increased fetal demand is unknown. Although there is a decrease in zinc concentration the total zinc mass maybe unchanged during pregnancy, (Tuttle et al, 1983). This maybe partly explained by the haemodilution producing a fall in zinc concentration without affecting the total zinc mass. Recently Hambidge et al, (1983) found that an early and progressive decline in plasma zinc was not influenced by zinc intake during gestation. Thus the fall in plasma zinc during pregnancy may not reflect an inadequate dietary intake.

Circumstantial evidence would suggest that zinc deficiency may have a profound effect on the outcome of a human pregnancy. The adverse effects of such a deficiency may occur early in gestation, before the mothers come under medical supervision. The possible existance of subclinical zinc deficiency during pregnancy requires investigation.

Of the 80 - 120mg of copper present in normal adult man most is located in the liver associated with copper binding proteins and metalloenzymes. Other copper rich tissues are hair, kidney, brain and heart (Widdowson, McCance and Spray, 1951). The distribution and amount of body copper varies with age and liver copper concentrations are at their maximum in the newborn infant (Widdowson, 1974). Most, (94%), plasma copper is a protein complex caeruloplasmin. A minute fraction is present as amino acid complexes

which may be important in the entry of copper into cells. Many copper - protein compounds have been isolated from tissues, several of which are enzymes with oxidative functions e.g. Superoxide dismutase, lysl oxidase, cytochrome oxidase, uricase, monoamine oxidase and ascorbic acid oxidase (Prasad, 1978).

Although copper and copper compounds were used to treat various human diseases as early as the nineteenth century it was not until 1930 that any suggestion of the clinical importance of copper in humans was made. Mills 1930 demonstrated an acceleration in haemoglobin synthesis in hypochromic anaemic human infants and adults in response to copper supplementation. These observations were confirmed by some workers but disputed by others. The controversy continues today as to whether copper deficiency exists in adult man. However, copper deficiency does occur in human infants in a number of circumstances. A copper responsive syndrome in Peruvian infants recovering from malnutrition has been described (Graham and Cardano, 1969). Other cases of copper deficiency in a premature, low birthweight infant have been reported (Griscom, Craig and Neuhauser, 1971, Ashkenazi et al, 1973, Yeun, Lia and Hutchison, 1979). During the latter part of pregnancy the accumulation of copper in the fetal liver is, presumably, sufficient to carry the infant from birth to weaning. Consequently rapidly growing premature infants with low copper hepatic stores maybe prone to copper deficiency if their copper intake is low. Al-Rashid and Spangler, (1971) reported the case of a premature infant who, at 3 months of age, presented with anaemia, neutropenia, hypocupremia and skeletal changes. The infant had received a milk formula with a low copper content since birth. Symptoms responded to copper sulphate therapy.

A congential clinical condition of copper deficiency described in male infants is Menke's kinky hair syndrome; a genetically determined defect in copper absorption. Infants present with blood, bone and cardiovascular abnormalities, severe mental retardation, and lack pigmentation of the hair and skin. The symptoms are unresponsive to oral or parenteral copper. Copper deficiency has been described in infants, older children and adults who are receiving parenteral nutrition, usually after gut resection or in chronic gut disease. The first report of copper deficiency during total parenteral nutrition (TPN) (Karpel and Peden, 1972) was in an infant who after surgical repair of an ileal atresia received TPN for the first seven months of life, at which time the first signs of copper deficiency appeared. Heller et al, 1978 reported skeletal changes of copper deficiency in two infants on TPN. These bone changes of osteoporosis, flaring of metaphyseals and fracturing at the end of long bones are similar to the changes seen in scurvy but develop in the presence of normal ascorbic acid levels. A copper responsive anaemia and hypocupremia occurred in a 14 year old boy receiving TPN for severe villous atrophy and mulutrition (McCarthy et al, 1978). Other cases of copper deficiency in patients receiving parenteral nutrition after extensive bowel surgery have been reported (Dunlap, James and Hume, 1974, Solomons Layden and Rosenburg 1976). The regimens used contained insufficient copper, and additional copper corrected the symptoms. Marginal copper deficiency has been reported in a patient with malabsorption given large doses of zinc (Porter et al, 1977). They reported the case of a coeliac patient who after receiving zinc supplementation for 14

weeks presented with profound hypochromic macrocytic anaemia. Her blood picture was responsive to withdrawal of the zinc supplementation and provision of oral copper sulphate. This may suggest an interaction between zinc and copper absorption.

Wilson's disease is an inherited disease of defective copper metabolism in which copper accumulates twenty to thirty fold in the tissues, predominately the liver, kidney and brain. If untreated the condition is generally fatal within two years. Treatment involves giving large doses of penicillamine to chelate intestinal copper, preventing its absorption.

The importance of copper during mammalian pregnancy has been described, (Hurley 1981) Little is known about copper intake and requirements during human pregnancy. It has been suggested the major source of copper during pregnancy is the maternal liver and lower concentrations of liver copper have been found in pregnant women as compared to non-pregnant (Mason, 1979). A tentative association between human maternal copper deficiency and malformations has been made. Morton, Elwood and Abernethy, (1976) reported neural tube defects and suggested that they may be associated with the copper content of water. Another study showed no correlation between maternal serum copper concentration and the incidence of anencephaly (Wald and Hambidge 1977). Rupp and Weser (1976), suggested that mothers treated with penicillamine may be at risk of maternal copper deficiency. Two cases have been reported of women who gave birth to infants presumed to have connective tissue abnormalities after receiving large doses of penicillamine during their pregnancy (Mjolnerod et al 1971, Solomon et al 1977).

The adult body contains approximately 12mg of manganese which is widely distributed in body tissues and fluids with the highest concentrations in the brain, kidney and liver. Circulating manganese is largely bound to a globulin, transmanganin (Cotzias and Bertinghamps, 1960).

A deficiency of manganese was observed in a human subject who was receiving an experimental diet deficient in Vitamin K (Doisy, 1973). Inadvertently, manganese was not included in the experimental diet, thus leading to Vitamin K and manganese deficiency in the subject. The subject suffered weight loss, transient dermatitis, occasional nausea and vomiting, slow growth and pigmentation of hair. Addition of manganese to the diet corrected this condition.

The importance of manganese in human pregnancy has not been established. Manganese levels in the fetal liver would suggest manganese is not stored there (Widdowson, Chan and Harrison 1972). The concentration of manganese in human milk is very low (Shaw, 1980), Therefore, the infant may obtain manganese from an alternative body store. It seems that although manganese is a small integral part of total nutrition, it plays a vital role in metabolic processes. Manganese deficiency has been described in animals but little knowledge of its biological action in man is available. Perhaps an adequate intake plus an efficient homeostatic control mechanism effectively combine to prevent human manganese deficiency.

Circumstantial evidence in man and animal studies would suggest that manganese, copper, and zinc are essential for prenatal development. Although this evidence is derived largely from animal

studies the essentiality of these elements for a successful gestation may also apply to human pregnancy. Growth and development of the fetus together with increases in maternal tissue impose greater nutritional demands during pregnancy. How these increased requirements are actually met is a matter of great interest. An increase in nutrient intake to meet the demands has not been shown nor disproven. The problem is largely one of technique in actually demonstrating an increased dietary intake from pre-pregnancy values. Previous studies have largely been concerned with energy and protein intake. Little is known about trace element intakes during human pregnancy. A number of reports of dietary intake would suggest normal intakes fall well below recommendations. Thus the following questions arise.:-

1. What are the dietary intakes of zinc and copper during human pregnancy?
2. How do they compare to recommendations/safe and adequate intakes?
3. Do they differ in women at risk of delivering small, light infants?
4. What happens to the absorption and retention of zinc, copper and manganese during pregnancy?
5. Does this differ in women at risk of delivering small, light infants?

This study evaluates dietary intakes of zinc. It investigates zinc, copper and manganese intake, absorption, and retention in normal primigravidae, in early and late pregnancy, and in those at risk of delivering small, light infants.

# SUBJECT, MATERIALS AND METHODS

## CHAPTER II

## MATERIALS

### Reagents

*Aristar Nitric Acid $HNO_3$ )

*Aristar Hydrochloric Acid HCl )

Analar Hydrochloric Acid ) BDH Laboratory Chemicals Limited,

Cupric Nitrate ) Poole.

Manganese Nitrate )

Zinc Nitrate )

* Maximum Limits of Impurities:-

| | |
|---|---|
| Zinc | 0.01ppm. |
| Copper | 0.002ppm. |
| Manganese | 0.005ppm. |

12 Ply Softer Swabs (Johnson & Johnson)

Fisons Fi-stream deionised water supply.

All containers used were of unpigmented polythene or polypropylene:-

5L containers for 6 x 24 hr urine collections) Stewarts Plastics Ltd.

Reject Diet )

1.5l containers for Faecal Collections ) Dines Ltd.

Faecal Swabs )

Urine Swabs )

Reject Diet Swabs )

15L containers for Duplicate Diet Pools 2 x 3 day pools )Boots Ltd.

Clear polythene gloves (Surgikos Ltd., Livingston)

Silverson Homogeniser

Homogeniser with Teflon coated head by P.S.B. Plastics Bordon Lindford

Silica Quartz Beakers (100ml)

Muffle furnace

## SUBJECTS

The pregnant women studied were healthy primigravidae. None of the women studied was on vitamin or mineral supplements prior to, or during, the study. All the women were Caucasian.

Group I (comprised Gp.I(a) & Gp.I(b))-selected, according to criteria established on local pregnant population, ten primigravidae of normal, weight for height (ie. between 25th & 75th centile) and weight gain 0.34-0.54kg between 20-30 weeks.
Group I(a)-Five completed a balance between 14-16 weeks.
Group I(b)-Seven, including two subjects from Group I(a), completed balances between 28-33 weeks gestation.

Group II - five primigravidae, selected as being at risk of delivering a small, light infant, completed a balance between 28 - 32 weeks gestation. These women were selected using the following criteria. Each woman exhibited at least two of the following four characteristics:

(1) Weight at 20 weeks $\leqslant$ 54kg

(2) Height $\leqslant$ 1.54m

(3) Weight for height $\leqslant$ 25 centile at 20 weeks gestation

(4) Weight gain between 20 and 30 weeks $\leqslant$ 0.35kg/week.

An association between low birthweight and these four indices has been demonstrated in Aberdeen primigravidae (Campbell-Brown 1982).

Non-Pregnant Group - four non-pregnant women aged between 20 - 25 years, not on any form of oral contraceptive.

## ATOMIC WEIGHTS

| | | |
|---|---|---|
| Zinc | Zn | 65.4 g |
| Copper | Cu | 63.5 g |
| Manganese | Mn | 54.94g |

## CONVERSION FACTORS

Gravimetric to Molar Quantification

| | | | | |
|---|---|---|---|---|
| (Zn mg) | x | 15.2 | = | umol |
| (Cu mg) | x | 15.7 | = | umol |
| ( Mn mg) | x | 18.2 | = | umol |
| $N_2$ g | x | 71.4 | = | mmol |

Individual details of all the women studied are given in Appendices I and II. Women were recruited at an ante-natal clinic where a detailed description of the entire study was given and agreement to participate obtained. A detailed dietary assessment was then taken from each patient. The aim was to obtain an accurate account of the type and quantity of food normally eaten. From this, a menu for the balance period was drawn up which simulated as near as possible the customary home diet. However, in attempting to do this certain problems arose. Firstly, the accuracy of recall dietary histories is subject to some uncertainty:- It is likely that, while recalling one's 'typical' food intake, certain food items will be omitted by the subject through simple forgetfulness or because the patient thinks they may be considered undesirable, organ meats and shellfish, both rich sources of zinc and copper, are consumed relatively infrequently and may, inadvertantly, be excluded from the dietary history; even if extreme care is taken the accuracy of recalling quantities of food normally consumed can be dubious. To minimise any error a check list was used to double check the intake of foods consumed, household measures were used to indicate quantities. Secondly, some commonly consumed foods eg. pies, stews, multi-ingredient dishes, are unsuitable for balances because an accurate duplicate cannot be obtained. This imposes limitations on the choice of foods for the balance diet. To overcome this each ingredient was weighed and duplicated separately.

The Clinical Research Unit has a specially designed metabolic ward comprising of a kitchen; with facilities for preparing, weighing and sampling food; 'dry toilets' for specimen collection and refrigerated lockers for storing specimens. The rooms are single or twin bedded. The research nursing staff are familiar with balance study procedures. Women were admitted to the metabolic ward for a ten day study period which consisted of an adjustment period of four days followed by a six day balance. Using the previously obtained dietary history, a ten day menu was planned representing as near as possible, the diet eaten by the patient at home. Any necessary adjustments to the quality or quantity of the balance diet were made during the adjustment period. This may have involved adjusting portion sizes, salt content or length of cooking. Major changes were avoided to prevent any significant fluctuations in mineral balance. As the women were on daily menus representing their home diet and not subject to any significant change in dietary intake a four day adjustment period was felt to be adequate. At the beginning and end of the balance period a non absorbable edicol blue faecal marker (Analysis Appendix I) was given to the volunteer. Faecal collections continued after the balance until the second faecal marker was passed. No blood samples were taken during the study. Volunteers were asked to avoid having very hot baths which may induce sweating and increase mineral losses. The importance of rinsing thoroughly after baths or showers to reduce contamination from soaps was stressed and talcum was not used during the balance period. Volunteers were asked to rinse their mouths out properly with deionised water after teeth cleaning and avoid swallowing any

toothpaste. They were encouraged to maintain normal activity and to go for short walks within the hospital grounds.

MATERIALS AND METHODS:- Extreme care was taken at every stage of the balance to avoid contamination of the specimens. All collection and storage containers were of unpigmented polythene or polypropylene type and had air tight lids; before use, each vessel was acid washed. Containers were soaked in 10% nitric acid (Analar) for 24 hours, rinsed six times in deionised water dried, weighed and stored sealed to prevent dust contamination; plastic containers for urine, faecal and diet collections, containers for swabs and all bottles for storing samples and reagents were acid washed; all acids and reagents used were of the highest purity available; deionised water was from a Fisons Fi-stream water still.

Talcum free polythene gloves were used for food preparation, handling and treating of all specimens in the laboratory. Volunteers also wore gloves for eating 'finger' foods and during the toilet procedure.

Preparation of deionised swabs:- The method described by Alexander and Delver (1972) was used. A 20L polythene vat was $^{2}/_{3}$ filled with deionised water in which 500g of Ethylenediamine Tetracetic Acid Disodium Salt (EDTA) was dissolved. The pH was adjusted to between 6 and 7 by the addition of ammonium hydroxide solution. The swabs were soaked in this solution for 24 hours, squeezed to remove the element rich EDTA and returned to solution for a further 24 hours. The swabs were then squeezed every 24 hours and returned to fresh deionised water for a total of six

rinses. The sodium content of the final rinse was checked before the swabs were finally squeezed and dried in an oven at $50^{0}$C, ready for use. Subsequent analysis of random swab samples from each batch showed negligible zinc, copper and manganese content.

Balance Diet:- During the balance period the protein content of the diets was maintained at ± 5g day. All food was prepared in the kitchen of the Research Unit. Cooking was carried out as in the home using ordinary cooking pots, pans and cutlery. Ordinary tap water was used in cooking. Only foods which could be duplicated accurately were used. Duplicates were from the same batch, tin or packet. Meat was cooked separately from vegetables, the same cut of meat was used for the diet and duplicate after all gristle or visible fat had been removed. Fish for the diet and duplicate were from the same fillet. Fish coated with ruskoline or batter were not suitable:- the amount of coating may vary and when fried may absorb different amounts of fat. All bread used had crusts removed before weighing. Only hard-boiled and scrambled eggs were used, yolk and white of hard boiled eggs were weighed and duplicated separately. Fresh oranges, bananas, apples had all inedible parts removed and the diet and duplicate sample were obtained from the same portion. Only soft chocolate or pastilles were suitable for homogenising.

Daily fluid allowances:- were weighed into plastic jugs and stored in the refrigerator. Milk was shaken well before sampling. Plastic cups were used throughout the balance.

SAMPLE COLLECTION

Duplicate Diet:- Each food item was weighed individually on a Salter R1 3000 balance and a duplicate sample obtained on a similar dish to ± 0.1g. Duplicate diet samples were pooled over 3 days and stored at -20°C throughout the balance. A spatula and deionised water were used to scrape and rinse duplicate diet plates to ensure the complete diet sample was collected. The patient's food was served on polythene covered trays and polythene cutlery provided.

Reject Diet:- Leftovers were also pooled and stored at -20°C. Plates were rinsed with deionised water into the reject diet bin. Plates and trays were wiped with deionised swabs and the swabs retained.

Urine:- Six 24 hour urine collections were made during the balance period. Deionised swabs replaced toilet paper. 10ml of concentrated hydrochloric acid were added daily to preserve the urine. A 10ml aliquot for nitrogen analysis was taken each day. Thereafter the six collections were pooled.

Faeces:-Faecal collections started from the appearance of the first marker and continued up to but excluding the second marker. Deionised swabs were used instead of toilet tissue. Used swabs were collected.

SAMPLE PREPARATION

Samples were collected and homogenised using a Silverson homogeniser with a teflon coated head and a plastic sampling tube. The head and sampling tube were acid washed and checked visually before use. No metal was exposed on the head and Teflon bushes reduced friction on the rotating column.

Duplicate Diet: Two three day pools were weighed and homogenised until a uniform consistency was obtained. A plastic spatula was used to check for any solid food particles remaining before a representative portion was taken via the sampling tube, during agitation.

Reject Diet: Reject diet swabs were soaked for 24 hours in 1% HCl. The eluate was removed and the swabs soaked for a further three hours in deionised water. The total eluate collected was added to the reject diet and the swabs counted and discarded. Reject diet was weighed, homogenised and sampled as for Duplicate Diet.

Urine: Pooled urine was weighed, homogenised and sampled as above.

Faeces: Faecal swabs were treated similarly to reject diet swabs. The eluate was added to the pooled faeces which was weighed, homogenised and samples obtained.

## A. SAMPLES FOR TRACE ELEMENT ANALYSIS

### I. Evaporation

Each sample was analysed in triplicate. All aliquots were weighed to the nearest 0.01g into acid washed quartz beakers. Diet, urine and faecal aliquots weighed 30 - 40g, 160 - 190g and 20 - 30g respectively. The aliquots were evaporated slowly on a sandbath and charred on a hotplate before ashing.

### II. Ashing

Charred aliquots were placed in a thermostatically controlled muffle furnace and the temperature increased gradually, over 3 - 4 hours, to 460$^{0}$C. This

temperature was maintained for 15 hours after which most of the carbon was burnt off. After cooling the aliquots were then treated with 2ml of concentrated nitric acid, slowly evaporated to dryness and returned to the muffle for a further ten hours. If necessary samples were retreated and returned to the muffle for a third time. After cooling 4ml. of 50% HCl were added to the aliquots. The aliquots were then slowly evaporated to dryness on a hot plate. The elements were now present as soluble chlorides. 5ml of 25% HCl was used to dissolve the residue and the aliquots made up to a final concentration of 2.5% HCl and final volume of 50ml with deionised water.

III. Analysis

The metals were measured on an Atomic Absorption Spectrophotometer (Instrumentation Laboratories 157). An optimal burner height of 5cm and a sample uptake rate by the nebuliser of 5ml/ minute were determined for each element prior to analysis. Zinc, copper and manganese determinations were made by spraying into an oxidising air-acetylene flame using a light source from a deuterium hollow cathode lamp. Aqueous standards in the linear range for each element were:- Zn 2 - 16 umol/litre, Cu 2 - 40 umol/litre and Mn 0.8 - 32 umol/litre. Background correction was used for all determinations. Absorption signals were measured with a two second integration time on microprocessor at wavelengths of 213.9, 324.7 and 279.5 for Zn, Cu and Mn respectively.

Standards

Commercial standards of 1mg/ml solution (BDH Laboratory) were used to make a 200 umol/litre mixed stock standard:-

| Standard | ml | |
|---|---|---|
| Zinc Nitrate | 6.54 ) | 5ml concentrated HCl ) |
| Copper Nitrate | 6.35 ) | deionised water to 500ml ) |
| Manganese Nitrate | 5.5 ) | |

Working standards were prepared every 6 - 8 weeks and compared with the previous set.

B. NITROGEN SAMPLES

Duplicate diet, reject diet, urine and faecel aliquots were taken from each homogenate.

Digestion

Each sample was analysed in duplicate. 6g aliquots were weighed into a glass cuvette. The samples were then poured into digestion tubes containing anti-bump granules and 1 kjeltab tablet. The cuvette was rinsed with 5ml hydrogen peroxide (30 vols) and emptied into the digestion tube. 10ml $H_2SO_4$ (conc.) was added to the digestion mixture and placed on a heating block. After digestion was complete, the samples were colourless. Samples were removed from the block and allowed to cool. Once cool, 40ml $H_2O$ and 20ml of 8% sodium thiosulphate were added.

Distillation

A 250ml graduated conical flask was prepared with 50ml of 2% boric acid and methyl red indicator to colour. A digestion tube was placed on the distillation apparatus. A controlled amount of 40% sodium hydroxide was added and the samples steam distilled.

When the contents of the flask were yellow and the volume was 150ml, distillation was complete.

Titration

Sulphuric acid (0.2ml) was titrated from a burette until the end point was indicated by the reappearance of the original pink colour.

NOTE: Titration figure (TF)

Calculated $N_2$ content mmol.

$$\frac{TF \times 14 \times \text{homogenate wt (g)}}{\text{acid conc.} \times \text{aliquot}} \times 71.4$$

IV. Analytical Quality Assurance

The scientific value of data obtained from balance studies is limited by the precision with which the intake and output of the nutrient under study can be determined. Trace Element balance studies are particularly prone to errors because of the problems inherent with estimating small concentrations of trace elements in a variety of biological material, with risks

of contamination from the environment at all stages. Contamination or loss can occur during sampling and collection of samples. The use of suitable containers i.e. of polythene or polypropylene, storage vessels and extreme care to avoid dust or sweat contamination while collecting samples will minimise errors at this stage.

The preparation of samples can result in changes in trace element content; contamination from air borne elements, particularly zinc and copper can occur during lengthy processes such as evaporation of samples on a sand bath. Dry ashing of organic matter may result in losses of zinc by volatilization if the sample reaches a temperature greater than 460$^{o}$C (Thiers, 1957). The interior walls and ceilings of the muffle furnace are often a source of serious contamination during dry ashing. The use of etched vessels for dry ashing can result in apparent losses of inorganic material due to adsorption of elements onto the surface of the vessel. In addition etched quartz glasses may carry contamination from sample to sample. Considering the potential errors associated with trace element technique it is necessary to monitor constantly the quality of the technique in order to assure the quality of the results. During this study the following were performed:-

The reproducability of the sampling method was assessed by taking 10 aliquots of the same homogenate for diet, urine and faeces, each aliquot was prepared and

analysed in triplicate. The results of ten aliquots of the same homogenate gave intrabatch variations for diet, urine and faeces of 3, 5 and 5% for zinc, 6, 10 and 6% for copper and 5, 5 and 6% respectively for manganese.

Recovery of Standard Additions

Information on the precision and self consistency of a method (although not on its accuracy or freedom from contamination) can be obtained by testing the recovery of known additives.

Spikes of 1/10, 1/100 and 1/1000 were prepared from zinc, copper and manganese commercial, (BDH) Standards of 1mg/ml solution. Additions were made to diet, faeces and urine aliquots. The amount of element added, shown below, was approximately equal to that already present in the aliquot.

TABLE 1 STANDARD ADDITIONS

| ALIQUOT | APPROX SAMPLE WT (g) | UMOL ADDED Zn | Cu | Mn |
|---|---|---|---|---|
| Duplicate diet | 35 | 2.30 | 0.236 | 0.567 |
| Reject diet | 35 | 0.459 | 0.110 | 0.151 |
| Urine | 180 | 0.918 | 0.314 | 0.151 |
| Faeces | 25 | 15.3 | 1.57 | 1.82 |

The aliquots were prepared and analysed using the same procedure as for samples (Page: 28), replicates of each homogenate, namely diet, reject diet, urine and faeces were analysed.

