ZINC STATUS OF LOCAL HUMAN POPULATION IN HEALTH AND DISEASES

THESIS

By

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In partial fulfilment of the requirements
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DEDICATED
TO
MY BELOVED PARENTS

CERTIFICATE-I

This is to certify that the thesis entitled, "ZINC STATUS OF LOCAL HUMAN POPULATION IN HEALTH AND DISEASES", submitted in partial fulfilment of the requirements for the award of the degree of Master of Science (Food Science and Nutrition) from the Department of Food Science and Nutrition of College of Home Science, Himachal Pradesh Krishi Vishvavidyalaya, Palampur (H.P.) is the bonafide research work carried out by Ms. Ruma Gulyani (P89-A-21M), daughter of Shri D.R. Gulyani under my guidance and supervision. All the assistance and help received during the course of this investigation has been fully acknowledged.

No part of this thesis has been submitted for any other degree.

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CERTIFICATE-II

This is to certify that the thesis entitled, "ZINC STATUS OF LOCAL HUMAN POPULATION IN HEALTH AND DISEASES", submitted by Ms.Ruma Gulyani (P89-A-21M), in partial fulfilment of the requirements for the degree of Master of Science (Food Science and Nutrition) has been approved by the Examining Committee after an oral examination of the same in collaboration with the External Examiner.

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INTRODUCTION

INTRODUCTION

The importance of zinc, a trace element which forms less than 0.01 per cent of the healthy body, for human health has been recognized only in the past two and a half decades. Requirement of zinc increases during the period of rapid growth, pregnancy and lactation. Low intake of zinc causes poor appetite, growth retardation, hypogonadism, congenital malformations, mental lethargy along with neuropsychiatric signs, rough skin and intercurrent infections.

Zinc is required for the normal production and action of insulin and the latter is related to glucose metabolism. Underwood (1971) observed increased uptake of glucose by adipose tissue in the presence of zinc as well as insulin and even more by a combination of the two. Zinc deficiency occurs in diabetic patients and zinc homeostasis alters as a consequence of glucose intolerance in diabetes.

Zinc is also vitally concerned with the fundamental process of protein synthesis. Zinc deficiency disturbs the protein metabolism and causes increased losses of protein

and other substances from the body especially during the diseases associated with zinc deficiency in human beings. Animals which received high protein but low zinc diet had a significant increase in non-essential free amino acid levels compared to those on zinc supplemented diet (Griffith and Alexander,1972). Tao and Hurley (1971) noticed significant decrease in the total plasma protein content of the zinc-deficient rats.

Cholesterol metabolism and high-density lipo protein (HDL) synthesis is also influenced by zinc status of the body. Barr *et al.*(1951) correlated low levels of HDL with increased risk of coronary heart disease and Pories *et al.* (1967) observed beneficial effect of zinc therapy in some cases of atherosclerosis.

Fluctuations in plasma zinc concentration have been reported during the menstrual cycle. The values were found higher during menses and follicular phases and lower during the ovulatory and the luteal phases (Deuster *et al.*,1987).

Pregnant women with marginal zinc status were found at increased risk of poor pregnancy outcome. Low maternal serum zinc level has been associated with congenital malformations in the fetus. Glover and Atherton (1988) detected symptomatic zinc deficiency in infants fed on breast milk having low zinc concentration.

Zinc depletion in human subjects is commonly observed during burns, major fractures, diabetes mellitus and protein deprivation as a result of hyperzincuria. Zinc status of

human beings has direct relation with the type of food they consume which in turn is influenced by the zinc status of the soil. There is ample evidence that zinc content of plants is affected by the soil type and the fertilizer treatment. Randhawa (1988) in his 5th convocational address to HPKV had pointed out wide ranging deficiencies of macro and micro nutrients in soils of Himachal Pradesh. Some pockets in low hill and mid hill regions of the state of Himachal Pradesh have been reported to be zinc-deficient (Kanwar *et al.*,1983). However, no systematic studies have been conducted either on zinc status of common food stuffs or on human population of the area.

Therefore, the present study was undertaken to elucidate the zinc status in human population during health and diseases with the given objectives:

1) to determine the concentration of zinc in locally consumed foods.

2) to monitor the zinc level in plasma, urine, hair, and saliva of diabetic, hypertensive and normal persons and also during different reproductive stages of human female.

3) to correlate the serum level of glucose, protein and cholesterol with zinc level in above subjects.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The literature has been reviewed under the following main and sub-titles:

1) SIGNIFICANCE AND DEFICIENCY SYNDROME OF ZINC

2) PARAMETERS FOR ASSESSING ZINC STATUS:

   a) Plasma or Serum

   b) Hair

   c) Urine

   d) Saliva

3) INFLUENCE OF ZINC DEFICIENCY ON PHYSIOLOGICAL CONDITIONS:

   a) Pregnancy

   i) Clinical syndrome

   ii) Biochemical changes in plasma, serum, hair and urine zinc.

   b) Lactation

   i) Breast milk zinc concentration and clinical changes in offspring.

   ii) Biochemical changes in plasma, serum and hair zinc.