Results

Mean % recoveries of zinc, copper and manganese added to aliquots of homogenate.

TAB:E: 2 PERCENTAGE RECOVERY OF STANDARD ADDITIONS

| | DUPLICATE DIET | URINE | FAECES |
|---|---|---|---|
| Zn | 95.2 ± 5.46 | 98.6 ± 5.33 | 99.3 ± 4.8 |
| Cu | 96.8 ± 8.97 | 87.6 ± 13.7 | 99.9 ± 2.9 |
| Mn | 98.6 ± 3.63 | 95.2 ± 4.68 | 103.9 ± 3.21 |

Values expressed as % recovers ± ISD

An externally controlled reference material for quality control of such determinations was not available. As internal control the same dietary sample from a pooled homogenate was analysed, on five separate occasions during the study. This gave interbatch variations of 5, 5 and 7% for zinc, copper and manganese respectively.

# Zn Balance

# Chapter III

Zinc requirements are particularly high during periods of rapid growth and development. The mechanism by which these extra requirements are met has not been shown but may depend partly on the mother's state of nutrition at the beginning of pregnancy, on her accustomed diet, and on economies in metabolism that may occur within her body as a result of pregnancy. The need for a increased energy or nutrient intake in order to meet all the requirements has not been shown but Sandstead (1973) suggested an extra intake of 5 mg/day dietary zinc is required during the last trimester. He calculated this from the estimated accretion of lean body tissue during pregnancy by estimating the concentration of zinc in that tissue, and assuming that the increased requirements are met by dietary means alone. This calculation is the basis for the additional 5mg of dietary zinc per day which is recommended during pregnancy (US Nat. Acac. Sci. 1980).

Intestinal absorption, secretion and reabsorption of zinc may contribute to zinc homeostatis and maintenance of zinc balance (Matseshe et al 1980, Cousins 1979). Dietary zinc is absorbed throughout the small intestine. Zinc in pancreatic and intestinal secretions may be reabsorbed and help maintain zinc balance (Matseshe et al, 1980). A number of studies in different species have shown both absorption and endogenous faecal excretion of zinc vary in relation to dietary zinc intake. (Weigand and Kirchgessner, 1980; Cotzias, Borg and Selleck, 1962, Underwood, 1977). Zinc absorption may be in accordance with the nutritional requirements of the host. Cousins (1979) postulated that a high plasma zinc concentration may induce synthesis of an intestinal protein which

subsequently binds with zinc increasing its concentration in the mucosal cell, and decreasing the amount of zinc entering the portal blood stream. Subsequently this enterocyte 'stored' zinc is lost during natural desquamation of mucosal cells.

Faecal zinc losses account for about 95% of total zinc loss, largely comprising of unabsorbed dietary zinc plus a small amount from intestinal secretions. Consequently faecal zinc output depends on dietary zinc intake. Adolescent girls lost significantly more zinc in their faeces when fed 14.7 mg than when fed 11.5 mg/day (Greger et al, 1978). McLeod et al, (1973), correlated dietary zinc intake and faecal zinc output.

Urinary zinc excretion in healthy adults is small, (0.3 - 0.6 mg/day), accounting for only 2 - 5% of total zinc loss (Schraer and Calloway, 1974). Elevated urinary zinc levels have been reported in the last trimester of pregnancy. - both groups given 20 mg of zinc per day a group of pregnant women showed a tendency to lose more zinc in their urine than a non-pregnant group. (Swanson and King, 1982). In contrast, Hambidge et al (1983) found urinary zinc excretion to be lower in pregnant women on normal home zinc intakes. Urinary zinc excretion was higher in women receiving zinc supplementation.

Sweat losses of zinc are rarely determined in balance studies and considerable variation is reported for both. Dermal losses in four pregnant teenagers were 2.8mg per day (Schraer and Calloway, 1974). In young women on a low zinc intake sweat losses of zinc were estimated to be 0.67mg per day. (Hess, King and Margen, 1977). Zinc losses in sweat may be related to the level of dietary

zinc and may be important when intakes are marginal (Milne et al, 1983). This may be of particular importance in tropical climates where sweat losses can be as high as 4 litres per day and as much as 4mg of zinc per day could be lost.

Zinc balance studies showed pregnant women excreted less zinc in their faeces than non pregnant women on a similar intake (Swanson and King (1982). Thus, the pregnant women had a higher apparent absorption which may reflect an increased absorption of dietary zinc or a decreased excretion of endogenous zinc. Animal studies showed an increased rate of zinc absorption at different stages in pregnancy, (Davies and Williams, 1976). The accretion of fetal and maternal tissues during pregnancy may result in an increased zinc requirement. How the maternal body obtains this extra zinc is, as yet, unknown. This study investigates zinc intake, apparent absorption and retention of pregnant women eating self selected and customary diets.

RESULTS

Group I primigravidae were of normal weight, height and weight for height. Balances on this group were carried out in early, Group 1(a), and late, Group I(b), pregnancy. Group II primigravidae were selected as being at risk of delivering a low birth weight infant. Mean weight, height, age and smoking habits of the groups are given; Table 3.

Table 3: Mean Weight, Height, Age and Smoking Habit of Study Group

| | Number Studied | Average Ages (Years) | Mean Ht (m) | Wt. at 20 wks. Pregnancy (Kg) | Number who Smoked |
|---|---|---|---|---|---|
| Group I (a) | 5 | 22.2 | 1.59 | 60.6 | 1 |
| (b) | 7 | 24.1 | 1.63 | 63.9 | 4 |
| Group II | 5 | 19.4 | 1.55 | 52.4 | 4 |
| Non Pregnant Group | 4 | 23.2 | 1.60 | 63.9 | 0 |

Mean weight gain during the balance period for the four groups Group I (a), Group I (b), Group II and the non-pregnant group were 0.29, 0.28, 0.56, -0.1Kg respectively (Appendix III). Mean daily protein intakes were 65.0, 77.2, 69.2 and 76.3g, and mean energy intakes 8.90, 9.59, 9.54 and 8.43 MJ in the four groups respectively.

Mean daily nitrogen balance was positive in all groups of primigravidae. Table: 4. Zinc intake was directly related to nitrogen intake (Fig: 1).

Table : 4

NITROGEN BALANCE mmol/day

| PATIENTS MEAN ± SD | INTAKE | OUTPUT | | | APPARENT ABSORP-TION | MAXIMUM RETEN-TION |
|---|---|---|---|---|---|---|
| | | TOTAL | URINE | FAECES | | |
| Group I (a) | 742.2 | 691.6 | 621.4 | 70.2 | +672.0 | + 50.6 |
| n = 5 | ±117.89 | ± 61.39 | ± 76.1 | ±24.24 | ±112.7 | ±113.5 |
| Range | 542to 823 | 648to 799 | 560to 753 | 46to 103 | +419to +777 | -122to +157 |
| Group I (b) | 882.6 | 779.7 | 690.3 | 89.4 | +793.2 | +102.8 |
| n = 7 | ±158.1 | ±157.4 | ±145.1 | ±21.9 | ±144.6 | ± 80.57 |
| Range | 649to 1147 | 591to 1049 | 507to 917 | 70to 132 | +579to +1015 | -1to +254 |
| Group II | 790.8 | 725.4 | 656.8 | 68.6 | +722.2 | + 65.4 |
| n = 5 | ±134.6 | ±134.8 | ±123.9 | ±18.3 | ±122.6 | ±50.7 |
| Range | 673to 1023 | 561to 929 | 489to 833 | 47to 96 | +601to +928 | +4to +112 |
| Non-Pregnant | 871.8 | 894.5 | 802.6 | 91.8 | +780.0 | - 22.7 |
| Group n = 4 | ±109.62 | ± 73.37 | ± 49.6 | ±31.12 | ± 85.07 | ± 53.47 |
| Range | 787to 1032 | 828to 999 | 763to 871 | 59to 128 | +713to +904 | -95to +33 |

Table : 5

ZINC BALANCE umol/day

| PATIENTS MEAN ± SD | INTAKE | OUTPUT | | | APPARENT ABSORP-TION | MAXIMUM RETEN-TION |
|---|---|---|---|---|---|---|
| | | TOTAL | URINE | FAECES | | |
| Group I (a) | 128.4 | 138.1 | 6.5 | 131.6 | - 3.2 | - 9.7 |
| n = 5 | ± 24.24 | ± 24.26 | ± 2.26 | ± 25.12 | ±16.51 | ±15.18 |
| Range | 93.7to 150.3 | 104.5to 172.2 | 4.2to 10.0 | 97.1to 167.0 | -23.3to +20.1 | -28.5to +10.1 |
| Group I (b) | 138.5 | 125.4 | 7.3 | 118.1 | +20.4 | +13.1 |
| n = 7 | ± 29.82 | ± 31.37 | ±1.83 | ± 30.64 | ±16.9 | ±18.36 |
| Range | 119.6to 203.6 | 87.5to 183.2 | 5.0to 10.1 | 80.6to 174.8 | -10.9to +39.6 | -21.1to +34.4 |
| Group II | 147.4 | 152.2 | 8.1 | 144.1 | + 3.3 | - 4.9 |
| n = 5 | ± 25.33 | ± 34.03 | ±1.41 | ± 33.19 | ±26.77 | ±26.50 |
| Range | 112.6to 181.2 | 101.7to 183.2 | 6.1to 9.9 | 93.9to 174.2 | -15.1to +44.9 | -24.2to +37.1 |
| Non-Pregnant | 192.7 | 170.9 | 9.2 | 161.7 | +31.0 | +21.8 |
| n = 4 | ± 53.60 | ±117.17 | ±1.72 | 117.50 | ±80.54 | ±79.58 |
| Range | 149.7to 266.0 | 89.1to 338.7 | 7.9to 11.6 | 77.3to 329.5 | -63.5to +122.0 | -72.7to +110.2 |

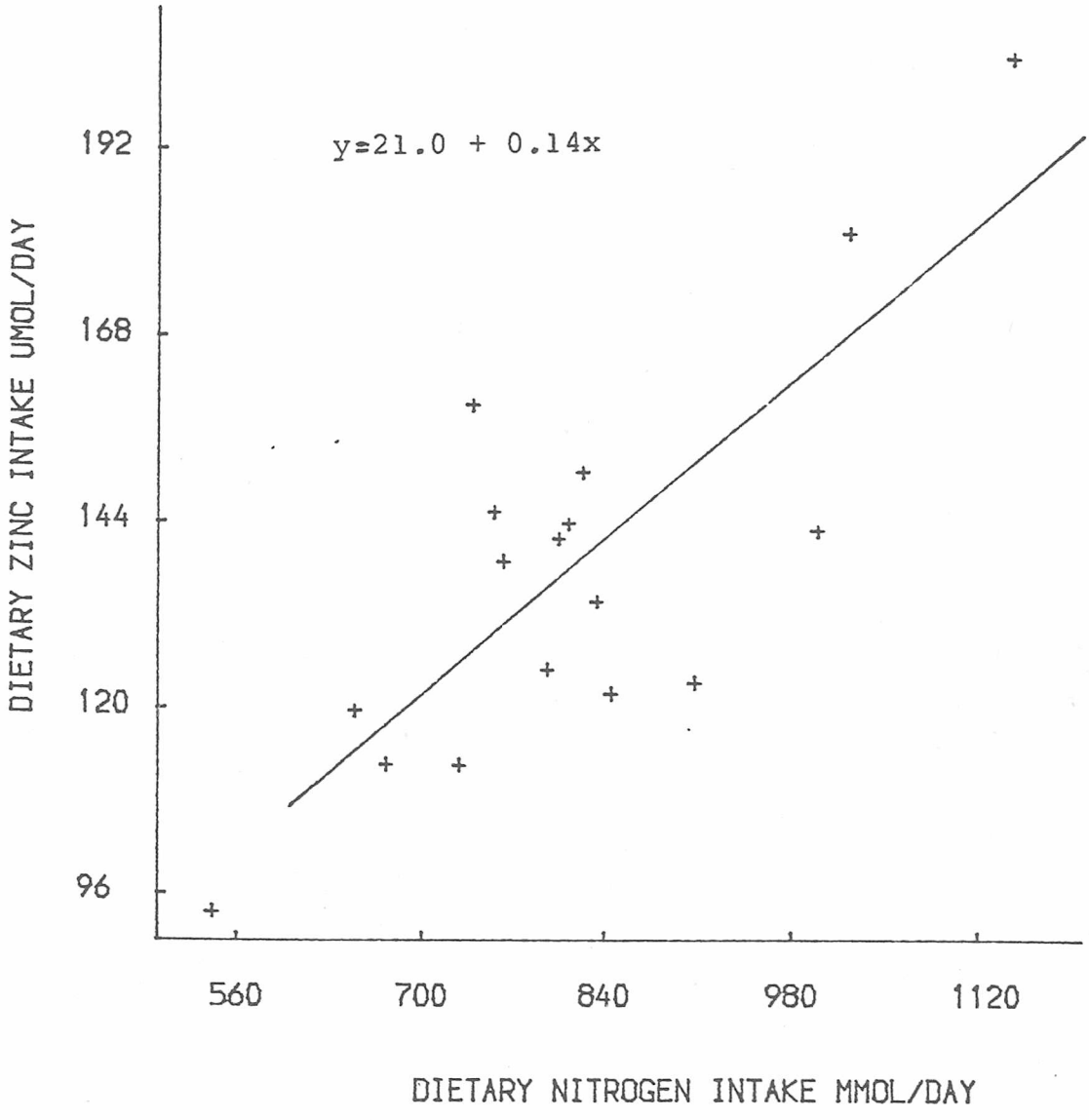


Fig. 1 Correlation between dietary nitrogen intake and zinc intake in primigravidae.

$r = 0.79 \quad P < 0.001$

Mean daily zinc intakes were similar in Group I (a), (b) and Group II (Table - : 5) and were less than half the recommended daily intake. None of the seventeen pregnant women had a zinc intake which met the recommendations and fourteen had intakes which were less than half (Table: 6) Mean daily urinary zinc excretion was marginally higher in late pregnancy, Group I (b) and Group II but lower than in non-pregnant women, (Table: 5).

Seven primigravidae had a faecal zinc output which exceeded intake. (Table: 6, Fig: 2). Figure 2 is a graphical representation of the balance data, based on the method of Reifenstein, Albright and Wells (1945). It is constructed as follows: (a) The scale for intake and balance, umol/24 hrs. is given as the axis; (b) The horizontal line at 0 of the axis is the base line to which intake and balance refer; (c) The intake is plotted as an area from the base line toward the top of the diagram; (d) The excretion is plotted from the top of the intake toward the bottom of the diagram, with faecal output followed by urinary output. If the excretion falls below the base line, this represents negative balance. If it does not reach the base line, the area between the bottom of the column and the base line represents a positive balance. If it reaches the base line the balance is in equilibrium. All data are plotted as mean daily balance for the individual women (initials shown below the columns).

Two non-pregnant women had a faecal zinc output exceeding intake (FIG: 2). Faecal zinc excretion was directly related to zinc intake (FIG: 3) and accounted for 95% of the measured zinc loss in both the pregnant and non-pregnant women. Two volunteers, (E.M., J.S.) Group

Table6 Mean daily zinc balance of pregnant and non-pregnant women.

GROUP I (a) ZINC BALANCE umol/day

| PATIENTS | INTAKE | OUTPUT | | | APPARENT ABSORP-TION | MAXIMUM RETEN-TION |
|---|---|---|---|---|---|---|
| | | TOTAL | URINE | FAECES | | |
| 1. AMa | 93.7 | 104.5 | 7.4 | 97.1 | - 3.4 | -10.8 |
| 2. EM | 141.8 | 142.3 | 4.2 | 138.1 | + 3.7 | - 0.5 |
| 3. JS | 143.7 | 172.2 | 5.1 | 167.0 | -23.3 | -28.5 |
| 4. SK | 112.5 | 131.7 | 6.1 | 125.5 | -13.0 | -19.2 |
| 5. KS | 150.3 | 140.2 | 10.0 | 130.2 | +20.1 | +10.1 |

GROUP I (b) ZINC BALANCE umol/day

| PATIENTS | INTAKE | OUTPUT | | | APPARENT ABSORP-TION | MAXIMUM RETEN-TION |
|---|---|---|---|---|---|---|
| | | TOTAL | URINE | FAECES | | |
| 6. AMc | 123.2 | 144.3 | 10.1 | 134.2 | -10.96 | -21.1 |
| 7. LD | 203.6 | 183.2 | 8.4 | 174.8 | +28.8 | +20.4 |
| 8. ES | 124.9 | 113.3 | 8.2 | 105.1 | +19.85 | +11.6 |
| 2. EM | 142.8 | 115.3 | 5.0 | 110.3 | +32.49 | +27.5 |
| 3. JS | 133.7 | 131.9 | 6.8 | 125.2 | +8.5 | + 1.8 |
| 9. LW | 121.8 | 87.4 | 5.15 | 80.6 | +39.6 | +34.4 |
| 10. SG | 119.6 | 103.7 | 7.47 | 96.3 | +23.3 | +15.9 |

GROUP II ZINC BALANCE umol/day

| PATIENTS | INTAKE | OUTPUT | | | APPARENT ABSORP-TION | MAXIMUM RETEN-TION |
|---|---|---|---|---|---|---|
| | | TOTAL | URINE | FAECES | | |
| 1. AG | 159.0 | 183.2 | 8.97 | 174.2 | -15.15 | -24.2 |
| 2. CR | 112.6 | 133.5 | 6.15 | 127.4 | -14.8 | -20.9 |
| 3. PR | 145.2 | 167.5 | 7.78 | 159.6. | -14.4 | -22.3 |
| 4. DMcD | 138.8 | 101.7 | 7.77 | 93.9 | +44.9 | +37.1 |
| 5. AA | 181.2 | 175.4 | 9.87 | 165.6 | +15.7 | + 5.8 |

ZINC BALANCE IN NON-PREGNANT WOMEN umol/day

| PATIENTS | INTAKE | OUTPUT | | | APPARENT ABSORP-TION | MAXIMUM RETEN-TION |
|---|---|---|---|---|---|---|
| | | TOTAL | URINE | FAECES | | |
| 1. DR | 266.0 | 338.7 | 9.2 | 329.5 | - 63.5 | - 72.7 |
| 2. AD | 199.3 | 89.15 | 11.6 | 77.3 | +122.0 | +110.2 |
| 3. SA | 149.7 | 91.5 | 8.0 | 83.5 | + 66.2 | + 58.2 |
| 4. JP | 155.9 | 164.6 | 7.9 | 156.7 | - 0.8 | - 8.7 |

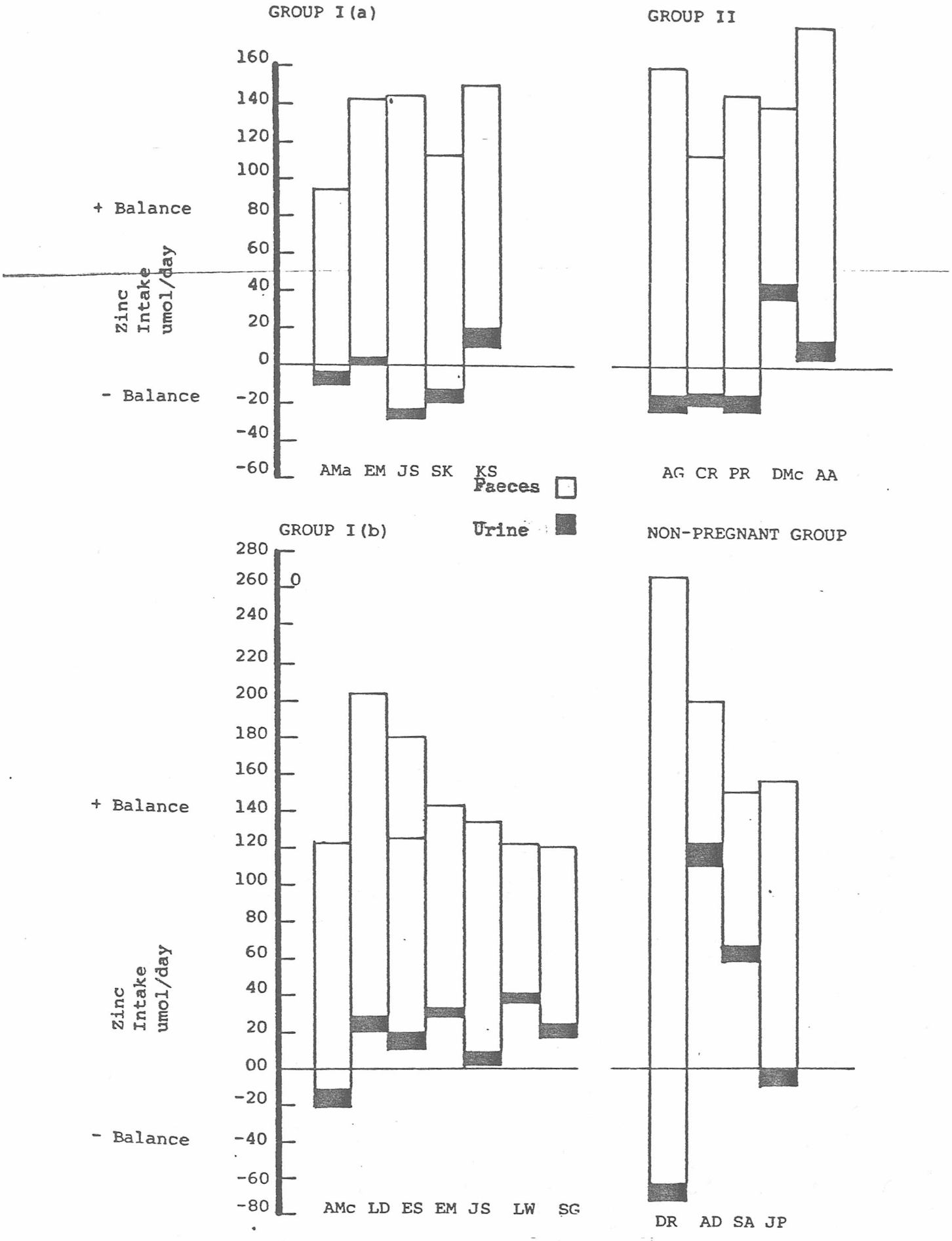


Fig.2 Graphical representation of zinc balance in pregnant and non-pregnant women.

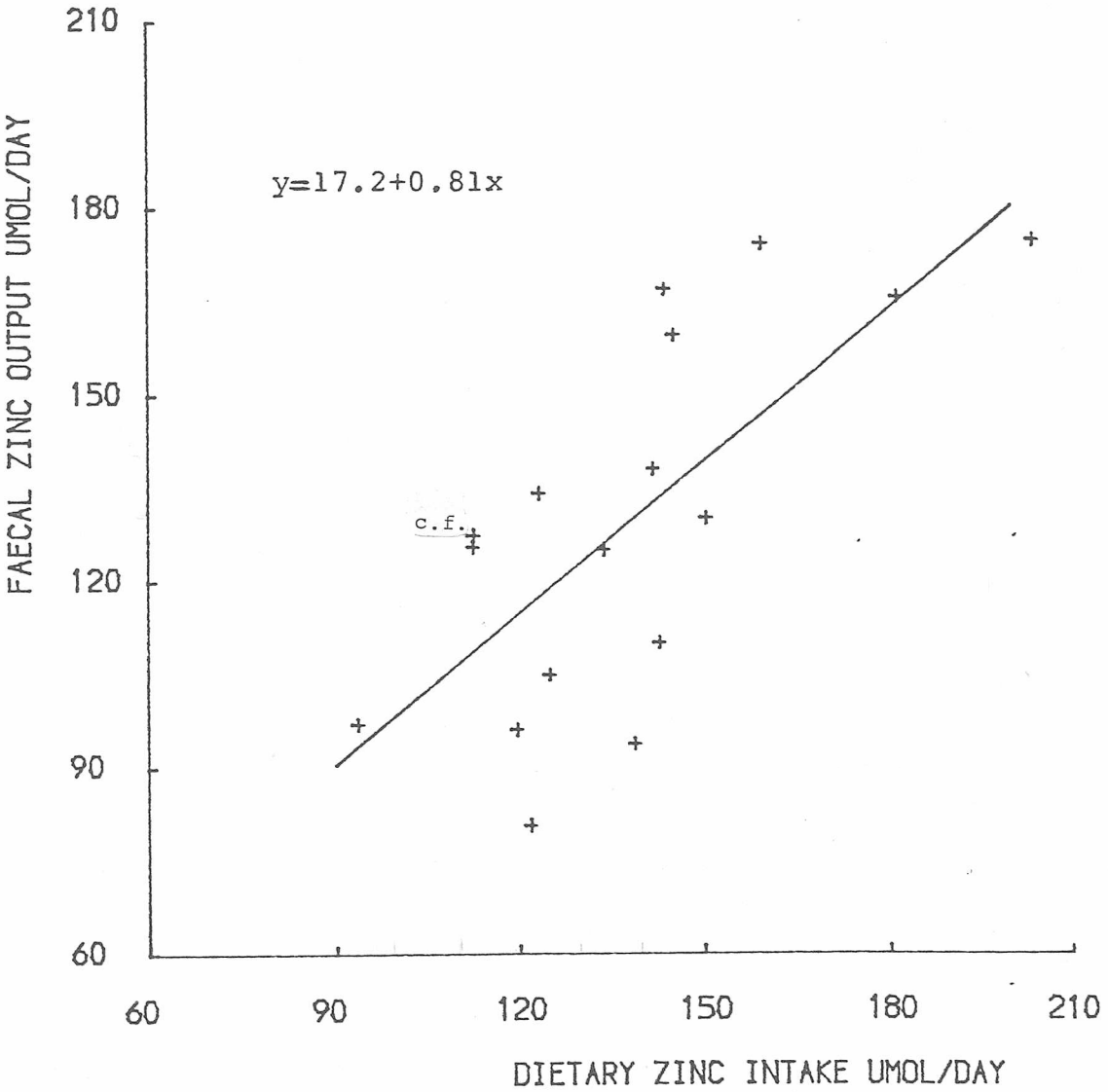


Fig.3 Faecal zinc output related to dietary zinc intake.

$r=0.71, P < 0.01$

c.f.-delivered infant with cystic fibrosis

I, completed an early and late balance. Without altering their dietary zinc intake significantly they showed a decrease in mean daily faecal excretion by 27.8 umol (1.82mg) and 41.8 umol (2.73mg) respectively between their 1st and 2nd balance (Table: 6).

Normal primigravidae in early pregnancy, had a mean daily zinc loss of -9.7 umol (0.64mg) (Table: 5), three women were in negative zinc balance. Normal primigravidae in late pregnancy had a mean daily zinc retention of +13.1 umol (0.86mg), one woman was in negative zinc balance (FIG. 2). The smaller, lighter women in Group II, had a mean daily zinc loss of -4.9 umol (0.32mg) per day, three were in negative zinc balance (Fig. 2). All women in Group I (a) and (b) delivered healthy infants $\geq$ 10th centile. Three women in Group II delivered an infant $<$ 10th centile,

DISCUSSION

In this study care was taken to ensure that each patient had a food intake which accurately represented her usual home intake. Mean daily energy intakes of the pregnant women are similar to those previously reported in two groups of Aberdeen primigravidae of 8.0 and 8.6MJ, (Armstrong 1982). Likewise the mean daily protein intakes of the pregnant women were similar to previously reported intakes as assessed by seven day weighed dietary surveys, (Armstrong 1982). This suggests the energy and protein intakes of the women in this study were typical of the customary intake of Aberdeen primigravidae consuming their home diets.

The recognised components of nitrogen storage during pregnancy are the fetus, placenta, liquor, uterus, breasts and blood. Hytten and Leitch (1971) calculated a total protein increment of 427g, equivalent to a daily retention of 6.1g protein, 0.98g nitrogen,

during the last ten weeks of pregnancy and 129g, equivalent to 1.84g protein, 0.29g nitrogen per day between 10 - 20 weeks. In this study the mean daily nitrogen retention of primigravidae around 30 weeks gestation was remarkably similar to this. Integumental nitrogen losses have not been accounted for in this study making the nitrogen retentions reported here high. From reported epithelial nitrogen losses in men and non-pregnant women, Johnstone, MacGillivray and Dennis, (1972) assumed a mean integumental nitrogen loss of 20.0 mmol (0.28g) per day for pregnant women. Applied to the data in this study this would give a mean daily retention of 30.6 mmol (0.43g) between 10 and 20 weeks, 45.4 mmol (0.63g) and 82.8 mmol (1.16g) in the smaller, lighter primigravidae and the normal primigravidae respectively. The positive nitrogen balance of these pregnant women and the weight gain during the balance period would suggest that the energy and protein intakes of the women during the balance were sufficient to maintain balance.

Mean daily zinc intakes in the present study confirm other reported intakes (Guthrie and Robinson 1977, Wolf, Holden and Green 1977, Hunt et al 1979) which are well below the recommendation. An earlier study in Aberdeen found mean daily zinc intake, calculated on an out-patient basis from seven day weighed dietary surveys, in two groups of primigravidae to be 155 umol (10.1mg) and 151 umol (9.9mg) (Armstrong, 1982)). The zinc intake of the women in this study was comparable to the customary intake of women eating their home diet.

The positive correlation between dietary nitrogen and zinc intake confirms an association between zinc and protein in foods, protein rich foods are the main sources of dietary zinc. The high mean daily zinc intake of the non-pregnant women reflected an

exceptionally high zinc intake of one subject, 266 umol (17.4mg) zinc per day. She had a mean daily zinc loss of 72.7 umol (4.7mg) per day. This negative zinc balance may have arisen due to a zinc intake during the balance which was greatly in excess of her accustomed intake and requirement or as a consequence of her high dietary fibre intake which exceeded 30g/day.

Ten primigravidae of normal weight and height delivered normal healthy infants. All of these women had dietary zinc intakes which were less than two thirds of the recommendation, only one woman had an intake exceeding 10 mg/day yet they all had a successful outcome to their pregnancy. Three women selected as being at risk of delivering a small infant, delivered an infant < 10th centile. On a similar dietary zinc intake to women of normal weight and height they had a tendency towards a negative zinc balance, and, two of the women who delivered infants < 10th centile were in negative zinc balance at 30 weeks gestation. A number of factors may have contributed to the outcome of these pregnancies. Firstly, one of the infants was diagnosed as having cystic fibrosis soon after birth - hereditary congential disorders are associated with small, light infants. Secondly, the three women smoked throughout their pregnancy - smoking is strongly associated with small, light infants (Scott, Moar and Ounsted, 1981, Metcoff et al, 1981). Thirdly, shorter and lighter women have smaller babies (Campbell-Brown, 1983, Dobbing, 1981). The negative zinc balance of these women may suggest their zinc intake was insufficient for their requirements. The existence of a nutritional zinc deficiency affecting fetal outcome cannot be excluded or, indeed, concluded without considering other maternal and extraneous

non-nutritional factors which influence fetal growth. The duration of gestation and sex of the baby have been taken into account. Maternal variables affecting fetal outcome which have been considered include race, parity and age. However, numerous other variables need to be considered before growth retardation can be attributed to a maternal nutritional zinc deficiency. It may be that total maternal nutritional balance needs to be considered rather than balance of a single nutrient (Metcoff et al, 1981).

The main determinant of zinc balance is faecal zinc excretion. Since Group I (b) women excreted less zinc in their faeces than in Group I (a), they had a greater mean apparent zinc absorption. Similarly the faecal zinc loss was less in Group I (b) than in Group II women. This balance method does not distinguish between unabsorbed dietary zinc and zinc of endogenous origin. Whether the higher mean apparent absorption values in Group I (b) reflect increased absorption of dietary zinc or decreased excretion of endogenous zinc cannot be shown. A fall in faecal zinc output demonstrated by the two women in Group I (EM, JS) resulted in an increase in apparent absorption by 20% and 23% respectively between early and late pregnancy. Their mean daily zinc intakes did not increase between their first and second balance. This increased apparent absorption attributable to a fall in their faecal zinc excretion between early and late pregnancy suggests that in normal pregnant women there is a tendency towards a positive zinc retention in late pregnancy. It seems, therefore, that there may be a tendency for apparent zinc absorption to increase, intestinal zinc excretion to decrease, or a combination of these during the course

of pregnancy. This may represent an adaption to increased zinc requirements during gestation. An increased zinc retention late in pregnancy would accompany the rapid increase in lean tissue which is predominent during the last ten weeks of pregnancy (Hytten and Chamberlain, 1980).

The mean daily urinary zinc output of the pregnant women was lower than non-pregnant women, confirming the findings of Hambidge et al (1983). In all groups the urinary zinc excretion was lower than that reported in earlier studies, (Swanson and King 1982 and King 1981). The slightly higher urinary zinc excretion of women in the 3rd trimester and the increased urinary zinc in the two women, (E.M. J.S), who completed an early and late balance may suggest urinary zinc excretion increases as pregnancy progresses. A similar observation was found by Hambidge et al, (1983).

Normal healthy primigravidae had a tendency towards an increased zinc retention in the 3rd trimester, concommitant with the rapid, accumulation of lean tissue at this stage. If this is true then the accumulation of zinc may be related to that of protein, hence zinc requirements may be calculated. The calculation shown in Table 7 is an estimate of the daily retention of zinc at different stages of human pregnancy, calculated as follows:- The zinc and protein content of a full term baby weighing 3.5 kg was found to be 53mg and 410g respectively (Widdowson, 1981). Thus, the total fetal tissue has a zinc and protein concentration of 15ug/g and 117mg/g, respectively from which a zinc/protein relationship of 128 ug zinc/g protein is calculated. If we then assume that this zinc/protein relationship applies to all lean tissue accumulated during

TABLE : 7

CALCULATED ACCUMULATION AND RETENTION
OF ZINC DURING PREGNANCY

| Weeks Gestation | 10 | 20 | 30 | 40 |
|---|---|---|---|---|
| Added protein (Hytten & Leitch, 1971) | 36 | 165 | 498 | 925 |
| Added zinc (ug) + (128ug/g Protein) | 4608 | 21120 | 63744 | 118400 |

| Weeks Gestation | | 1 - 10 | 11 - 20 | 21 - 30 | 30 - 40 |
|---|---|---|---|---|---|
| Daily increased zinc retention | ug | 66 | 236 | 609 | 780 |
| | umol | (1.01) | (3.61) | (9.32) | (11.93) |
| *Increased daily Zinc Intake | mg | 0.32 | 1.18 | 3.04 | 3.90 |
| | umol | 4.9 | (18.0) | (46.5) | (59.7) |

(* Assuming 20% availability of Zinc from the diet)

+ = Assuming tissue Protein concentration 117mg/g and zinc 15ug/g (Widdowson, 1981)

pregnancy, not just that of the fetus and, from the total added protein at different stages of pregnancy Hytten and Leitch (1971) we can calculate the accumulation and retention of zinc during pregnancy. Assuming that 20% of dietary zinc is available for absorption the increase in daily zinc intake necessary to meet this requirement can also be estimated (Table 7).

This calculation is based on the assumption that zinc accumulated during pregnancy is directly related to the addition of protein in the maternal and fetal body. Any increase in dietary zinc intake required to meet the daily zinc retention will, of course, depend on the availability of the element. Assuming a 20% availability the additional daily dietary zinc, calculated here, which is necessary to meet the zinc retention is lower than the extra 5mg per day recommended throughout pregnancy (U.S. Nat. Acad. Sci, 1980). The present calculation would suggest an additional 46.5 umol (3.04mg) dietary zinc per day between 20 and 30 weeks of pregnancy and 59.7 umol (3.90mg) dietary zinc per day during the last ten weeks of pregnancy would meet the increased retention. The zinc retention for the last ten weeks of pregnancy, is calculated to be 11.9 umol per day (780ug). This is similar to the mean daily zinc retention of normal primigravidae in this study during the third trimiester, 13.1 umol, 856ug.

# Cu Balance

# Chapter IV

The suggested estimated safe and adequate' intake for copper, 31.4 to 47.1 umol, (2 to 3mg) per day, is derived from balance studies by early investigators who found intakes between 31.4 and 39.2 umol (2 to 2.5mg/day) were sufficient to maintain balance in adult females. (Leverton, 1939, Leverton and Binkley, 1944). As yet insufficient data is available to suggest a recommended daily amount or additional requirement for pregnancy. The level of copper intake thought to be sufficient to maintain balance in adults is 31.4 to 39.2 umol (2.0 to 2.5mg) (Mason 1979). Copper balance studies in women report negative copper balance at intakes of 32.9 umol (2.1mg) or less per day (Crews, Taper and Ritchey, 1980, Taper Hinners and Ritchey 1980 and White and Gynne 1971). Hartley, Dawson and Hodgkinson (1974) correlated copper intake and retention in patients with various bone and gastro-intestinal disorders and found a mean daily copper intake of 26.0umol (1.65mg) was necessary to maintain balance in these patients. The copper content in some commonly consumed diets may be marginal; Klevay (1978) found that a high percentage of diets in the United States provide less than 31.4 umol per day (2mg). Intakes of New Zealand women were reported to be less than 31.4 umol (2mg) per day, except in women who regularly consumed liver, (Guthrie and Robinson 1977). Intakes of 28.3 umol (1.8mg) and 24.4 umol (1.5mg) were found in two groups of primigravidae. (Armstrong 1982). Reports of copper deficiency in humans have been confined to those with special nutritional requirements, e.g. total parenteral nutrition, premature infants. In those groups of the population where copper requirements are high the possibility of copper deficiency arising should be recognised.

The deleterious effects of copper deficiency on fetal development in mammalian species have been discussed. No such effects have been observed in human pregnancy but copper requirements may be higher during gestation. The human fetal liver shows a marked ability to accumulate copper, (Widdowson 1972), particularly in the latter half of pregnancy. Copper is stored in the liver and it has been suggested that the maternal liver may serve as a source of copper during pregnancy, (Mason 1979).

Copper absorbed in the small intestine enters the mucosal cell where it is bound to a specific protein, thought to be similar to that involved in zinc absorption. An interaction between zinc and copper absorption has been suggested when zinc intakes are high (Porter et al 1977). A zinc to copper weight ratio of (500 : 1) markedly depressed copper absorption in the rat, while a ratio of 50 : 1 had no effect on copper absorption, (Van Campen 1966). Thus an antagonistic effect in zinc and copper absorption is suggested. However, in most human diets, the ratio of zinc to copper is in the range of 10 : 1 to 40 : 1 and rarely, except when therapeutic amounts of zinc are provided would it reach a higher ratio. Zinc intakes of 275 to 366 umol (18 to 24mg) per day had no effect on copper retention in adult women, (Taper, Hinners and Ritchey 1980).

Data on urinary output is extremely variable. The urinary copper output of four young women on copper intakes of 23.5 to 31.4 umol (1.5 - 2mg) per day ranged from 0.1 - 0.7 umol (11 - 48ug) (Robinson et al 1973, Taper, Hinners and Ritchey, 1980 Greger et al, 1978). Urinary copper was significantly higher in women on oral contraceptives, (Crews, Taper and Ritchey, 1980)

The major route of endogenous copper excretion is in the bile (Gollan and Dellar 1973). Intravenous injection of copper produces an increase in bilary excretion but did not affect urinary copper output (Prasad 1978). The proportion of copper appearing in the faeces is largely unabsorbed dietary copper plus copper actively excreted in the bile. Endogenous copper excretion is predominantly, 75% in the form of bilary copper. Of the remaining copper excreted 20% passes directly into the bowel as intestinal secretions and 3 to 4% appears in the urine (Cartwright and Wintrobe, 1964).

The only report of copper balance in pregnant women was by Kyer and Bethal (1936). A healthy young woman was maintained during the last three months of her pregnancy on 34.5 umol (2.2mg) of copper per day and a daily retention of 0.78 umol (0.05mg). This study investigates copper absorption and retention in Aberdeen primigravidae at different stages of gestation.

RESULTS

There was a large variation in dietary copper intakes ranging from 12.0 umol (0.76mg) to 35.4 umol (2.25mg) per day, (Table 8). Women who consumed liver during their balance period had a higher copper intake.Two pregnant women, (EM,JS) and two non-pregnant women, (DR, SA) had a mean daily copper intake which exceeded 31.4 umol (2mg). All of these women consumed liver during the balance period.

Faecal copper output was directly related to copper intake (FIG: 4). There was a mean daily apparent absorption of copper in all the groups studied, (Table: 9). Four women had mean daily faecal excretion which exceeded their intake (Table 8, FIG: 5).

# Table:8 Mean daily copper balance of pregnant

GROUP I (a) COPPER BALANCE umol/DAY

| PATIENTS | INTAKE | OUTPUT | | | APPARENT ABSORP-TION | MAXIMUM RETEN-TION |
|---|---|---|---|---|---|---|
| | | TOTAL | URINE | FAECES | | |
| 1. AMa | 12.0 | 14.3 | 1.18 | 13.1 | - 1.1 | - 2.3 |
| 2. EM | 35.4 | 22.5 | 1.12 | 21.4 | +14.0 | +12.9 |
| 3. JS | 34.1 | 29.3 | 1.07 | 28.2 | + 5.9 | + 4.8 |
| 4. SK | 17.9 | 16.4 | 1.43 | 14.9 | + 3.0 | + 1.5 |
| 5. KS | 19.2 | 15.7 | 2.59 | 13.1 | + 6.1 | + 3.5 |



GROUP I (b) COPPER BALANCE umol/DAY

| PATIENTS | INTAKE | OUTPUT | | | APPARENT ABSORP-TION | MAXIMUM RETEN-TION |
|---|---|---|---|---|---|---|
| | | TOTAL | URINE | FAECES | | |
| 7. AMc | 13.3 | 14.5 | 0.68 | 13.8 | -0.5 | -1.2 |
| 8. LD | 20.3 | 22.2 | 1.87 | 20.3 | 0. | -1.9 |
| 9. ES | 14.0 | 14.0 | 1.80 | 12.2 | +1.8 | 0. |
| 2. EM | 20.4 | 17.9 | 2.58 | 15.3 | +5.1 | +2.5 |
| 3. JS | 17.3 | 21.1 | 2.45 | 18.6 | -1.3 | -3.8 |
| 10. LW | 16.4 | 9.0 | 1.16 | 7.9 | +8.5 | +7.7 |
| 12. SG | 18.8 | 12.7 | 1.17 | 11.5 | +7.3 | +6.0 |

and non-pregnant women.

GROUP II COPPER BALANCE umol/DAY

| PATIENTS | INTAKE | OUTPUT | | | APPARENT ABSORPTION | MAXIMUM RETENTION |
|---|---|---|---|---|---|---|
| | | TOTAL | URINE | FAECES | | |
| 1. AG | 19.3 | 19.5 | 2.44 | 17.1 | +2.2@ | -0.2 |
| 2. CR | 12.8 | 15.3 | 3.17 | 12.2 | +0.60 | -2.5 |
| 3. PR | 14.7 | 18.2 | 1.00 | 17.2 | -2.58 | -3.6 |
| 4. DMcD | 20.2 | 18.2 | 1.77 | 16.5 | +3.7@ | +1.9 |
| 5. AA | 21.1 | 22.4 | 2.10 | 20.3 | +0.79 | -1.3 |

COPPER BALANCE IN NON-PREGNANT WOMEN umol/DAY

| PATIENTS | INTAKE | OUTPUT | | | APPARENT ABSORPTION | MAXIMUM RETENTION |
|---|---|---|---|---|---|---|
| | | TOTAL | URINE | FAECES | | |
| 1. DR | 33.4 | 41.7 | 2.43 | 39.2 | - 5.8 | - 8.3 |
| 2. AD | 20.3 | 12.3 | 1.88 | 10.3 | +10.0 | + 8.0 |
| 3. SA | 32.6 | 21.6 | 3.93 | 17.6 | +14.9 | +11.0 |
| 4. JP | 23.9 | 20.3 | 1.63 | 18.7 | + 5.2 | + 3.6 |

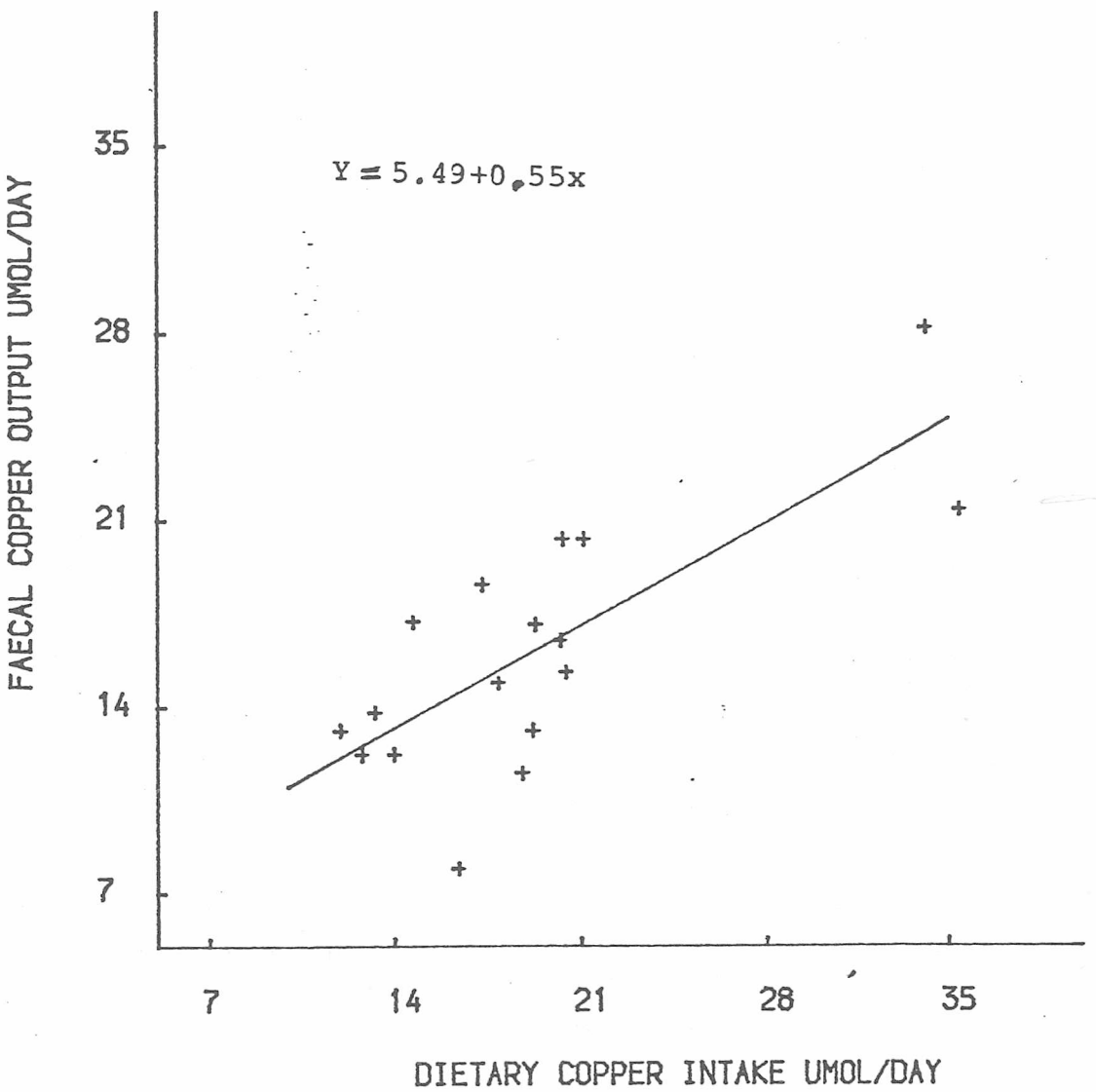


Fig. 4 Faecal copper output related to dietary copper intake.

$r = 0.75$ $P<0.001$

TABLE : 9

COPPER BALANCE umol/day

| PATIENTS MEAN ± SD | INTAKE | OUTPUT | | | APPARENT ABSORP-TION | MAXIMUM RETEN-TION |
|---|---|---|---|---|---|---|
| | | TOTAL | URINE | FAECES | | |
| Group I (a) | 23.72 | 19.62 | 1.48 | 18.14 | +5.58 | +4.08 |
| n = 5 | ±10.44 | ± 6.24 | ±0.64 | ± 6.58 | ±5.53 | ±5.61 |
| Range | 12.0to 35.4 | 14.3to 29.3 | 1.0to 2.5 | 13.1to 28.2 | -1.1to +14.0 | -2.3to +12.9 |
| Group I (b) | 17.21 | 15.90 | 1.67 | 14.23 | +2.98 | + 1.32 |
| n = 7 | ± 2.84 | ± 4.71 | ±0.70 | ± 4.27 | ±3.67 | ±4.26 |
| Range | 13.3to 20.4 | 9.0to 22.2 | 0.68to 2.6 | 7.9to 20.3 | -1.3to +8.5 | -3.8to +7.7 |
| Group II | 17.62 | 18.78 | 2.10 | 16.69 | +0.94 | -1.15 |
| n = 5 | ± 3.65 | ± 2.57 | ±0.80 | ±2.92 | ±2.33 | ±2.12 |
| Range | 12.8to 21.2 | 15.3to 22.4 | 1.0to 3.2 | 12.2to 20.3 | -2.5to +3.7 | -3.6to +1.9 |
| Non-Pregnant | 27.55 | 23.97 | 2.46 | 21.45 | +6.08 | +3.58 |
| Group n = 4 | ± 6.47 | ±12.51 | ±1.03 | ±12.40 | ±8.85 | ±8.48 |
| Range | 20.3to 33.4 | 12.3to 41.7 | 1.6to 3.9 | 10.3to 39.2 | -5.8to +14.9 | -8.3to +11.0 |

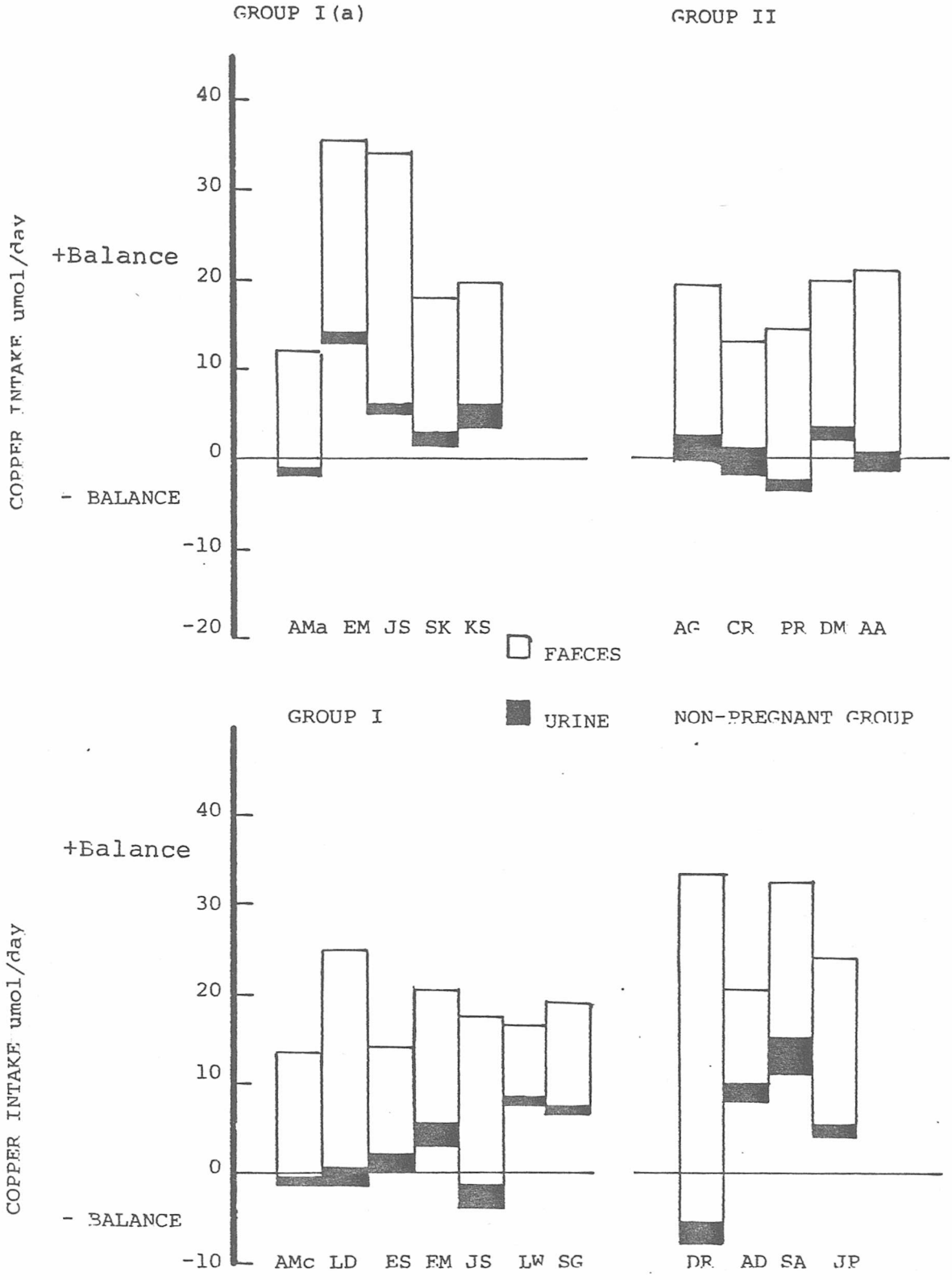


Fig.5 Graphical representation of copper balance in pregnant and non-pregnant women.

Urinary copper outputs were higher than the normal range for adults of 0.16 to 0.94 umol per day (0.01 - 0.69mg/day), (Table: 9), EM and JS had more than doubled their urinary copper output by the 3rd trimester (Table : 8).

Mean daily copper balance was positive in normal primigravidae in early and late pregnancy. Figure 5 gives a graphical representation of copper balance in the individual women. In early pregnancy one woman was in negative copper balance. In the 3rd trimester, three normal primigravidae, Group I(b) were in negative copper balance. The two women who completed an early and late balance, (EM, JS) showed a reduction in daily copper intake by 42% and 49% respectively. The decrease in their faecal copper output was less marked. Consequently copper balance was reduced in one subject, (EM), and was negative in the other (JS). The small lighter primigravidae had a mean daily copper loss of -1.15 umol (73ug), Table 9, four were in negative copper balance (Fig: 5).

Copper retention correlated with copper intake in the pregnant women. (Fig: 6).

Three of the non-pregnant women were in positive copper balance, one had a faecal copper excretion which exceeded her intake (Table: 8, Fig: 5).

DISCUSSION

Reports of dietary copper intakes in developed countries suggest intakes are lower than the suggested safe and adequate intake (US Nat. Acad. Sci. 1980). As copper is widely distributed in food, day to day variations in intake are small except when a food of particularly high copper content is included in the diet eg: liver,

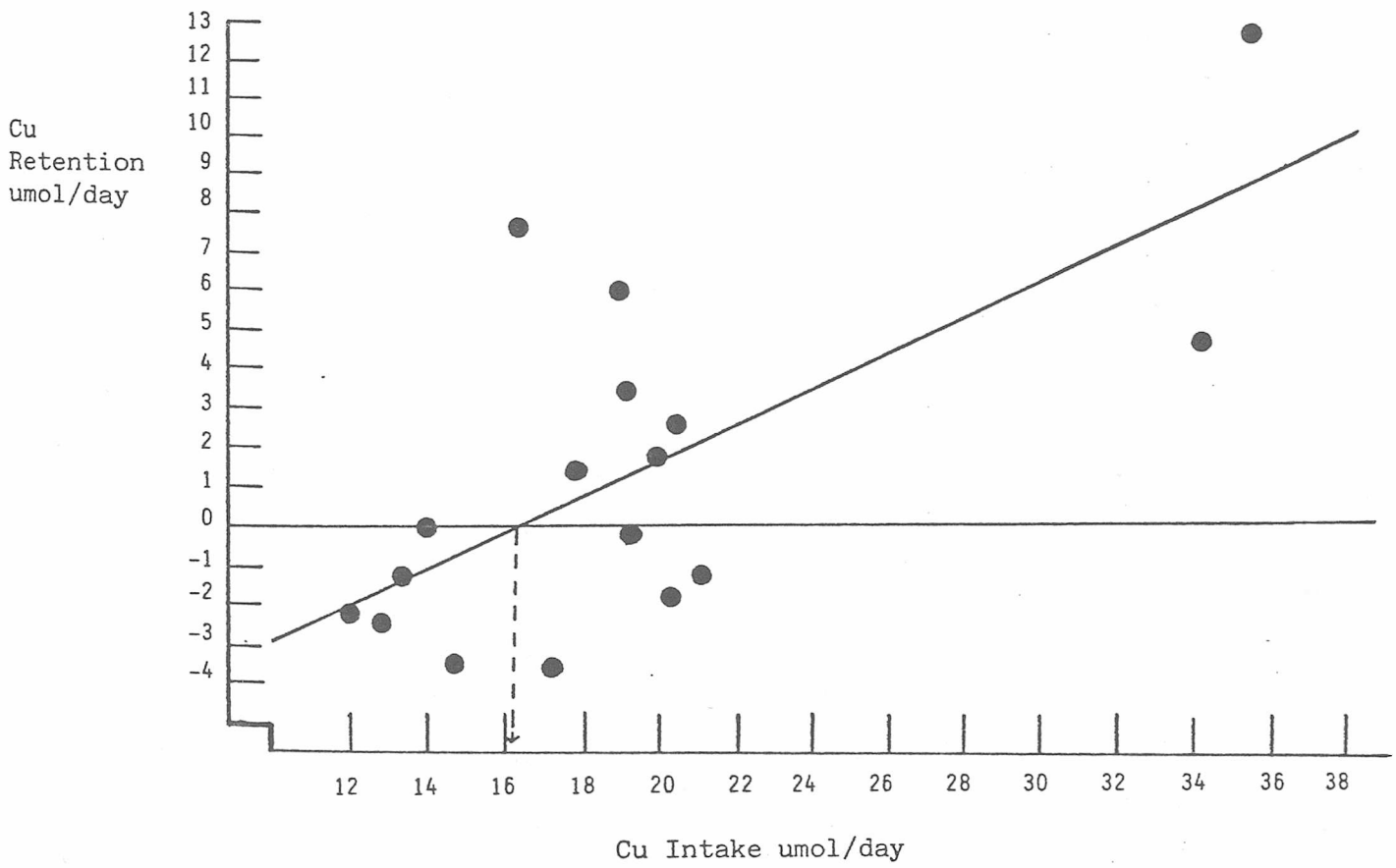


FIG: 6 Relationship between Copper Retention and Copper Intake in 17 Primigravida.

$Y = -7.56 + 0.47\ X$ $r = 0.68$

$P < 0.01$

oysters. In this study 76% of the women had a mean copper intake of less than 23.6 umol (1.5mg) per day. These intakes correspond with those of another group of primigravidae who weighed customary home diet for seven days, (Armstrong 1982). Refined cereals and cereal products contain less copper than wholegrain cereals and products. A diet in which the cereal, cereal products are largely of unrefined source will tend to contain more copper than when these products are refined. Liver contributed considerably to dietary copper, those women with copper intakes greater than 31.4 umol (2mg) ate liver during the balance period. Those women who almost halved their copper intake between early and late pregnancy ate liver during their first but not their second balance.

From this study the relationship of copper retention to copper intake (Fig: 6) suggests that a dietary copper intake of 16.1 umol (1.02mg) would maintain copper balance in primigravidae. However, copper requirement would vary at different stages of pregnancy depending on the rate and the type of tissue accumulated at that particular time. Most studies of copper balance in young women report negative copper balance at intakes of 31.5 umol (2.0mg) per day or less. The dietary copper intakes of women in this study were similar to previously reported intakes for young non-pregnant women eating home diets and each group was in positive balance. Any additional copper required during pregnancy may be obtained by an increase in dietary copper intake or by conserving body stores. The maternal liver copper store may serve as a source of copper during pregnancy and lower copper concentrations in the livers of pregnant women compared to non-pregnant women have been found, (Mason, 1979). Endogenous copper is largely excreted via the bile.

If there was an increased copper requirement then the enterohepatic circulation of copper would represent an efficient system for conservation of body stores. A reduction in copper excretion into the bile or reabsorption of biliary copper would help to conserve liver copper stores.

Urinary copper output in both pregnant and non-pregnant women was considerably higher than reported losses for young women and men on similar intakes. Urinary copper output doubled between early and late pregnancy in the two women studied. The rise in plasma copper during pregnancy may be associated witn an increase in urinary copper output. During pregnancy the increase in urinary amino acid excretion, (Hytten and LEitch 1971), may result in a greater loss of urinary copper as amino acids complexes (Henkin 1974).

The contribution of drinking water to dietary copper intake can reach significant levels (WHO Chron. 1978). The copper content of the hospital water supply may differ from supplies to other areas in Aberdeen. This was not considered when calculating total copper intakes and copper balance in this study.

Table 10: shows the calculated accumulation and daily retention of copper during different stages of pregnancy. It assumes that the protein and copper concentration of lean tissue accumulated during pregnancy is 117mg/g and 3.9ug/g respectively (Widdowson 1981). Hence the daily copper retention is related to the protein added at different stages of pregnancy (Hytten and Leitch, 1971).

TABLE : 10

CALCULATED ACCUMULATION AND DAILY RETENTION OF COPPER DURING PREGNANCY

| Weeks Gestation | 10 | 20 | 30 | 40 |
|---|---|---|---|---|
| Added protein (Hytten & Leitch, 1971) | 36 | 165 | 498 | 925 |
| Added Copper (ug) + (33.3ug/g Protein) | 1199 | 5494 | 16583 | 30802 |

| Weeks Gestation | 1 - 10 | 11 - 20 | 21 - 30 | 30 - 40 |
|---|---|---|---|---|
| Daily increased ug | 17.1 | 61.4 | 158 | 203 |
| Copper retention umol | (0.269) | (0.96) | (2.49) | (3.19) |
| *Increased daily ug | 57 | 205 | 527 | 677 |
| Copper intake umol | (0.89) | (3.21) | (8.27) | (10.65) |

+ = Assuming lean tissue is 117mg/g Protein and 3.9ug/g copper (Widdowson, 1981)

* = Assuming 30% availability of dietary Copper (Cartwright and Wintrobe, 1964)

An increase in daily copper retention, concomitant with the protein increments, of 2.49umol (158ug) between 20 and 30 weeks gestation and 3.19 umol (203ug) for the last ten weeks of pregnancy would be necessary. If this was obtained from dietary sources alone and assuming a 30% availability, this would require an increase in copper intake of 10.6umol (0.67mg) per day for the last ten weeks. A recent report on absorption of copper using stable isotope $^{65}Cu$ found mean apparent copper absorption to be 27.7% of the daily intake of 3.3mg (Turnland et al 1982).

# MANGANESE BALANCE

## CHAPTER V

Manganese deficiency has been demonstrated in a number of animal species. Depressed reproductive function has been found in manganese deficient animals (Orent and McCollum, 1931, Everson, Hurley and Greiger, 1959). In the female, three stages of manganese deficiency can be recognised according to the degree and duration of the deficiency. In the least severe stage the animals give birth to viable young, some or all of which exhibit ataxia. In the second, more severe stage the young are born dead or die shortly after birth and in the most severe stage reproductive cycles are absent or irregular. An association between normal brain function and manganese has been suggested; manganese deficient rats were more susceptible to convulsions than normal animals (Hurley, 1963). Tanaka (1978) found some children with convulsive disorders of unknown aetiology had whole blood manganese concentrations significantly below normal. These findings were confirmed by Papavasilion et al (1979). Manifestations found in manganese deficient animals suggest its essentiality for normal growth, reproduction, skeletal development and brain function. The natural occurrence of manganese deficiency is rare, the normal diet of most species seems to contain sufficient manganese to prevent deficiency.

There is insufficient information on manganese requirements to establish a recommended daily intake. From balance studies which suggest manganese equilibrium is achieved at an intake of 45 umol (2.5mg) per day, the U.S. National Academy of Science, (1980) suggest a 'safe and adequate intake' of 45.5 - 91.0 umol (2.5 - 5mg) per day for adults. Using data from dietary surveys the World Health Organisation (1973), suggest an intake of 36.4 umol - 54.6 umol (2 - 3 mg) per day for adults.

The average daily manganese intake of two adults in which 40 - 50% of the calories came from white flour were 40 - 49 umol (2.2 - 2.7 mg) the corresponding intakes for two individuals in which the same proportion of calories came from 92% extraction flour were 155 - 160 umol (8.5 - 8.8 mg) manganese (Kent and McCance, 1941). Monier - Williams (1949) calculated the manganese content of a 'typical' winter English diet to be 127 umol (7 mg), 60 umol (3.3 mg) of which was estimated to come from tea. Mean daily manganese intake of nine college women in the USA was 67 umol (3.7 mg) (North Leichsenning and Norris, 1960) and four young adult women in New Zealand had intakes ranging from 47 - 56 umol (2.6 - 3.1 mg) (McCleod and Robinson 1972). Hamilton and Minski (1972) found similar intakes for adult diets in the United Kingdom of 49 umol (2.7mg) per day. A later study from New Zealand reported manganese intake to be 49 umol (2.7 mg) in adult women (Guthrie and Robinson 1977). Manganese intake of Canadian women was 56 umol (3.1 mg) per day. (Gibson and Scythes 1982). Lower intakes have been reported in American College women (White 1969).

Neither the mechanism nor the main site for manganese absorption has been demonstrated. It is suggested that manganese homeostasis is regulated largely by manganese excretion (Leach and Lilburn, 1978). Manganese is excreted almost totally in the faeces and very little is eliminated in the urine. The main excretory pathway for endogenous manganese is in the bile, small amounts are excreted directly into the gastrointestinal tract. Faecal excretion represented 91% of the total excretion in young adult women (North, Leichsenning and Norris, 1972). The renal excretion

of manganese tends to be widely variable, studies report outputs between 0.05 and 2.9 umol (3 and 160 ug) (White and Gynne 1971, McLeod and Robinson 1972, Spencer et al 1979).

There are few reports of manganese intakes and balance in humans. Positive manganese balance occurred when manganese intakes were greater than 2mg (36.4 umol) (Greger et al 1978, McLeod and Robinson 1972, and North, Leichsenning and Norris 1960). A daily manganese intake of 1.2 mg (22.2 umol) was insufficient to maintain balance in nine young women (White and Gynne 1971) and 2.1 mg (38.2 umol) did not maintain balance in adult men (Spencer et al 1979).

This study reports manganese intake, absorption and retention in groups of pregnant and non-pregnant young women.

RESULTS

The mean daily manganese intake of primigravidae in early pregnancy was 47.24 umol (2.59 mg) per day, intakes during the 3rd trimester were 43.13 umol (2.37 mg) and 54.44 umol (2.99 mg) per day. (Table II). There was considerable variation in individual intakes of these women, ranging from 25.8 umol (1.42 mg) to 76.2 umol (4.19 mg) per day, (Table 12). Mean daily manganese intake of the non-pregnant women tended to be higher ranging from 54.9 umol (3.0 mg) to 153.4 umol (8.4 mg), (Table 12). Urinary manganese output represented 2 - 5% of the total manganese output. Faecal manganese output was directly related to manganese intake (FIG: 7). Faecal manganese output exceeded intake in ten of the pregnant women (FIG: 8). All groups of primigravidae were in negative manganese balance. There was a tendency toward a negative manganese balance in early pregnancy, Group I(a), two of the women in this group were

TABLE : 11 MANGANESE BALANCE umol/day

| PATIENTS MEAN ± SD | INTAKE | OUTPUT | | | APPARENT ABSORP-TION | MAXIMUM RETEN-TION |
|---|---|---|---|---|---|---|
| | | TOTAL | URINE | FAECES | | |
| Group I (a) | 47.24 | 52.61 | 1.20 | 51.42 | -4.18 | -5.37 |
| n = 5 | ±12.40 | ±32.23 | ±0.39 | ±31.93 | ±21.87 | ±22.17 |
| Range | 28.9to 62.8 | 26.0to 104.7 | 0.79to 1.54 | 25.2to 103.2 | -40.4to +12.7 | -41.9to +11.6 |
| Group I (b) | 43.13 | 47.36 | 1.42 | 45.94 | -2.81 | - 4.23 |
| n = 7 | ±12.27 | ±16.72 | ±0.77 | ±16.70 | ±13.72 | ±13.85 |
| Range | 25.8to 56.4 | 28.6to 79.0 | 0.45to 2.2 | 30.8to 77.2 | -31.2to +9.8 | -33.1to +8.2 |
| Group II | 54.44 | 63.06 | 1.80 | 61.27 | - 6.82 | - 8.62 |
| n = 5 | ±17.28 | ±13.62 | ±0.79 | ±13.2 | ±11.37 | ±11.94 |
| Range | 35.8to 76.2 | 48.6to 78.1 | 0.89to 3.0 | 48.7to 76.1 | -25.1to +4.7 | -28.1to +3.4 |
| Non-Pregnant | 98.20 | 85.73 | 1.87 | 83.87 | +14.33 | +12.47 |
| Group n = 4 | ±44.59 | ±53.65 | ±0.62 | ±53.74 | ±25.9 | ±26.36 |
| Range | 54.9to 153.4 | 48.4to 165.4 | 1.0to 2.3 | 46.1to 163.6 | -10.2to +50.9 | -12.0to +49.9 |

Table:12 Mean daily manganese balance of pregnant and non-pregnant women

GROUP I (a) MANGANESE BALANCE umol/DAY

| PATIENTS | INTAKE | OUTPUT | | | APPARENT ABSORP-TION | MAXIMUM RETEN-TION |
|---|---|---|---|---|---|---|
| | | TOTAL | URINE | FAECES | | |
| 1. AMa | 44.6 | 33.9 | 0.79 | 33.2 | +11.40 | +10.62 |
| 2. EM | 28.9 | 26.0 | 0.91 | 25.2 | +3.40 | +2.81 |
| 3. JS | 62.8 | 104.7 | 1.54 | 103.2 | -40.4 | -41.9 |
| 4. SK | 52.7 | 62.7 | 1.70 | 61.0 | - 8.3 | -10.0 |
| 5. KS | 47.2 | 35.6 | 1.07 | 34.5 | +12.7 | +11.6 |

GROUP I (b) MANGANESE BALANCE umol/DAY

| PATIENTS | INTAKE | OUTPUT | | | APPARENT ABSORP-TION | MAXIMUM RETEN-TION |
|---|---|---|---|---|---|---|
| | | TOTAL | URINE | FAECES | | |
| 7. AMc | 45.9 | 79.0 | 1.8 | 77.2 | -31.2 | -33.1 |
| 8. LD | 56.4 | 49.1 | 2.09 | 46.9 | + 9.42 | +7.3 |
| 9. ES | 49.9 | 41.7 | 1.91 | 40.0 | +9.85 | + 8.2 |
| 2. EM | 27.0 | 28.6 | 1.02 | 27.6 | -0.60 | -1.6 |
| 3. JS | 53.8 | 55.9 | 0.46 | 55.4 | -1.60 | -2.1 |
| 10. LW | 43.1 | 44.2 | 0.45 | 43.7 | -0.60 | -1.10 |
| 12. SG | 25.8 | 33.0 | 2.25 | 30.8 | -4.97 | -7.20 |

GROUP II MANGANESE BALANCE umol/DAY

| PATIENTS | INTAKE | OUTPUT | | | APPARENT ABSORP-TION | MAXIMUM RETEN-TION |
|---|---|---|---|---|---|---|
| | | TOTAL | URINE | FAECES | | |
| 1. AG | 67.0 | 74.4 | 1.78 | 72.9 | - 5.90 | - 7.75 |
| 2. CR | 53.4 | 50.0 | 1.33 | 48.7 | + 4.70 | + 3.4 |
| 3. PR | 76.2 | 78.1 | 1.99 | 76.1 | + 0.12 | - 1.87 |
| 4. DMc | 39.8 | 48.6 | 0.89 | 47.7 | - 7.9 | - 8.79 |
| 5. AA | 35.8 | 63.9 | 3.00 | 60.9 | -25.1 | -28.11 |

MANGANESE BALANCE IN NON-PREGNANT WOMEN umol/DAY

| PATIENTS | INTAKE | OUTPUT | | | APPARENT ABSORP-TION | MAXIMUM RETEN-TION |
|---|---|---|---|---|---|---|
| | | TOTAL | URINE | FAECES | | |
| 1. DR | 153.4 | 165.4 | 1.84 | 163.6 | -10.2 | -12.0 |
| 2. AD | 114.3 | 64.4 | 1.00 | 63.4 | +50.9 | +49.9 |
| 3. SA | 54.9 | 48.4 | 2.35 | 46.1 | + 8.8 | + 6.5 |
| 4. JP | 70.2 | 64.7 | 2.28 | 62.4 | + 7.8 | + 5.5 |

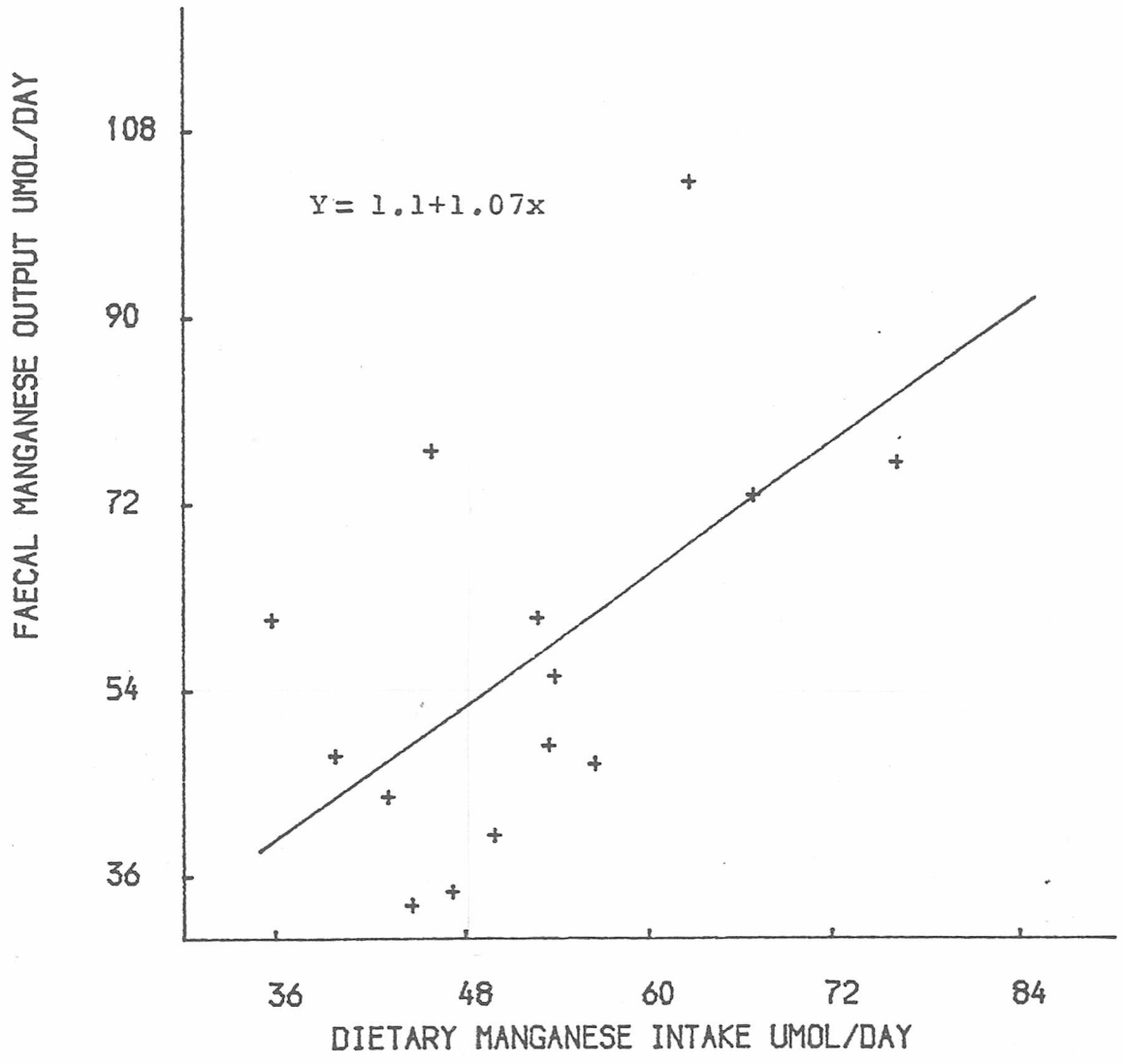


Fig.7 Faecal manganese output related to dietary manganese intake.

$r = 0.70$ $P<0.01$

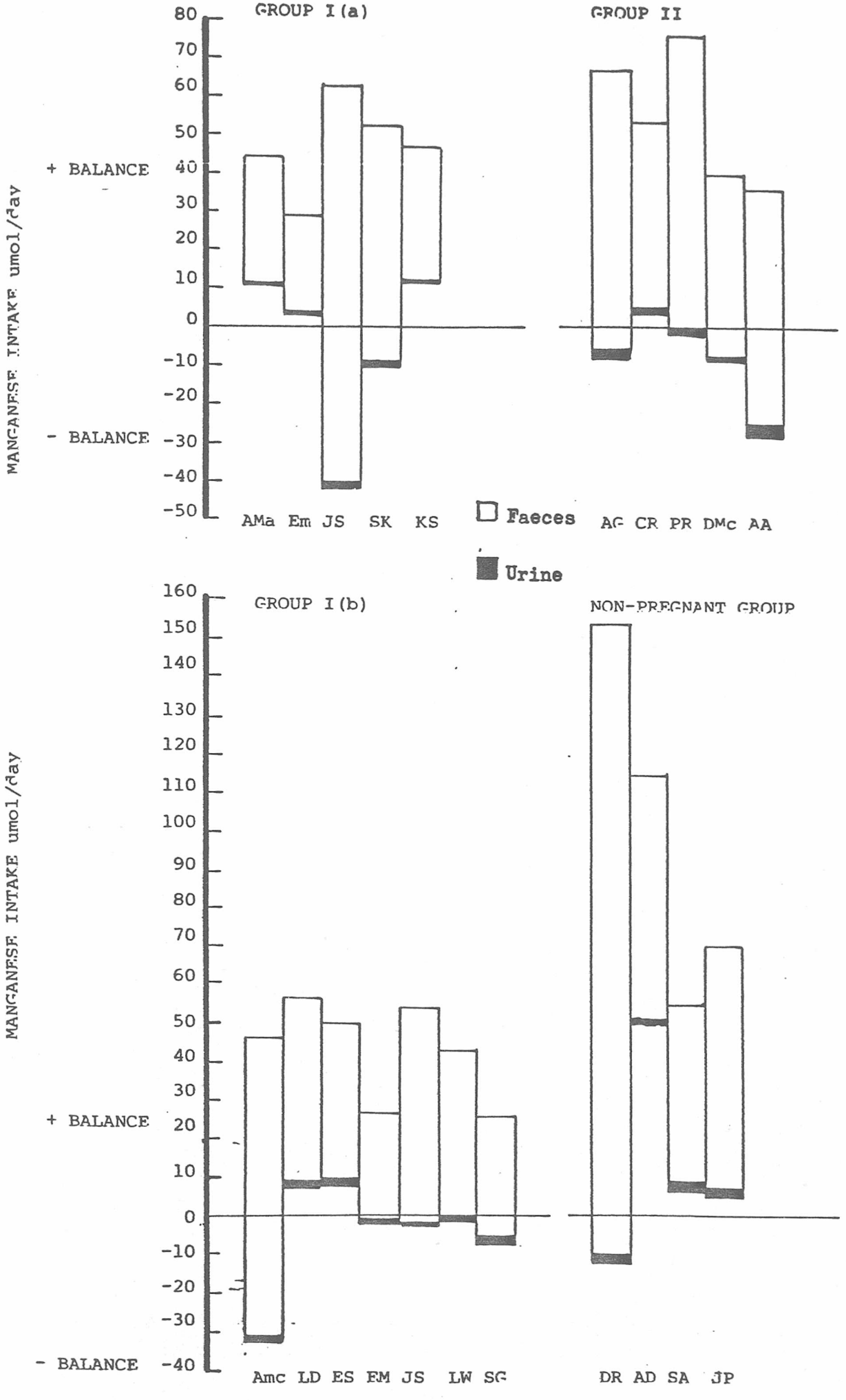


Fig.8 Graphical representation of manganese balance in pregnant and non-pregnant women.

in negative balance and in the 3rd trimester five from Group I(b) and four from Group II were in negative balance (FIG :8). Three of the four non-pregnant women were in a positive manganese balance (FIG : 8).

DISCUSSION

The wide range of manganese intakes in this study reflect how the manganese concentrations of dietary constituents are extremely variable. On the whole, nuts, whole grain cereals, legumes and leafy vegetables have a relatively high manganese content while animal tissues, dairy products, meat, poultry, seafood and refined cereals are poor sources (Schroeder, Balassa and Tipton 1966). Tea is exceptionally rich in manganese, one cup of tea can contribute as much as 23.7 umol (1.3 mg) of manganese (Wenlock, Buss and Dixon, 1979). McLeod and Robinson (1972) found soluble coffee and tea accounted for 10% of total dietary manganese intake in young women. North, Leichsenning and Norris (1960) found a cup of tea contained 5.2 umol (0.29 mg), therefore, an average of four cups per day could contribute as much as 21.1 umol (1.16 mg) to the daily manganese intake. Milling of wheat greatly reduces the manganese content as most of the manganese is contained in the bran and germ (Underwood 1977). Diets high in milk, meat, sugar and refined cereals are characteristically low in manganese while those high in whole cereals, nuts, legume seed and green leafy vegetables are high. In developing countries, where unrefined cereals are the staple food, dietary intakes of manganese will be invariably high.

In this study pregnant women who had low manganese intakes below 36.4 umol (2.0 mg) per day (E.M., S.G. A.A.) were either non tea drinkers or drank less than four cups during the balance. The

higher manganese intakes of the non-pregnant women may be explained by the nature of their diets. They habitually consumed wholemeal bread and cereals, in contrast, to the pregnant women who chose white bread and refined breakfast cereals. Unrefined and partially refined cereals such as brown and wholemeal flour, wheat germ and unpolished rice are rich in manganese. White flour and cornflakes contain very little manganese.

Renal excretion of urinary manganese outputs in the present study are similar to those reported in other studies. The higher urinary manganese excretion in the non pregnant group may reflect the higher manganese intakes of these women.

There was a tendency for pregnant women to be in negative manganese balance, manganese loss was not a reflection of a low intake. However, it may be the manganese requirements of these pregnant women vary as much as the intakes which, in some instances may be insufficient to maintain a positive balance. Manganese balance may be regulated over longer periods than the duration of the balances in this study. The results of this study show a large variation in daily manganese intake and balance.

## SUMMARY AND CONCLUSION OF ZINC, COPPER AND MANGANESE BALANCE

The object of this work was to investigate the absorption and retention of zinc, copper and manganese by 'normal' primigravidae, primigravidae at risk of delivering a small,light infant and non-pregnant women.

Fifteen primigravidae and four non-pregnant women participated in a six day metabolic balance of zinc, copper and manganese. Of the fifteen women ten were 'normal' primigravidae - five of whom completed a balance in early pregnancy and seven in the third trimester (two completed both an early and third trimester balance). The other five primigravidae were selected as being at risk of delivering a small,light infant, balance studies were completed in the 3rd trimester.

During the six day balance the primigravidae were fed a diet which was similar to their customary home diet. Results were expressed in amounts per day with no correction for body size. The composition and pattern of weight gain at a given gestation will vary within an individual. In respect of this, the use of maternal weight to correct for body size at a given gestation would be inaccurate.

Zinc and copper balance were positive in normal primigravidae in the third trimester. If we consider the pattern of fetal weight gain during pregnancy, it is greatest during the last ten weeks. Accumulation of zinc and copper may be associated with the accretion of lean body tissue, necessitating the greatest retention of these elements during the latter half of pregnancy. Retention of zinc and copper in these primigravida occurred on a dietary intake remarkably below the recommended intake and 'suggested intake' for these elements. A positive zinc and copper balance in the 3rd trimester would suggest the dietary intakes were sufficient to maintain the balance and the recommendations may be over estimated.

Primigravidae at risk of delivering a small,light baby were in negative zinc and copper balance.

Urinary zinc and copper are partly in the form of amino acid complexes and it is possible they follow a similar pattern of excretion during pregnancy. Increased excretion of these elements in the third trimester may accompany the increased loss of amino acid complexes.

Net loss of zinc and copper may occur if dietary intake is insufficient. 'Normal' primigravidae retained zinc and copper on an intake which was similar to women at risk of delivering growth retarded infants.

A net intestinal loss of manganese occurred in all groups of primigravidae. Faecal output exceeding dietary intake may be due to an increased bilary or intestinal manganese excretion. Using the conventional balance method as in this study it is impossible to distinguish the proportion of faecal metal which is unabsorbed dietary metal and that which is from endogenous secretions. Consequently, true intestinal absorption of the dietary metal cannot be shown. The future use of stable isotopes in trace element balance studies will expand our knowledge of their true absorption and excretion and improve our understanding of their homeostasis in healthy humans at different stages of growth and development and in disease states.

# COMPARISON BETWEEN VALUES OBTAINED FROM FOOD TABLES AND CHEMICAL ANALYSIS OF DIETS

## CHAPTER VI

A method commonly employed to estimate the nutrient intake of groups or individuals is to weigh the food consumed daily and to calculate its nutritive value from food composition tables. It is assumed that the error introduced by the use of tables is small and consistent enough for valid comparisons to be made between the dietary intakes of different groups and recommended daily amounts. The food tables used most frequently in this Country are McCance and Widdowson's, "The Composition of Foods", 4th Edition (Paul and Southgate 1978). A comparison was made between protein intake calculated using the 1st edition (1940) of these tables and analysed intakes for mixed metabolic ward diets, (Widdowson and McCance, 1943). The analysed and calculated protein intakes agreed within $\pm$ 20%. Stock and Wheeler (1972) analysed 54 daily diets and compared them to calculated values using the 3rd edition (1960) of the tables and found that 78% of the analysed daily protein intakes agreed within $\pm$ 20% of the calculated. The range of percentage differences from 9 day dietary surveys was less than those from 3 day surveys, suggesting that discrepancies between calculated and analysed nutrient intakes for groups of individuals decrease as the duration of the survey increases. Lawson et al (1982) correlated analysed and calculated values for nitrogen using the most recent edition of the tables, 1978; of the six comparisons made during this study, five agreed within $\pm$ 20%. Analysed and calculated intakes of zinc and copper correlated despite large variations in individual comparisons.

## MATERIALS AND METHODS

To assess the accuracy of using food tables to calculate the trace element content of diets twenty-one calculated intakes of nitrogen, zinc and copper have been compared to direct analysis of the diets. The methods of chemical analysis, (A), for dietary nitrogen, zinc and copper were those described in Chapter II. Calculated intakes, (C), were determined by computer analysis from the recent edition of "The Composition of Food Tables" (Paul and Southgate, 1978).

## RESULTS

Four of the calculated nitrogen intakes were higher than analysed (Table 13). All of the calculated values agreed within ± 20% of the analysed ones and 57% agreed within ± 10% (Appendix VI). The difference between calculated and analysed nitrogen intakes was significant, (A) being consistently higher than (C), (Table 13). Figure 9 shows the correlation between nitrogen intakes calculated by using food tables and by direct analysis.

Calculated zinc intakes had a tendency to be lower than the analysed, fifteen, (71%), of the calculated values were lower (Table 13). ALl of the calculated values agreed with ± 25% of analysed values and, 81% agreed within ± 20%, (Appendix VII). Figure 9 shown the correlation between the two methods of assessing zinc intake.

There was a considerable variation in comparisons of copper intake, (Table 13). Thirteen (62%) of the values agreed within ± 20% (Appendix VIII). Figure 9 shows the relationship between the calculated and analysed intakes. Calculated intakes were not significantly higher than analysed, (Table 13).

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Table:13
Mean difference of daily nutrient intakes determined by direct analysis (A) and by using food tables (C).

| NUTRIENT | MEAN INTAKE/DAY ± SD CALCULATED (C) | ANALYSED (A) | MEAN DIFFERENCE C - A | % DIFFERENCE $\frac{C-A}{A}$ X 100 RANGE | PAIRED t TEST |
|---|---|---|---|---|---|
| NITROGEN MMOL | 954.9 ± 141.05 | 994.4 ± 137.03 | -39.5 ± 97.04 | -18.5 to +18.1 | 2.59 * |
| ZINC UMOL | 165.1 ± 27.36 | 173.7 ± 32.20 | -8.6 ± 24.64 | -24.9 to 23.2 | 1.62 (NS) |
| COPPER UMOL | 25.43 ± 6.04 | 25.02 ± 7.31 | + 0.41 ± 6.32 | -30.4 to 72.5 | -0.11 (NS) |

Differences Significance * ($P < 0.01$)

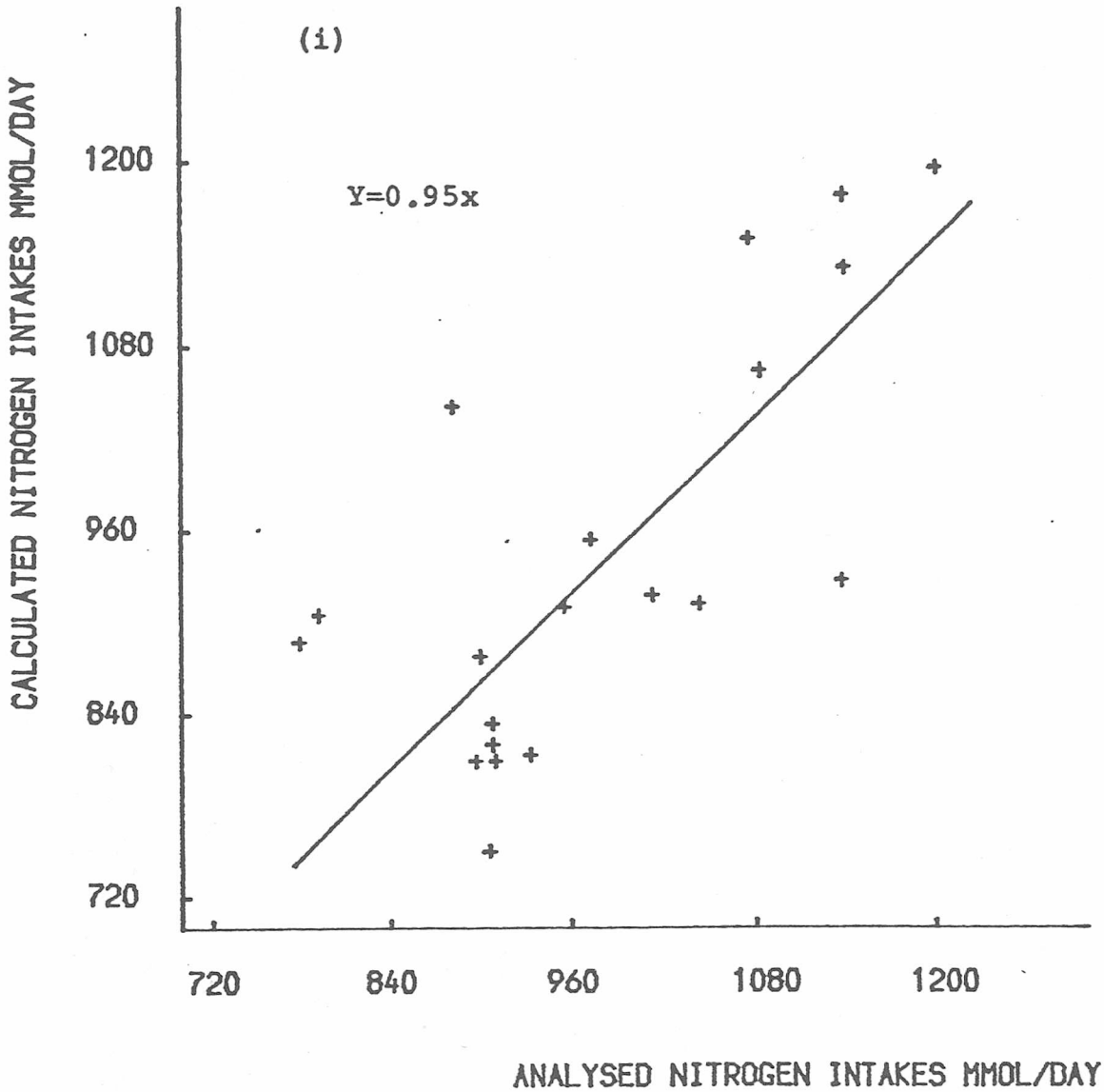
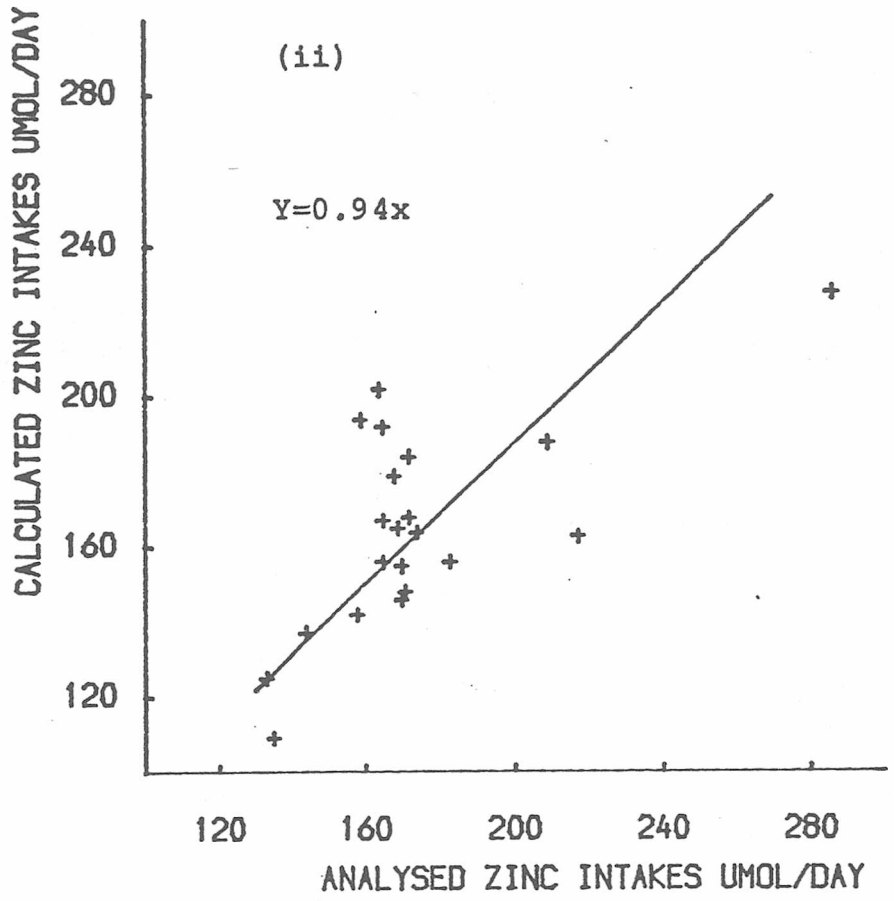
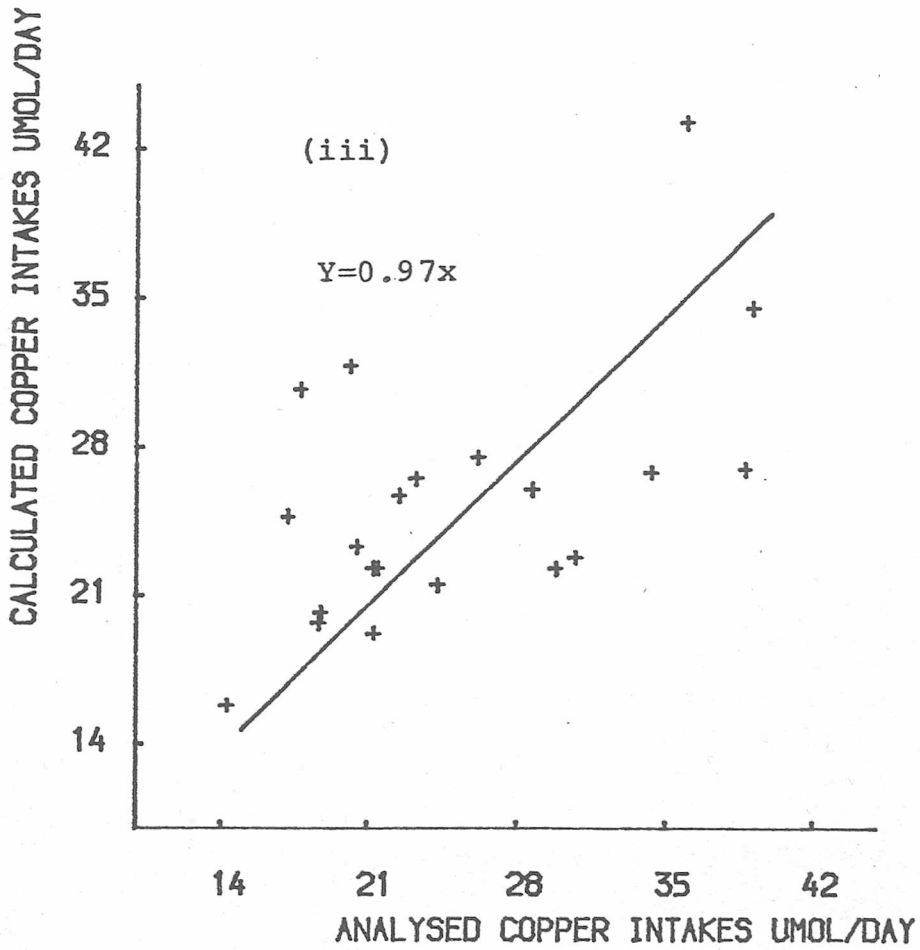



Fig.9 (i),(ii),(iii)
Relationship between dietary nitrogen,zinc and copper intake determined by direct analysis and by using food tables.


(ii)
Y=0.94x
CALCULATED ZINC INTAKES UMOL/DAY
280
240
200
160
120
120
160
200
240
280
ANALYSED ZINC INTAKES UMOL/DAY


(iii)
Y=0.97x
CALCULATED COPPER INTAKES UMOL/DAY
42
35
28
21
14
14
21
28
35
42
ANALYSED COPPER INTAKES UMOL/DAY

## DISCUSSION

The significant tendency for the calculated nitrogen intakes to be lower than the analysed values suggests that nitrogen intakes calculated from food tables may be underestimated. Lawson et al, (1982) found 4 of 6 calculated values were lower than the analysed. Discrepancies between direct analysis and calculated intakes may partly be explained by the varying composition of multi-ingredient dishes such as sauces, bakeries, pies, etc. The analysis of these dishes for inclusion in the food tables are based on the recipes provided in the tables. Unless these recipes are used to prepare these dishes within a dietary survey the composition and nutrient content differ. In addition the food tables are composed of average values of particular food items and isolated food samples may not be comparable, eg. the concentration of protein in meat products such as hamburgers and sausages will vary according to the ingredients used by the particular supplier. Some of these factors may explain inaccuracies which can arise from using food tables.

Although there is a tendency for the analysed zinc intakes to be higher than the calculated ones the difference was not significant.

Zinc:- There were six diets in which calculated zinc intakes exceeded analysed intakes. Different water sources and varying degrees of environmental contamination may influence the zinc content of food. Zinc levels in dust from domestic homes is high (Hamilton, 1976). The contamination of food by air-borne dust particles may be important when assessing total zinc intake, the significance of such contamination on total trace element intakes has not been established.

The zinc content of the water supply may vary according to corrosion from plumbing materials, (Obrecht and Pourbaix, 1967), which in turn will influence zinc in water used in cooking, for making beverages, or for drinking direct from the tap, none of which are taken into account using the food tables. Further, no consideration is given to the possible contribution of cooking utensils to the abundance of zinc in the prepared diet.

Copper:- There was a wide range of discrepancies for copper intakes. Overestimated and underestimated intakes were almost equally distributed so there was no consistent tendency for calculated copper intakes to be 'high' or 'low'. The individual variation of these results would suggest that the use of such tables to assess the dietary copper intake of individuals may give rise to important errors. The large discrepancies between the analysed and calculated figures may be attributed to numerous factors which influence the amount of copper in food consumed. Copper content may vary according to genetics, eg. the variety of orange or tomato may determine the copper content of fresh or canned fruit and juice. The variety of wheat, rye or oats may influence the copper content of bread, cereal and other baked goods. (Greaves and Anderson, 1936). Younger animals have a higher copper concentration in organs and muscle meats than do other animals of the same species (Pennington and Calloway, 1973, Schroeder, 1966). The copper content of soil and soil type may determine the amount which a plant absorbs which, in turn, affects the concentration in fruits, vegetables and cereals, as well as the amount available to herbivorous and ominivorous animals in forage, (Miller and Mitchell, 1931, Schroeder, 1966). The copper contamination of water, air and

soil from industry and metal works results in geographical variations in copper concentrations (Hamilton, 1974). The copper content of oysters from waters contaminated with metallic waste is higher than those from cleaner waters (Abdulla, Royle and Morris, 1972). The copper content of drinking water depends on the hardness of the water and the type of pipes it is transported along (Hamilton, 1974). Some copper lined hot water systems may increase copper content of water 100 fold in areas with soft water supplies. (Golse, 1936). Food cooked in naturally soft water may lose minerals. The water in the area of this study is naturally soft and therefore copper may have been lost in the cooking fluid of vegetables. The level of copper may be altered by manufacturing processes (Masiron, Koirtyphann and Pierce, 1977, Hamilton, 1972).

Considering the numerous factors which influence the trace element content of foods eaten by man it is likely that inaccuracies will arise from the use of food tables to assess nutrient intakes of a small population.

## SUMMARY

Nitrogen, zinc and copper content of 21 diets have been determined and compared with calculated intakes using food composition tables. Nitrogen calculated from food tables gave consistently lower results than analysed and there was a tendency for calculated zinc intakes to be lower. Mean daily intake of nitrogen and zinc in a group of twenty individuals, assessed using food tables, was reasonably accurate. The numerous factors affecting the copper content of foods in different areas may lead to inaccuracies when the dietary copper intakes of a small group of individuals is assessed.

# DIETARY INTAKES OF PRIMIGRAVIDAE
# CHAPTER VII

It was thought the ubiquitous presence of zinc and copper as a natural constituent or contaminant in foods meant that requirements were invariably met under normal dietary conditions. However, today zinc deficiency has been recognized in the human population in both children and adults in developing and developed countries. Human copper deficiency has also been described associated with conditions requiring special dietary management.

DIETARY REQUIREMENTS AND RECOMMENDATIONS:- When considering the total dietary intake of an element, it should be remembered that interactions between elements themselves and other components of the diet will affect the bioavailability of that element. It is meaningless to stipulate minimum dietary requirements or maximum safe intakes for individual trace elements and the use of safe and adequate ranges, depending on the nature of the whole diet and the environment may be most suitable.

ZINC:- Dietary requirements for zinc have been advised by the World Health Organization (WHO), 1973. These requirements are calculated on a factorial basis from limited information on growth requirements, tissue repair and obligatory excretion. Required intakes during pregnancy are given depending on the availability of zinc (Table: 14)

Table 14: Estimated requirements of dietary Zinc (WHO, 1973)

| Pregnant Women | Milligrams necessary in daily diet if content of available zinc is:- | | |
|---|---|---|---|
| | 10% | 20% | 40% |
| 0 - 20 weeks | 25.5 (390 umol) | 12.8 (196 umol) | 6.4 ( 98 umol) |
| 20 - 30 weeks | 29.0 (444 umol) | 14.5 (222 umol) | 7.3 (112 umol) |
| 30 - 40 weeks | 30.0 (459 umol) | 15.0 (229 umol) | 7.5 (115 umol) |

The U.S. Nat. Acad. Sci., 1980 recommended a daily zinc intake of 20mg during pregnancy.

Evidence from a number of studies may suggest that customary dietary zinc intakes are well below the above recommendations in a number of countries. Slorach and Gustaffsson, (1982) found Swedish adults diets contained 131 umol (8.6mg) per day. Mean daily intake of young New Zealand women was 153 umol (10.0mg) (Guthrie and Robinson, 1977) and the analysis of self selected diets of teenage and college women revealed intakes of 184 umol (12.0mg) and 211 umol (13.8mg) respectively, (White, 1969). The daily zinc intake of young women living in the West of Scotland was 116 umol (7.6mg). (Lyon, Smith and Smith, 1979). Other workers have reported similar intakes (Wolf, Holden and Green 1977, Osis et al, 1972) all falling

well below the recommendation of 229 umol (15mg) for adults (U.S. Nat. Acad. Sci, 1980). Similarly, reported intakes of pregnant women are also remarkably less than those recommended. The mean daily zinc intake of healthy Swedish primigravidae was 144 umol (9.4mg) (Jameson1976 ) and that of Mexican women was 144 umol (9.4mg) in early pregnancy and 153 umol (10mg) in the third trimester. (Hunt et al, 1979).

Zinc is most abundant in high protein foods, particularly those of animal origin and certain seafoods. Some workers have reported a correlation between protein and zinc intake, (Brown et al, 1976, Murphy, Page and Watt, 1971, Hunt et al, 1979).

COPPER:- The WHO (1973) suggested an intake of 30ug/kg body weight per day for adults; equivalent to a daily copper intake of 25umol(1.6mg) for a 55kg woman and 33 umol (2.1mg) for a 70kg man. The U.S. National Academy of Science (1980) propose an estimated 'safe and adequate' intake of 31.5 - 47.2 umol (2 - 3mg) per day for adults. Dietary surveys in the USA suggest that over two thirds of the population may have a daily copper intake of less than 31.4 umol (2mg) (Mason 1979). Daily copper intakes from mixed western diets ranged from 15 - 47 umol (1 - 3mg)/day (Hartley, Dawson and Hodgkinson 1974; Robinson et al 1973). Analysis of twenty-two self selected diets gave a mean daily copper intake of 15.8 umol (1.01mg) (Wolf, Holden and Green, 1977). Young Canadian women had a copper intake of 29.9 umol, (1.9mg) (Gibson and Scythes, 1982) and intake of Swedish adults was 22 umol (1.4mg)/day (Abdulla and Swensson, 1981). A study in New Zealand showed liver included in the diet contributed considerably to copper intake, (Guthrie and Robinson, 1977): non liver eaters had copper intakes of 24 umol (1.5mg)

compared to 119 umol (7.6mg) when liver was included.

Reports of zinc and copper intake in numerous groups of people are less than that recommended for zinc and suggested for copper. There is little information on intakes of zinc and copper during pregnancy, we have investigated dietary intakes of zinc and copper in Aberdeen primigravidae during the third trimester.

PATIENTS AND METHODS

Dietary intakes of twenty primigravidae of normal weight, height and weight for height (Group A), and twenty eight primigravidae selected as being at risk of delivering a small, light baby, (Group B), were investigated. The criteria for patient selection was as described in Chapter II. Each patient completed a 7 day weighed dietary survey around thirty weeks of pregnancy. During the survey one 24 hour urine sample was collected by each subject. This was subsequently analysed for nitrogen by the Micro-Kjeldhal method described in Chapter II. Patients were recruited at an Antenatal Clinic where a detailed description and demonstration of weighing and recording food intake was given. Each patient was provided with a 7 day dietary diary and a Salter (Model 512) scale. They were shown how to operate the scale and were seen to be competent before beginning the survey. For seven consecutive days everything consumed except water was weighed and recorded. They detailed the type of foods, method of cooking and brand names where appropriate. Any plate waste or inedible parts of food were also weighed. On completion each diary was checked in the presence of the patient for obvious weighing errors, absence of weights or insufficient detail of the food items. The information from the

diet surveys was coded on computer sheets and the nutrient content calculated from the recent Composition of Food Tables (Paul and Southgate 1978). Each computer output was checked before being accepted.

RESULTS

Mean weight at 20 weeks, age and height of Group A was 60.6Kg, 22.2 years and 163cm respectively. Four women in Group A smoked during their pregnancy. Mean weight at 20 weeks, age and height of Group B was 51.3 Kg, 24.2 years and 155cm respectively. Nine women in Group B smoked during their pregnancy. Details of the individual women in the study are given in Appendix : IX and X. All 48 women completed a seven day weighed dietary survey. Forty completed a 24 hour urinary collection. Urinary nitrogen excretion is directly related to dietary nitrogen intake (Johnstone et al, 1981). Mean daily nitrogen intake correlated with 24 hour urinary nitrogen output, (FIG : 10) There was no significant difference in mean daily intake of energy, protein, zinc and copper between Group A and Group B (Table 15). Mean daily energy intake in Group A and B were less than the 10.0MJ recommended for pregnant women, (DHSS, 1979) only 25% of the women in Group A and 32% from Group B had an energy intake which met the recommendation. Mean daily protein intake of both groups was higher than the 60.0g recommended for pregnancy (DHSS, 1979). Zinc intake correlated with protein intake (FIG : 11). Four women in Group A and seven in Group B had liver during their weeks survey. Mean daily copper intakes of liver eaters were 31.2 umol compared to 21.4 umol of non-liver eaters (see Appendix:

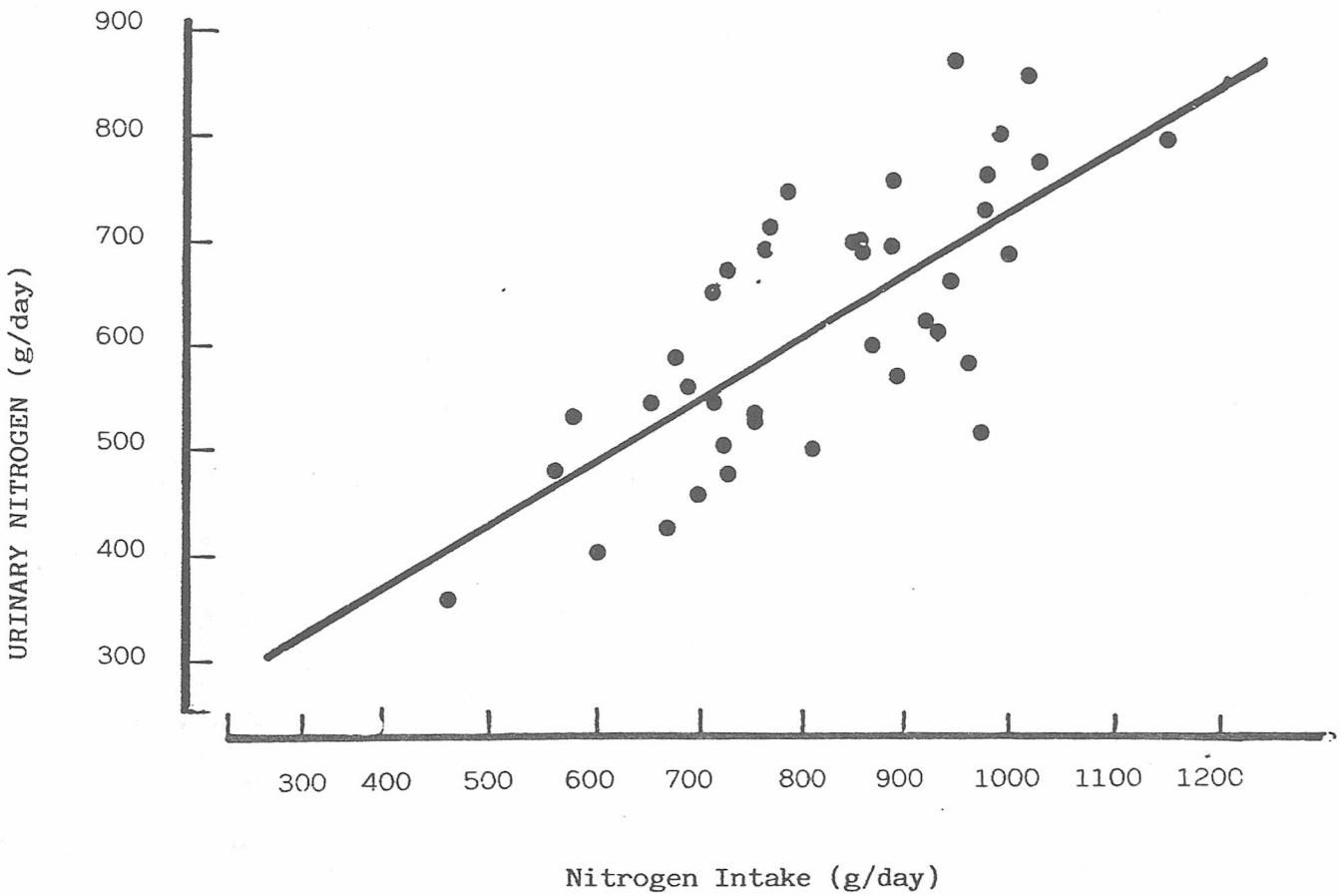



Fig: 10

Relationship between Nitrogen Intake and Urinary Nitrogen output.

$Y = 130 + 0.6X$

$r = 0.73$; $P < 0.001$

TABLE : 15

DAILY INTAKES IN GROUP A
AND GROUP B

| MEAN DAILY INTAKE | GROUP A n = 20 MEAN ± ISD | GROUP B n = 28 MEAN ± ISD | df = 46 t | SIGN |
|---|---|---|---|---|
| ENERGY MJ | 8.37 ± 1.97 | 8.93 ± 1.75 | 1.05 | NS |
| PROTEIN g | 69.7 ± 13.91 | 74.7 ± 17.15 | 1.05 | NS |
| ZINC umol | 136.6 ± 31.93 | 148.9 ± 35.86 | 1.23 | NS |
| COPPER umol | 22.64 ± 5.38 | 24.53 ± 10.16 | 0.75 | NS |

TABLE : 16

DAILY INTAKES OF GROUP B WOMEN WHO DELIVERED INFANTS > 10th CENTILE GROUP B (i) AND THOSE WHO DELIVERED INFANTS < 10th CENTILE GROUP B (ii)

| MEAN DAILY INTAKE | GROUP B (i) n = 22 MEAN ± ISD | GROUP B (ii) n = 6 MEAN ± ISD | df = 26 t | SIGN |
|---|---|---|---|---|
| ENERGY MJ | 9.31 ± 1.57 | 7.32 ± 1.37 | 2.81 | P < 0.01 |
| PROTEIN g | 75.6 ± 18.81 | 71.1 ± 9.21 | 0.56 | NS |
| ZINC umol | 149.4 ± 38.47 | 147.5 ± 26.98 | 0.72 | NS |
| COPPER umol | 25.5 ± 10.68 | 20.7 ± 7.50 | 1.03 | NS |

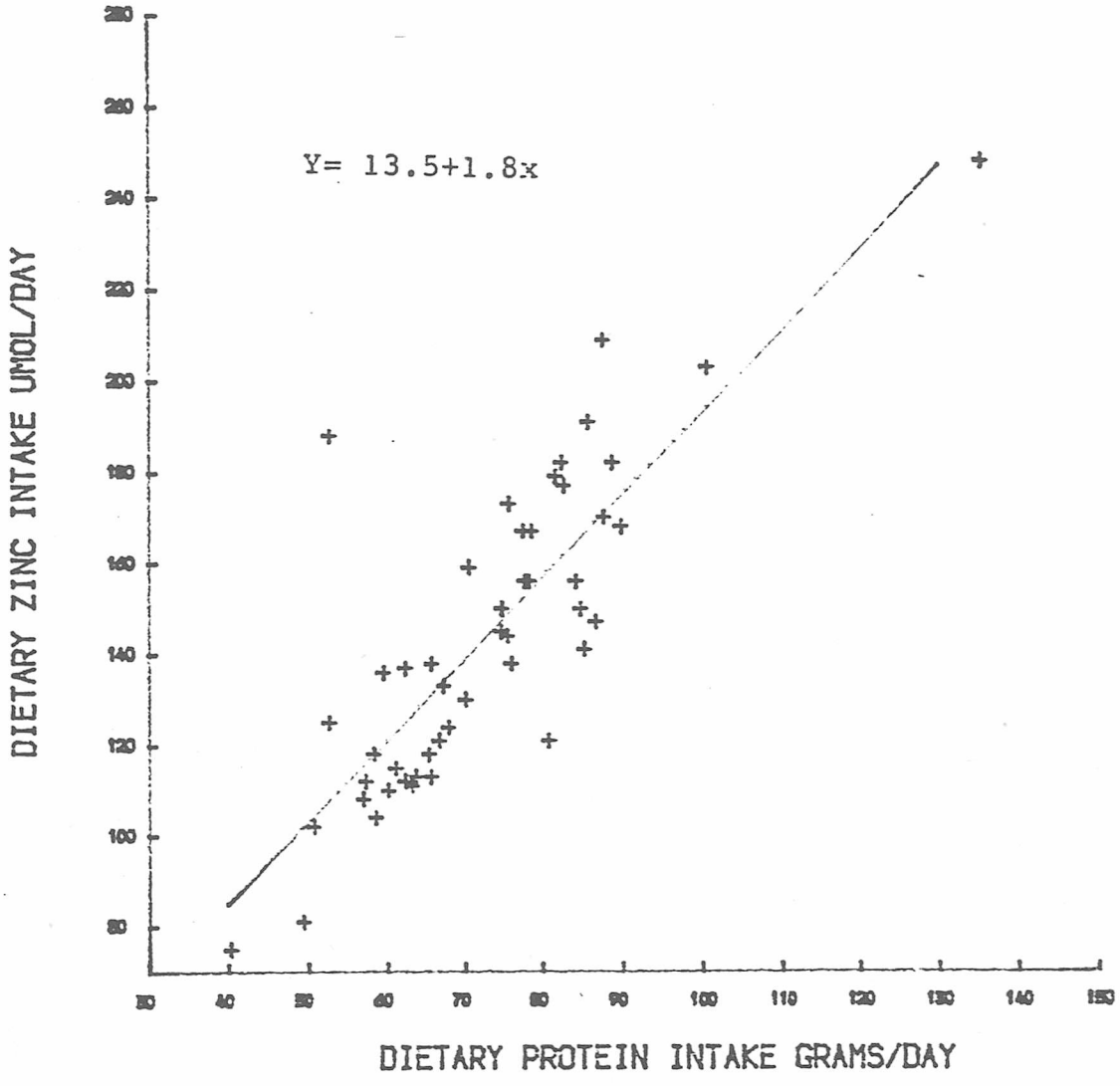



Fig.11:Correlation between dietary protein intake and zinc intake.

r=0.83 P< 0.001

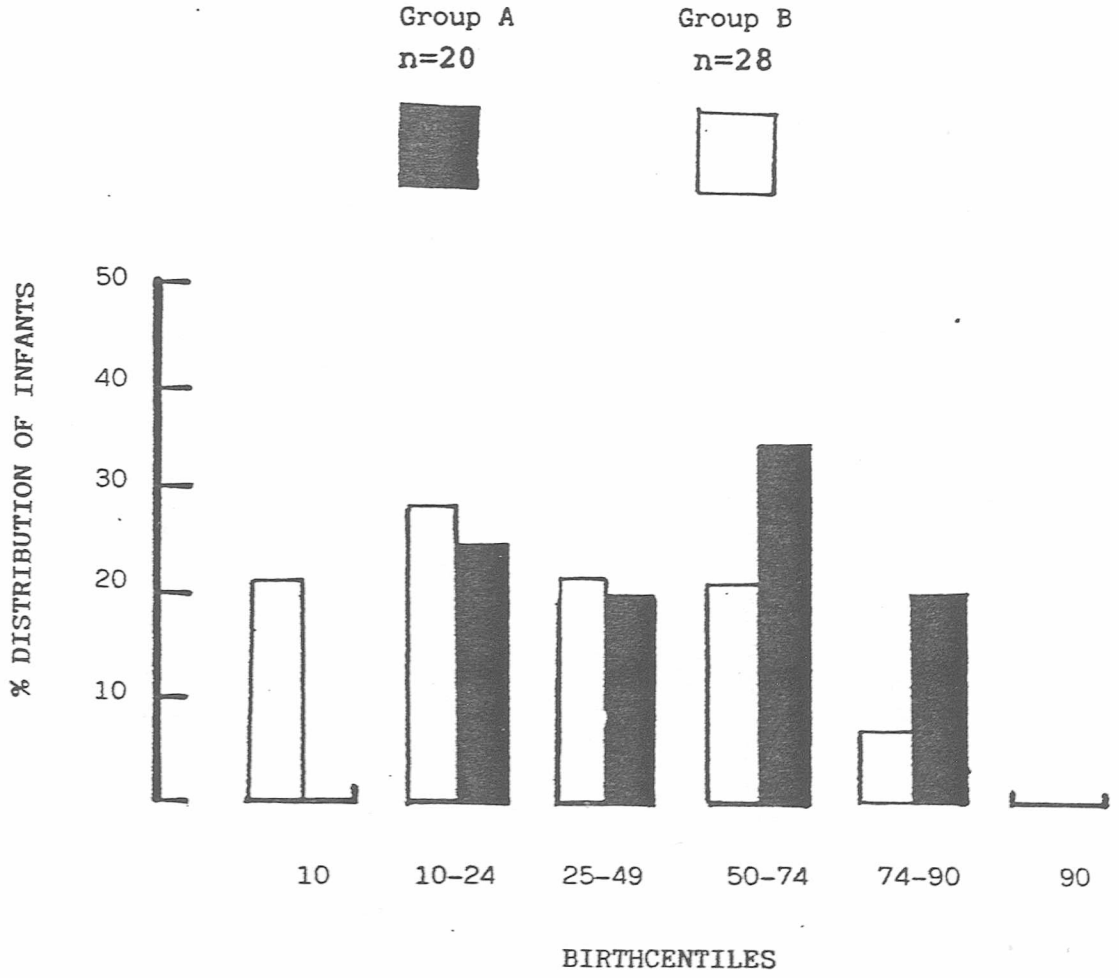
XI and XII). Protein, carbohydrate and fat provided 14.2%, 47% and 38.8% of the energy intake in Group A and 14.2, 46.7% and 39.1% respectively in Group B.

The distribution of infant birthweights in the two groups studied is shown (FIG : 12). As anticipated from the selection criteria the distribution shows a tendency for women in Group B to deliver infants of lighter birthweight than women in Group A. Birthcentiles were used in order to take the mothers gestation at delivery and the sex of the infant into account. Half of the women in Group B delivered infants < 25th birthweight centile, six delivered infants < 10th centile. None of the mothers in Group A delivered an infant < 10th centile. Group B mothers who delivered infants > 10th centile constitute Group B(i), n = 22, and women who delivered the infants < 10th centile, constitute Group B(ii), n = 6. Mean maternal weight at 20 weeks, age and height were similar in the two groups. Six women in Group B(i) and three in (ii) smoked during their pregnancy. Dietary intakes of these two groups were compared.

Mean daily energy intake of women who delivered infants < 10th centile was significantly, ($P < 0.01$), lower than that of the group who delivered infants > 10th centile, (Table 16), protein intake was marginally but not significantly lower and zinc intake was similar in both Groups. Copper intake was lower but not significantly lower in the group who delivered infants < 10th centile, (see Appendix XIII and XIV).

Fig: 12

Birthcentile distribution of infants delivered by women in Group A and Group B.



## DISCUSSION

In this study, dietary intakes of primigravidae were calculated from 7 day weighed surveys. The accuracy of the information obtained from a dietary survey depends on the reliability of the subject to keep a record which reflects his or her customary diet. Errors from dietary surveys can arise from significant but unapparent weighing errors. Patients may omit to record foods consumed outwith the home or simply forget to complete the diary, subjects may record what they think they ought to eat instead of what they actually eat. A method of verifying dietary assessments has been established by Johnstone, (1977), who showed a close correlation between nitrogen in the diet and urinary nitrogen excretion. It was further demonstrated that this relationship could confirm the daily nitrogen intake as measured from weighed dietary surveys (Johnstone et al 1981). In this study 24 hour urinary nitrogen output correlated with nitrogen intake in 40 subjects confirming the survey data. Mean daily energy intakes in this study are consistant with these reported for other groups of Aberdeen primigravidae, 8.78 MJ and 8.45 MJ (Campbell et al, 1982). Protein intakes were higher than recommended which is consistent with other reported intakes of pregnant women (Doyle, Crawford and Lawrance 1982; Abraham, 1982; King, Stein and Doyle, 1981).

Mean daily zinc intakes of the primigravidae were considerably lower than the recommendations for the third trimester. The U.S.Nat. Acad. Sci. recommendation, 1980 is estimated from the daily retention of zinc during human pregnancy by relating it to the increase of weight in the components of pregnancy (Sandstead 1973).

The calculation assumes that the zinc concentration of components other than the fetus are similar to that of the fetus. On this basis a daily retention of 750ug is calculated during the last twenty weeks of gestation, and, assuming 15% of dietary zinc is available,an extra intake of 5mg zinc per day is recommended during pregnancy. If we use the association between protein and zinc intake found in this study (FIG : 11) - in order to meet this extra 5mg of zinc per day, by normal dietary means, an extra 35g of protein would be required daily. Mean daily protein intakes in the present study are more than adequate by recommended standards. In order to meet the zinc recommendation for pregnancy an abnormally high protein intake would be necessary, a protein intake of 162g per day would provide 20mg of zinc (Fig: 11). Thus it would seem to be impossible to meet the recommended daily intake for zinc during pregnancy by normal dietary means alone, without a protein intake far in excess of its own recommended intake. In the present study none of the diets provided 306 umol, (20mg) of zinc per day, 62% provided less than half of this, one woman had an intake greater than 15mg/day and eleven intakes were less than 115 umol (7.5mg)/day. The recommended daily intake for protein during pregnancy is 60g, this is equivalent to 121 umol (7.9mg) of dietary zinc (FIG: 11). By definition the RDA for all nutrients is intended to cover the needs of most healthy individuals i.e. intakes which are less than the RDA do not necessarily mean they are inadequate. However, 'the greater the proportion of people with intakes below those recommended the greater the possibility that some individuals may be undernourished with respect to the nutrient in question'

(DHSS, 1979a). The discrepancy between the recommendation for zinc and the observations in this study is considerable and would suggest the recommended intake is far in excess of the requirement. Of the forty eight primigravidae studied forty two delivered normal healthy infants. In the absence of any symptoms of a dietary zinc deficiency and the successful outcome of their pregnancy it would seem their apparently low zinc intakes were adequate for their requirements. Dietary zinc intake in women who delivered infants < 10th centile was not significantly different. Assuming the dietary zinc requirements of these women was not higher it would seem factors in addition to, or other than this were important in the outcome of their pregnancy.

Copper intakes in the present study are similar to those reported for other mixed western diets. No significant difference was found in daily copper intake between women who delivered infants < 10th centile and those who delivered infants > 10th centile. The mean intake was below that advised, (U.S. National Academy of Science, 1980 WHO, 1973).

Energy intake was significantly lower in the group of women who delivered small infants, protein intakes were similar. The difference in energy intake was due to a significantly higher carbohydrate ($P < 0.01$) intake in women who delivered the normal weight babies. It is well known that the demand for energy takes first priority and if carbohydrate and fat are not consumed in sufficient amounts to meet energy requirements protein will be used as a source of energy. In this study the energy intake of mothers who delivered growth retarded babies may have been one of a number

of factors affecting fetal growth. However, the aetiology of growth retardation is complex and may involve economic factors, environmental conditions, human behaviour, hereditary influences as well as prepregnancy nutritional status (Metcoff et al, 1981), none of which are considered in the present study. A relationship between smoking and growth retardation is well established, half of the women who delivered small babies smoked during their pregnancy. A retrospective study found no difference in energy and protein intake between a group of women who delivered small, light infants and those who delivered normal infants (Armstrong 1982). With the small number of women in this study and, without excluding the numerous other factors which may have influenced the outcome of these pregnancies it would be rash to attribute them to an insufficient energy intake alone. The number and complexity of factors affecting infantile birthweight make it difficult to isolate individual factors.

The difference in copper intake between liver eaters and non liver eaters was not as marked as reported in the New Zealand study when mean daily copper intake was five times higher when liver was included in the diet. (Guthrie and Robinson, 1977). However, it does indicate the marked contribution of liver to dietary copper intake. The main sources of copper in the British Household diet were found to be meat and meat products and cereal and cereal products, providing 28% and 24% of the total intake respectively. (Spring Robertson and Buss, 1979). Absence of any reports of naturally occurring dietary copper deficiency in adults would suggest that dietary copper intakes are sufficient in this country.

The contribution of drinking water to dietary copper intake was not accounted for in this study. The average person takes in about one and a half litres of water every day and some 60% of this is tap water. Even if the inorganic elements are present in low concentrations the total amount of water ingested can make this source appreciable. It has been estimated that tap water may provide 10% of daily copper intake (WHO Chron., 1978). However, the tendency for the general population to consume more refined foods, particularly cereals and cereal products at the expense of more 'whole foods' may result in a decrease in copper and indeed zinc intakes. The refining of food tends to reduce the trace element content. The milling of flour results in a loss of 68% and 78% of zinc and copper respectively. Consequently, white bread as compared with wholewheat bread contains 69.8% and 77.4% less copper and zinc respectively (Schroeder, 1971). However, a reduction in zinc and copper content through processing may be compensated for by an increase in their availability, particularly if the process removes the fibres and phytins. Copper may be present in food as a dietary contaminant from copper cooking vessels, in industry and homes copper water storage vessels and pipes. More recently, however, copper vessels may be replaced by plastic, aluminium and steel with a concomitant reduction in dietary contamination. In view of this it is important to recognise that a reduction in zinc and copper intake may occur when refined and processed foods replace wholemeal, wholegrain and fresh foods. This may not result in suboptimal intakes in the general population but those groups with higher requirements e.g. children and pregnancy may be particularly at risk.

## SUMMARY

The aim of this study was to expand present knowledge of trace element nutrition during human pregnancy. With this in mind the object was to try and answer the following:-

i. What are the customary zinc and copper intakes of primigravidae in the area studied?
ii. How do they compare to current recommendations and safe and adequate intakes?
iii. Do they differ in primigravidae at risk of delivering small infants?
iv. How accurate are food tables for assessing dietary intakes of trace elements?
v. What happens to zinc, copper and manganese absorption and retention during the course of pregnancy?
vi. Does the absorption and retention of the elements differ in primigraviedae at risk of delivering a small infant?

Dietary intakes of zinc and copper by a group of normal primigravidae and a group at risk of delivering a small infant have been assessed from 7-day weighed surveys. The absorption and retention of zinc, copper and manganese by groups of primigravidae have been investigated in early and late pregnancy.

The validity of using food composition tables to estimate zinc, copper and nitrogen intakes has been assessed by comparing calculated intakes with the direct analysis.

1. All of the calculated nitrogen intakes and 81% of the zinc intakes agreed within ±20% of the analysed. Calculated copper intakes were more variable; 62% agreed within ± 20%
2. Dietary zinc intake of primigravidae in this study was less than half the recommendation (US Nat. Acad. Sci., 1980). This suggests it may be greatly in excess of requirements and should be reconsidered. Dietary copper intake was less than advised (WHO, 1973, Nat. Acad. Sci., 1980).

3. Daily dietary energy, protein, zinc and copper intake were assessed during the 3rd trmester in two groups of primigravidae. Group I were women of normal weight for height and weight gain, and Group II were women at risk, according to criterion established on local population, of delivering a small infant. There was no significant difference in the dietary intakes of the nutrients studied.

4. Group II primigravidae were followed up to delivery. Subsequently, six went on to deliver a small infant, < 10th centile. The energy intake of these women, when assessed during the 3rd trimester had been significantly lower than those who went on to deliver infants > 10th centile. However, dietary protein, zinc and copper intakes did not differ.

5. Zinc and copper were retained in a group of normal primigravidae during the third trimester. This may be related to the accretion of lean body tissue by the products of conception during the latter half of pregnancy. Net intestinal loss of manganese was apparent in all groups of primigravidae.

6. A group of primigravidae at risk of delivering infants with small, light infants ardation were in negative zinc and copper balance in the third trimester. The possible consequences of zinc and copper loss in women at risk of delivering a small infant requires further investigation.

APPENDIX I

## GROUP I PRIMIGRAVIDAE

| PATIENT | AGE YRS | HEIGHT M | SMOKING | WEIGHT AT 20 WKS Kg | GESTATION AT DELIVERY | BIRTH WEIGHT (g) | SEX | BIRTH WEIGHT CENTILE |
|---|---|---|---|---|---|---|---|---|
| 1. AMa | 23 | 1.595 | S | 64.9 | 40 | 3480 | M | <75 |
| 2. EM (2 Balances) | 20 | 1.63 | - | 67.1 | 41 | 3350 | M | <50 |
| 3. JS (2 Balances) | 22 | 1.54 | - | 54.9 | 39 | 3340 | M | <75 |
| 4. SK | 23 | 1.60 | - | 56.5 | 41 | 4000 | F | <95 |
| 5. KS | 23 | 1.62 | - | 59.65 | 40 | 3970 | M | <90 |
| 6. AMc | 28 | 1.67 | - | 68.2 | 39 | 3160 | M | <50 |
| 7. LD | 27 | 1.66 | - | 65.75 | 41 | 3980 | F | < 95 |
| 8. ES | 28 | 1.65 | S | 62.7 | 40 | 2770 | F | < 25 |
| 9. LW | 25 | 1.63 | - | 58.4 | 40 | 3450 | F | <75 |
| 10. SG | 19 | 1.62 | - | 70.7 | 40 | 3610 | M | <75 |

ANALYSIS OF EDICOL BLUE MARKER
umol/marker (100mg)
Zn 0.18, Cu 0.08, Mn 0.04

APPENDIX II

Group II PRIMIGRAVIDAE

| PATIENT | AGE YRS | HEIGHT M | SMO-KING | WEIGHT AT 20 WKS Kg | WEIGHT AT 30 WKS Kg | GESTATION AT DELIVERY WEEKS | BIRTH-WEIGHT (g) | SEX | BIRTH WEIGHT CENTILE |
|---|---|---|---|---|---|---|---|---|---|
| 1. AG | 19 | 1.57 | S | 54.8 | 57.2 | 40 | 3030 | F | <50 |
| 2. PR | 23 | 1.56 | S | 52.6 | 60.9 | 40 | 2650 | F | <10 |
| 3. CR | 18 | 1.52 | S | 47.15 | 54.45 | 37 | 2380 | M | <10 |
| 4. DMc | 17 | 1.54 | - | 55.5 | 59.65 | 38 | 3350 | F | <75 |
| 5. AA | 20 | 1.57 | S | 52.0 | 57.10 | 36 | 2200 | M | <10 |

GROUP I (a) PRIMIGRAVIDAE (EARLY BALANCES)

| PATIENT | GESTATION DURING BALANCE | (DAY 1) WEIGHT Kg | (DAY 6) WEIGHT Kg | WEIGHT CHANGE Kg |
|---|---|---|---|---|
| 1. AMa | 16 | 63.8 | 63.8 | 0 |
| 2. EM | 14 | 65.9 | 66.55 | +0.65 |
| 3. JS | 16 | 52.35 | 52.60 | +0.25 |
| 4. SK | 15 | 53.35 | 54.0 | +0.65 |
| 5. KS | 16 | 57.65 | 57.55 | -0.10 |

GROUP I (b) PRIMIGRAVIDAE (LATE BALANCES)

| PATIENT | GESTATION DURING BALANCE | (DAY 1) WEIGHT Kg | (DAY 6) WEIGHT Kg | WEIGHT CHANGE Kg |
|---|---|---|---|---|
| 2. EM | 32 | 75.45 | 76.15 | +0.7 |
| 3. JS | 30 | 59.9 | 60.0 | +0.1 |
| 6. AMc | 31 | 73.8 | 74.15 | +0.35 |
| 7. LD | 30 | 70.8 | 71.15 | +0.35 |
| 8. ES | 31 | 65.65 | 66.25 | +0.6 |
| 9. LW | 32 | 59.1 | 59.25 | +0.15 |
| 10. SG | 29 | 74.0 | 74.55 | +0.55 |

GROUP II

| PATIENT | GESTATION | DAY 1 WEIGHT Kg | DAY 6 WEIGHT Kg | WEIGHT CHANGE Kg |
|---|---|---|---|---|
| 1. AG | 26 | 55.55 | 56.00 | +0.45 |
| 2. PR | 31 | 61.0 | 61.55 | +0.55 |
| 3. CR | 30 | 53.6 | 54.7 | +1.10 |
| 4. DMc | 30 | 59.3 | 59.7 | +0.40 |
| 5. AA | 31 | 57.0 | 57.3 | +0.30 |

NON-PREGNANT BALANCE PATIENTS

| PATIENT | AGE | DAY 1 WEIGHT Kg | DAY 6 WEIGHT Kg | WEIGHT CHANGE Kg |
|---|---|---|---|---|
| 1. DR | 21 | 64.2 | 64.65 | +0.45 |
| 2. AD | 21 | 53.8 | 53.8 | 0 |
| 3. SA | 25 | 69.5 | 68.0 | -1.5 |
| 4. JP | 26 | 68.25 | 68.9 | +0.65 |

# APPENDIX III

## MEAN DAILY ENERGY AND PROTEIN INTAKE

### GROUP I(a)

| PATENT | ENERGY MJ | PROTEIN (g) |
|---|---|---|
| 1. AMa | 7.90 | 47.4 |
| 2. Em | 8.14 | 70.5 |
| 3. JS | 9.71 | 71.1 |
| 4. SK | 9.30 | 63.8 |
| 5. KS | 9.43 | 72.0 |
| Mean | 8.90 | 65.0 |
| SD | ±0.82 | ±10.34 |

### GROUP I(b)

| PATIENT | ENERGY MJ | PROTEIN (g) |
|---|---|---|
| 6. AMc | 8.76 | 79.5 |
| 7. LD | 10.79 | 100.4 |
| 8. ES | 8.74 | 69.7 |
| 2. EM | 10.86 | 87.4 |
| 3. JS | 8.81 | 73.0 |
| 9. LW | 8.77 | 74.0 |
| 10. SG | 10.42 | 56.8 |
| MEAN | 9.59 | 77.2 |
| SD | ±1.03 | ±13.83 |

### GROUP II

| PATENT | ENERGY MJ | PROTEIN (g) |
|---|---|---|
| 1. AG | 8.53 | 64.7 |
| 2. CR | 7.82 | 58.9 |
| 3. PR | 10.99 | 66.2 |
| 4. DMcD | 9.32 | 66.8 |
| 5. AA | 11.03 | 89.5 |
| Mean | 9.54 | 69.2 |
| SD | ±1.44 | ±11.76 |

### NON-PREGNANT GROUP

| PATIENT | ENERGY MJ | PROTEIN (g) |
|---|---|---|
| 1. DR | 9.22 | 90.3 |
| 2. AD | 9.42 | 68.9 |
| 3. SA | 7.15 | 7.15 |
| 4. JP | 7.95 | 74.1 |
| Mean | 8.43 | 76.3 |
| SD | ±1.07 | ±9.57 |

## APPENDIX IV

GROUP I(a) NITROGEN BALANCE

mmol/DAY

| PATIENT | INTAKE | OUTPUT | | | APPARENT ABSORPTION | MAXIMUM RETENTION |
|---|---|---|---|---|---|---|
| | | TOTAL | URINE | FAECES | | |
| AMa | 542 | 664 | 613 | 51 | 419 | -122 |
| EM | 805 | 648 | 584 | 64 | 741 | +157 |
| JS | 812 | 663 | 560 | 103 | 709 | +149 |
| SK | 729 | 684 | 597 | 87 | 642 | + 45 |
| RS | 823 | 799 | 753 | 46 | 777 | + 24 |

GROUP I(b) NITROGEN BALANCE

mmol/DAY

| PATIENT | INTAKE | OUTPUT | | | APPARENT ABSORPTION | MAXIMUM RETENTION |
|---|---|---|---|---|---|---|
| | | TOTAL | URINE | FAECES | | |
| AMc | 908 | 866 | 783 | 83 | + 836 | + 42 |
| LD | 1147 | 1049 | 917 | 132 | +1015 | + 98 |
| ES | 796 | 718 | 639 | 79 | + 717 | + 78 |
| EM | 999 | 870 | 797 | 73 | + 926 | +129 |
| JS | 834 | 714 | 609 | 105 | + 729 | +120 |
| LW | 845 | 591 | 507 | 84 | + 761 | +254 |
| SG | 649 | 650 | 580 | 70 | + 579 | - 1 |

GROUP II NITROGEN BALANCE

mmol/DAY

| PATIENT | INTAKE | OUTPUT | | | APPARENT ABSORPTION | MAXIMUM RETENTION |
|---|---|---|---|---|---|---|
| | | TOTAL | URINE | FAECES | | |
| AG | 739 | 735 | 677 | 58 | + 681 | + 4 |
| CR | 673 | 561 | 489 | 72 | + 601 | +112 |
| PR | 756 | 739 | 669 | 70 | + 686 | + 17 |
| DMcD | 763 | 663 | 616 | 47 | + 716 | +100 |
| AA | 1023 | 929 | 833 | 96 | + 928 | + 94 |

NITROGEN BALANCE IN NON-PREGNANT WOMEN

mmol/DAY

| PATIENT | INTAKE | OUTPUT | | | APPARENT ABSORPTION | MAXIMUM RETENTION |
|---|---|---|---|---|---|---|
| | | TOTAL | URINE | FAECES | | |
| DR | 1032 | 999 | 871 | 128 | 904 | + 33 |
| AD | 787 | 882 | 802 | 74 | 713 | - 95 |
| SA | 821 | 828 | 769 | 59 | 762 | - 7 |
| JP | 847 | 869 | 763 | 106 | 741 | - 22 |

## APPENDIX V

## COMPARISON OF ANALYSED AND CALCULATED DETERMINATIONS OF DIETARY NITROGEN (mmol)

| | ANALYSED (A) | CALCULATED (C) | C - A | PERCENTAGE ERROR $\frac{C-A}{A} \times 100$ |
|---|---|---|---|---|
| 1 | 1137 | 927 | - 210 | -18.5 |
| 2 | 1201 | 1196 | - 5 | - 0.4 |
| 3 | 792 | 905 | + 113 | +14.3 |
| 4 | 897 | 809 | - 88 | - 9.8 |
| 5 | 908 | 820 | - 88 | - 9.7 |
| 6 | 1139 | 1131 | - 8 | - 0.7 |
| 7 | 973 | 954 | - 19 | - 1.95 |
| 8 | 1043 | 912 | - 131 | -12.5 |
| 9 | 1138 | 1178 | + 40 | + 3.5 |
| 10 | 1076 | 1150 | + 74 | + 6.9 |
| 11 | 933 | 813 | - 120 | -12.9 |
| 12 | 1013 | 918 | - 95 | - 9.4 |
| 13 | 910 | 809 | - 101 | -11.1 |
| 14 | 779 | 887 | + 108 | +13.9 |
| 15 | 1083 | 1064 | - 19 | - 1.8 |
| 16 | 906 | 750 | - 156 | -17.2 |
| 17 | 908 | 834 | - 74 | - 8.1 |
| 18 | 900 | 878 | - 22 | - 2.4 |
| 19 | 881 | 1041 | + 160 | +18.1 |
| 20 | 1310 | 1167 | - 143 | -10.9 |
| 21 | 956 | 910 | - 46 | - 4.8 |
| MEAN | 994.4 | 954.9 | - 39.5 | |
| $\pm$ 1 SD | $\pm$ 137.03 | $\pm$ 141.03 | $\pm$ 97.04 | |

APPENDIX VI

## COMPARISON OF ANALYSED AND CALCULATED DETERMINATIONS OF DIETARY ZINC (umol)

| | ANALYSED (A) | CALCULATED (C) | C - A | PERCENTAGE ERROR $\frac{C-A}{A} \times 100$ |
|---|---|---|---|---|
| 1 | 169 | 165 | - 4 | - 2.4 |
| 2 | 209 | 188 | - 21 | -10.0 |
| 3 | 183 | 156 | - 27 | -14.8 |
| 4 | 158 | 142 | - 16 | -10.1 |
| 5 | 171 | 148 | - 23 | -13.4 |
| 6 | 164 | 202 | + 38 | +23.2 |
| 7 | 172 | 184 | + 12 | + 6.9 |
| 8 | 165 | 167 | + 2 | + 1.2 |
| 9 | 159 | 194 | + 35 | +22.0 |
| 10 | 165 | 192 | + 27 | +16.4 |
| 11 | 144 | 137 | - 7 | - 4.9 |
| 12 | 172 | 168 | - 4 | - 2.3 |
| 13 | 133 | 125 | - 8 | - 6.0 |
| 14 | 170 | 146 | - 24 | -14.1 |
| 15 | 168 | 179 | + 11 | + 6.5 |
| 16 | 135 | 109 | - 26 | -19.3 |
| 17 | 170 | 155 | - 15 | - 8.8 |
| 18 | 165 | 156 | - 9 | - 5.4 |
| 19 | 217 | 163 | - 54 | -24.9 |
| 20 | 286 | 228 | - 58 | -20.3 |
| 21 | 174 | 164 | - 10 | - 5.7 |
| MEAN | 173.7 | 165.1 | - 8.6 | |
| $\pm$ 1 SD | $\pm$ 32.20 | $\pm$ 27.36 | $\pm$ 24.64 | |

## COMPARISON OF ANALYSED AND CALCULATED DETERMINATIONS OF DIETARY COPPER (umol)

| | ANALYSED (A) | CALCULATED (C) | C - A | PERCENTAGE ERROR $\frac{C-A}{A} \times 100$ |
|---|---|---|---|---|
| 1 | 17.2 | 24.7 | + 7.5 | +43.6 |
| 2 | 21.5 | 22.3 | + 0.8 | + 3.7 |
| 3 | 18.8 | 20.2 | + 1.4 | + 7.4 |
| 4 | 29.8 | 22.3 | - 7.5 | -25.2 |
| 5 | 38.8 | 27.0 | -11.8 | -30.4 |
| 6 | 20.5 | 23.3 | + 2.8 | +13.7 |
| 7 | 39.2 | 34.6 | - 4.6 | -11.6 |
| 8 | 20.2 | 31.8 | +11.6 | +57.4 |
| 9 | 26.2 | 27.5 | + 1.3 | + 5.0 |
| 10 | 22.5 | 25.7 | + 3.2 | +14.2 |
| 11 | 21.3 | 19.2 | - 2.1 | - 9.9 |
| 12 | 24.3 | 21.5 | - 2.8 | -11.5 |
| 13 | 18.7 | 19.7 | + 1.0 | + 5.3 |
| 14 | 21.3 | 22.3 | + 1.0 | + 4.7 |
| 15 | 17.8 | 30.7 | +12.9 | +72.5 |
| 16 | 14.3 | 15.8 | + 1.5 | +10.5 |
| 17 | 28.7 | 26.0 | - 2.7 | - 9.4 |
| 18 | 30.7 | 22.8 | - 7.9 | -25.7 |
| 19 | 23.3 | 26.5 | + 3.2 | +13.7 |
| 20 | 36.0 | 43.3 | + 7.3 | +20.3 |
| 21 | 34.3 | 26.8 | - 7.5 | -21.9 |
| MEAN | 25.02 | 25.43 | +0.41 | |
| $\pm$ 1 SD | $\pm$ 7.31 | $\pm$ 6.04 | $\pm$ 6.32 | |

APPENDIX IX

| PATIENT | AGE (Yrs.) | HEIGHT CM | SMOKING | GESTATION (Ws) | WEIGHT Kg |
|---|---|---|---|---|---|
| E.M. | 23 | 160 | - | 20 | 64.0 |
| M.C. | 21 | 159 | - | 20 | 62.3 |
| MGMcK | 21 | 166 | S | 20 | 64.2 |
| G.M. | 22 | 165 | - | 20 | 61.45 |
| F.S. | 31 | 156 | - | 20 | 57.95 |
| M.McK | 26 | 160 | - | 20 | 55.9 |
| L.M. | 23 | 169 | - | 20 | 57.0 |
| J.D. | 24 | 174 | - | 20 | 69.85 |
| A.M. | 23 | 158 | - | 20 | 67.8 |
| J.M. | 22 | 165 | - | 20 | 56.15 |
| C.F. | 21 | 163 | - | 20 | 57.9 |
| M.Gu. | 18 | 160 | - | 20 | 57.0 |
| M.Ge. | 21 | 158 | - | 20 | 54.7 |
| A.G. | 24 | 161.5 | - | 20 | 57.5 |
| C.S. | 20 | 157 | - | 20 | 64.1 |
| D.R. | 20 | 167 | S | 20 | 56.6 |
| L.B. | 22 | 160 | S | 20 | 55.0 |
| R.T. | 21 | 166 | - | 20 | 63.5 |
| A.A. | 22 | 164 | - | 20 | 60.4 |
| P.K. | 19 | 170 | S | 19 | 68.8 |

GROUP A

| GESTATION (Ws.) | WEIGHT Kg | GESTATION at Delivery | SEX | BABIES BIRTHWEIGHT (g) | Birth CENTILE |
|---|---|---|---|---|---|
| 30 | 72.4 | 41 | M | 3460 | < 50 |
| 30 | 65.1 | 39 | F | 2830 | < 25 |
| 30 | 70.2 | 39 | M | 2940 | < 25 |
| 30 | 65.4 | 40 | F | 3350 | < 75 |
| 30 | 65.0 | 39 | F | 3430 | < 75 |
| 30 | 60.4 | 36 | M | 2800 | < 50 |
| 30 | 61.95 | 40 | F | 3340 | < 75 |
| 30 | 74.70 | 41 | F | 3800 | < 75 |
| 30 | 72.8 | 40 | M | 3700 | < 75 |
| 30 | 59.5 | 40 | F | 3300 | < 75 |
| 30 | 62.0 | 39 | F | 3120 | < 50 |
| 30 | 62.8 | 40 | F | 2930 | < 25 |
| 30 | 60.1 | 41 | F | 3680 | < 75 |
| 30 | 62.45 | 39 | M | 3650 | < 75 |
| 30 | 68.60 | 41 | M | 3680 | < 75 |
| 30 | 58.2 | 40 | F | 2740 | < 25 |
| 30 | 59.7 | 40 | F | 3190 | < 50 |
| 29 | 69.6 | 41 | F | 3340 | < 75 |
| 29 | 64.2 | 41 | M | 4070 | < 75 |
| 30 | 71.4 | 40 | F | 2870 | < 25 |

APPENDIX (X)

GROUP B

| | PATIENT | AGE Yrs. | HEIGHT CM | SMOKING | WEEKS GESTATION | WEIGHT Kg | WEEKS GESTATION |
|---|---|---|---|---|---|---|---|
| 1 | C.B. | 26 | 150 | - | 20 | 51.8 | 30 |
| 2 | J.W. | 32 | 155 | - | 20 | 50.1 | 30 |
| 3 | K.H. | 17 | 155 | - | 20 | 48.6 | 30 |
| 4 | L.Pa | 22 | 154 | S | 20 | 49.2 | 30 |
| 5 | G.C. | 25 | 153 | S | 20 | 51.9 | 30 |
| 6 | L.B.(D) | 27 | 146 | S | 20 | 45.0 | 30 |
| 7 | M.F. | 32 | 152.5 | - | 16 | 53.6 | 29 |
| 8 | L.Pe | 27 | 152 | - | 17 | 53.1 | 29 |
| 9 | A.H. | 26 | 158 | - | 20 | 47.05 | 30 |
| 10 | S.S. | 26 | 161 | - | 20 | 54.45 | 30 |
| 11 | A.W. | 33 | 153 | - | 14 | 47.6 | 30 |
| 12 | S.H. | 22 | 154 | - | 20 | 60.2 | 31 |
| 13 | C.F. | 21 | 164 | - | 16 | 50.3 | 30 |
| 14 | L.H. | 23 | 147 | - | 20 | 44.7 | 30 |
| 15 | D.F. | 28 | 152 | - | 20 | 54.1 | 30 |
| 16 | H.S. | 17 | 154.5 | S | 22 | 55.3 | 30 |
| 17 | M.R. | 25 | 165.5 | - | 20 | 56.9 | 29 |
| 18 | H.C. | 27 | 156.5 | - | 21 | 51.75 | 30 |
| 19 | M.B. | 25 | 166 | S | 20 | 51.8 | 30 |

| WEIGHT Kg | GESTATION AT DELIVERY | SEX | BABIES BIRTHWEIGHT (g) | Birth CENTILE |
|---|---|---|---|---|
| 55.45 | 41 | F | 3660 | < 75 |
| 53.35 | 40 | F | 3250 | < 50 |
| 52.10 | 40 | M | 3260 | < 50 |
| 51.9 | 40 | F | 2670 | < 10 |
| 55.4 | 41 | M | 3030 | < 25 |
| 48.5 | 36 | F | 2000 | < 10 |
| 59.1 | 40 | F | 3420 | < 75 |
| 60.3 | 36 | F | 2540 | < 50 |
| 49.5 | 40 | M | 3080 | < 25 |
| 55.5 | 40 | F | 3070 | < 50 |
| 53.85 | 39 | M | 2840 | <25 |
| 61.4 | 40 | F | 3690 | <75 |
| 56.55 | 39 | M | 3420 | <75 |
| 48.3 | 40 | F | 2900 | < 25 |
| 56.6 | 39 | F | 2880 | <25 |
| 58.25 | 39 | M | 3200 | < 50 |
| 59.9 | 39 | M | 3230 | < 50 |
| 54.45 | 40 | F | 3280 | <75 |
| 56.05 | 39 | M | 2830 | <25 |

continued

APPENDIX X

| PATIENT | AGE Yrs. | HEIGHT CM | SMOKING | WEEKS GESTATION | WEIGHT Kg |
|---|---|---|---|---|---|
| 20 K.C. | 19 | 152 | S | 16 | 44.7 |
| 21 C.D. | 23 | 153 | - | 16 | 60.5 |
| 22 J.H. | 20 | 159 | S | 20 | 54.0 |
| 23 J.S. | 18 | 161 | S | 20 | 49.9 |
| 24 Y.Mc | 19 | 159.5 | - | 15 | 49.3 |
| 25 H.Mc | 23 | 154 | - | 25 | 54.6 |
| 26 M.A. | 33 | 156 | S | 17 | 53.9 |
| 27 A.G. | 23 | 156 | - | 17 | 45.8 |
| 28 T.H. | 20 | 154 | - | 16 | 46.6 |
| MEAN ± SD | 24.2 ± 4.65 | 155.5 ± 4.89 | | 18.1 ± 4.31 | 51.31 ± 4.28 |

| WEEKS GESTATION | WEIGHT Kg | GESTATION AT DELIVERY | SEX | BABIES BIRTHWEIGHT (g) | Birth CENTILE |
|---|---|---|---|---|---|
| 31 | 50.4 | 39 | F | 2820 | < 25 |
| 30 | 61.8 | 40 | M | 2650 | < 10 |
| 29 | 56.95 | 41 | M | 3700 | < 75 |
| 30 | 55.0 | 41 | M | 3520 | < 75 |
| 27 | 57.9 | 41 | F | 2650 | < 10 |
| 30 | 58.45 | 40 | F | 3340 | < 75 |
| 30 | 57.9 | 40 | F | 2720 | < 10 |
| 30 | 53.3 | 39 | M | 2490 | < 10 |
| 31 | 57.7 | 40 | M | 2920 | < 25 |
| 29.82 ± 0.77 | 55.92 ± 3.44 | 39.6 ± 1.23 | | 3038 ± 405 | |

## MEAN DAILY INTAKES OF GROUP A WOMEN UNITS/DAY

| | PATIENT | DATE OF SURVEY | ENERGY MJ | PROTEIN g | CHO g | FAT g | ZINC umol | COPPER umol | DIETARY FIBRE g |
|---|---|---|---|---|---|---|---|---|---|
| 1 | EM | 13.03.81 | 10.94 | 75.8 | 301 | 125.5 | 173 | 24.7 | 16.8 |
| 2 | MC * | 26.07.81 | 6.21 | 63.6 | 174 | 64.4 | 113 | 26.8 | 9.5 |
| 3 | MgMcK | 08.08.81 | 11.15 | 74.8 | 337 | 115.4 | 145 | 26.8 | 14.6 |
| 4 | GM | 10.08.81 | 10.10 | 85.9 | 259 | 122.0 | 191 | 28.4 | 19.3 |
| 5 | FS | 01.08.81 | 9.08 | 63.3 | 261 | 100.9 | 111 | 22.1 | 14.6 |
| 6 | MMcK | 27.07.81 | 6.95 | 52.8 | 234 | 53.8 | 125 | 20.5 | 19.2 |
| 7 | LM | 30.09.81 | 9.55 | 82.8 | 285 | 96.5 | 177 | 25.2 | 14.1 |
| 8 | JD | 30.09.81 | 8.34 | 87.9 | 191 | 100.0 | 170 | 20.5 | 14.6 |
| 9 | AM | 08.11.81 | 7.07 | 50.8 | 233 | 67.6 | 102 | 20.5 | 17.8 |
| 10 | JM | 06.10.81 | 8.37 | 65.8 | 239 | 93.5 | 113 | 22.1 | 16.6 |
| 11 | CF | 23.03.82 | 7.06 | 62.4 | 196 | 77.6 | 137 | 18.9 | 12.8 |
| 12 | MGv | 23.02.82 | 5.65 | 49.5 | 196 | 46.6 | 81 | 13.1 | 13.8 |
| 13 | MGe | 22.02.82 | 7.47 | 67.3 | 234 | 71.4 | 133 | 18.9 | 15.8 |

continued

* Liver Eaters 31.2 ± 13.94

21.4 ± 4.03

APPENDIX XI

| PATIENT | DATE OF SURVEY | ENERGY MJ | PROTEIN g |
|---|---|---|---|
| 14 AG * | 09.04.82 | 12.12 | 100.7 |
| 15 CS | 24.05.82 | 6.32 | 59.6 |
| 16 DR * | 26.06.82 | 7.90 | 84.9 |
| 17 LB | 12.06.82 | 8.10 | 61.2 |
| 18 RT | 24.05.82 | 11.67 | 80.9 |
| 19 AA * | 26.06.82 | 6.06 | 68.0 |
| 20 PK | 12.07.82 | 7.28 | 57.4 |

| CHO g | FAT g | ZINC umol | COPPER umol | DIETARY FIBRE g |
|---|---|---|---|---|
| 375 | 120.9 | 203 | 36.2 | 17.9 |
| 222 | 66.6 | 136 | 17.3 | 10.4 |
| 210 | 84.2 | 150 | 29.9 | 22.9 |
| 254 | 106.1 | 115 | 22.0 | 9.1 |
| 365 | 121.9 | 121 | 25.2 | 19.1 |
| 144 | 68.4 | 124 | 17.3 | 6.9 |
| 207 | 80.9 | 112 | 16.5 | 11.2 |

APPENDIX XII

MEAN DAILY INTAKES

| | PATIENT | DATE OF SURVEY | ENERGY MJ | PROTEIN g |
|---|---|---|---|---|
| 1 | CB | 15.11.81 | 10.65 | 89.0 |
| 2 | JW * | 13.10.81 | 11.41 | 135.5 |
| 3 | KH | 06.04.81 | 10.54 | 82.5 |
| 4 | LPa | 06.04.81 | 6.76 | 58.4 |
| 5 | GC | 20.04.81 | 8.17 | 60.2 |
| 6 | LB(D) | 16.04.81 | 5.82 | 78.7 |
| 7 | MF * | 26.04.81 | 9.43 | 77.6 |
| 8 | LPe | 30.04.81 | 11.24 | 86.9 |
| 9 | AM | 07.05.81 | 6.93 | 58.6 |
| 10 | SSm | 08.05.81 | 9.08 | 78.3 |
| 11 | AW | 11.05.81 | 9.40 | 85.4 |
| 12 | SH | 16.06.81 | 10.92 | 84.3 |
| 13 | CF | 27.06.81 | 8.30 | 77.8 |
| 14 | LH | 10.07.81 | 7.26 | 52.8 |
| 15 | DF * | 18.08.81 | 6.42 | 66.8 |
| 16 | HS * | 09.08.81 | 9.14 | 65.8 |

* Liver Eaters

OF GROUP B WOMEN UNITS/DAY

| CHO g | FAT g | ZINC umol | COPPER umol | DIETARY FIBRE g |
|---|---|---|---|---|
| 300 | 104.2 | 182 | 26.8 | 18.9 |
| 251 | 138.6 | 248 | 66.1 | 15.5 |
| 330 | 105.9 | 182 | 25.2 | 12.3 |
| 198 | 71.1 | 118 | 18.9 | 11.6 |
| 246 | 87.8 | 110 | 17.3 | 10.3 |
| 129 | 65.5 | 167 | 12.6 | 6.3 |
| 270 | 102.3 | 167 | 31.5 | 30.2 |
| 334 | 118.7 | 147 | 23.6 | 14.2 |
| 244 | 55.8 | 104 | 18.9 | 15.2 |
| 262 | 94.7 | 156 | 22.1 | 14.1 |
| 258 | 100.6 | 141 | 25.2 | 22.3 |
| 334 | 112.5 | 156 | 29.9 | 20.2 |
| 201 | 102.0 | 156 | 25.2 | 20.8 |
| 243 | 60.1 | 188 | 18.9 | 13.2 |
| 176 | 65.1 | 121 | 17.3 | 18.7 |
| 269 | 101.5 | 138 | 39.4 | 11.0 |

continued

APPENDIX XII

| PATIENT | DATE OF SURVEY | ENERGY MJ | PROTEIN g | CHO g |
|---|---|---|---|---|
| 17 MR | 18.07.81 | 7.98 | 57.1 | 208 |
| 18 MC | 02.10.81 | 9.00 | 76.1 | 228 |
| 19 MB | 13.11.81 | 10.30 | 90.0 | 271 |
| 20 KC | 23.01.82 | 9.06 | 70.2 | 293 |
| 21 CD | 04.02.82 | 6.55 | 74.9 | 179 |
| 22 JH | 24.12.81 | 7.17 | 40.3 | 269 |
| 23 JS | 18.02.82 | 10.88 | 65.5 | 405 |
| 24 YMc | 26.01.82 | 8.32 | 62.4 | 261 |
| 25 HMc | 01.03.82 | 12.05 | 87.8 | 362 |
| 26 MA * | 08.03.82 | 6.98 | 70.6 | 186 |
| 27 AG * | 26.03.82 | 9.58 | 81.8 | 268 |
| 28 TH * | 30 05.82 | 10.69 | 75.6 | 319 |

| FAT g | ZINC umol | COPPER umol | DIETARY FIBRE g |
|---|---|---|---|
| 95.0 | 108 | 20.5 | 11.9 |
| 105.3 | 138 | 18.9 | 13.4 |
| 117.0 | 168 | 25.2 | 15.0 |
| 85.6 | 130 | 22.1 | 14.9 |
| 65.6 | 150 | 18.9 | 12.9 |
| 60.9 | 75 | 12.6 | 3.1 |
| 90.9 | 118 | 22.1 | 13.9 |
| 83.2 | 112 | 22.1 | 19.0 |
| 124.5 | 209 | 26.8 | 18.8 |
| 74.0 | 159 | 17.3 | 9.4 |
| 103.1 | 179 | 34.7 | 18.0 |
| 114.3 | 144 | 26.8 | 8.3 |

APPENDIX XIII

Mean Daily Intakes of
GROUP B (i), DELIVERED Infants >10th Centile

| PATIENT | ENERGY MJ | PROTEIN g | CHO g | FAT g | ZINC umol | COPPER umol | Dietary Fibre (g) |
|---|---|---|---|---|---|---|---|
| 1 CB | 10.65 | 89.0 | 300 | 104.2 | 182 | 26.8 | 18.9 |
| 2 JW | 11.41 | 135.5 | 251 | 138.6 | 248 | 66.1 | 15.5 |
| 3 KH | 10.54 | 82.5 | 330 | 105.9 | 182 | 25.2 | 12.3 |
| 5 GC | 8.17 | 60.2 | 246 | 87.8 | 110 | 17.3 | 10.3 |
| 7 MF | 9.43 | 77.6 | 270 | 102.3 | 167 | 31.5 | 30.2 |
| 8 LPe | 11.24 | 86.9 | 334 | 118.7 | 147 | 23.6 | 14.2 |
| 9 AH | 6.93 | 58.6 | 244 | 55.8 | 104 | 18.9 | 15.2 |
| 10 SS | 9.08 | 78.6 | 262 | 94.7 | 156 | 22.1 | 14.1 |
| 11 AW | 9.40 | 85.4 | 258 | 100.6 | 141 | 25.2 | 22.3 |
| 12 SH | 10.92 | 84.3 | 334 | 112.5 | 156 | 29.9 | 20.2 |
| 13 CF | 8.30 | 77.8 | 201 | 102.0 | 156 | 25.2 | 20.8 |

continued

APPENDIX XIII

GROUP B (i)

| PATIENT | ENERGY MJ | PROTEIN g |
|---|---|---|
| 14 LH | 7.26 | 52.8 |
| 15 DF | 6.42 | 66.8 |
| 16 HS | 9.14 | 65.8 |
| 17 MR | 7.98 | 57.1 |
| 18 HC | 9.00 | 76.1 |
| 19 MB | 10.30 | 90.0 |
| 20 KC | 9.06 | 70.2 |
| 22 JH | 7.17 | 40.3 |
| 23 JS | 10.88 | 65.5 |
| 25 HMc | 12.05 | 87.8 |
| 28 TH | 9.58 | 75.6 |

MEAN DAILY INTAKE
DELIVERED INFANTS >10th centile

| CHO g | FAT g | ZINC umol | COPPER umol | DIETARY FIBRE (g) |
|---|---|---|---|---|
| 243 | 60.1 | 188 | 18.9 | 13.2 |
| 176 | 65.1 | 121 | 17.3 | 18.7 |
| 269 | 101.5 | 138 | 39.4 | 11.0 |
| 208 | 95.0 | 108 | 20.5 | 11.9 |
| 228 | 105.3 | 138 | 18.9 | 13.4 |
| 271 | 117.0 | 168 | 25.2 | 15.0 |
| 293 | 85.6 | 130 | 22.1 | 14.9 |
| 269 | 60.9 | 75 | 12.6 | 3.1 |
| 405 | 90.9 | 118 | 22.1 | 13.9 |
| 362 | 124.5 | 209 | 26.8 | 18.8 |
| 319 | 114.3 | 144 | 26.8 | 8.3 |

MEAN DAILY INTAKE
GROUP B (ii) DELIVERED INFANTS < 10th Centile

| PATIENT | ENERGY MJ | PROTEIN g | CHO g | FAT g | ZINC umol | COPPER umol | DIETARY FIBRE (g) |
|---|---|---|---|---|---|---|---|
| 4 LPa | 6.67 | 58.4 | 198 | 71.1 | 118 | 18.9 | 11.6 |
| 6 LB(D) | 5.82 | 78.7 | 129 | 65.5 | 167 | 12.6 | 6.3 |
| 21 CD | 6.55 | 74.9 | 179 | 65.6 | 150 | 18.9 | 12.9 |
| 24 YMc | 8.32 | 62.4 | 261 | 83.2 | 112 | 22.1 | 19.0 |
| 26 MA | 6.98 | 70.6 | 186 | 74.0 | 159 | 17.3 | 9.4 |
| 27 AG | 9.58 | 81.8 | 268 | 103.1 | 179 | 34.7 | 18.0 |

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