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4) INFLUENCE OF ZINC DEFICIENCY ON:

a) **Glucose metabolism**

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ii) Biochemical changes in serum, plasma, urine and hair zinc.

iii) Others

b) **Protein metabolism**

c) **Cholesterol metabolism**

5) ZINC CONTENT OF FOOD PRODUCTS

2.1 SIGNIFICANCE AND DEFICIENCY SYNDROME OF ZINC:

Todd et al.(1934) were successful in demonstrating that zinc was essential for the growth and development of rats. The disease of swine, porcine parakeratosis, was shown to be the result of zinc deficiency by Tucker and Salmon (1955). Later O'Dell and Savage (1957) showed that zinc was essential for growth in birds also.

The manifestations of zinc deficiency in animals included growth retardation, loss of hair, thickening and hyper-keratinization of the epidermis, and testicular atrophy. Deficiency of zinc in breeding hens resulted in lowered hatchability, gross embryonic anomalies characterized by impaired skeletal development, and varying degrees of weakness in chicks that hatch (Blamberg *et al.*,1960).

Zinc deficiency in human subjects had been reported to occur in conditions where there was an increased requirement of zinc which included infants and children during the

rapid-growth-rate period and women who were pregnant and lactating (Prasad, 1989). Halsted *et al.*(1974) described the clinical aspects of zinc deficiency in man to consist of severe iron deficiency anemia, hepatosplenomegaly, short stature, infantile testes, open epiphyses, spoon nails, frequently a history of geophagia, and rough skin with hyperpigmentation.

A dwarfism syndrome in man due to zinc deficiency was first described by Lemann in 1910. Later Halsted and Prasad (1960) suggested growth and sexual retardation in dwarfs to be the result of zinc deficiency. Daily supplementation with zinc sulphate resulted in significantly more rapid growth and sexual development.

Reports by Henkin *et al.*(1971) and Schechter *et al.* (1972) indicated correction of a decrease in acuity of taste and smell (idiopathic hypogeusia and hyposmia) by the administration of zinc. Hambidge *et al.*(1972) also observed that growth retardation, poor appetite, and impaired taste acuity due to zinc deficiency was corrected when zinc was supplemented.

In 1975, Kay and Tasman-Jones reported the occurrence of severe zinc deficiency in subjects receiving total parenteral nutrition (TPN) for prolonged periods without zinc. Prasad (1989) stated severe deficiency of zinc to occur in patients with acrodermatitis enteropathica, following TPN without zinc, following excessive use of alcohol, and following penicillamine therapy.

A high incidence of congenital malformation of fetuses and infants born of pregnant women with acrodermatitis enteropathica (AE) were reported by Hambidge et al.(1978). Similar malformations had been reported in the offspring of maternal rats that were zinc deficient which suggested the susceptibility of human fetus to teratogenic effects of maternal zinc deficiency (Hurley, 1976).

A moderate level of zinc deficiency has been reported by Prasad (1989) to include growth retardation, hypogonadism in the males, poor appetite, mental lethargy, rough skin and intercurrent infections. All these features were corrected by zinc supplementation.

The clinical manifestations of a mild level of deficiency of zinc in humans decreased serum testosterone level and produced oligospermia in males, decreased lean body mass, hyperammonemia, neurosensory changes, and decreased serum thymulin activity (Prasad, 1989).

2.2 PARAMETERS FOR ASSESSING ZINC STATUS:

2.2.1 Plasma or Serum:

Plasma or serum zinc were the standard diagnostic parameters of zinc status but were more useful for detecting severe deficiencies of zinc (Freeland - Graves et al.,1981). Halsted *et al.*(1974) reported demonstrable zinc deficiency in man to be accompanied by a low plasma zinc concentration.

The observation of a higher serum zinc concentration in normal male subjects (15.3 $\pm$ 2.1 S.D. µmol/l) than in female controls (14.1 $\pm$ 1.7 S.D µmol/l) had been reported by

Lindeman *et al.*(1971) and Rose *et al.*(1972). Rea (1989) noticed greater serum zinc values in men, old and young, than women of the same age and differences were significant between young men and women.

Mean zinc in serum of normal male and female subjects 11-54 years old was 84 ± 20 µg/dl with no functional relation between zinc in serum and age or sex of subjects (Monceda *et al.*,1989).

Khalil *et al.*(1981) reported serum zinc in Egyptian men and women to be 162.5 ± 22.7 µg/dl and 135.0 ± 21.2 µg/dl, respectively. Serum zinc concentration for Finn men and women were 12.7 µmol/l (82 µg/dl) and 11.7 µmol/l (77 µg/dl), respectively, with variations in zinc values with sex, age, length of fast, time of day and geographical area (Bjorksten *et al.*1978). Karlinskii (1973) gave the concentration of zinc in serum of healthy subjects 15-69 years old as 115.1 ± 14.2 (range 90-154) µg/dl with no sex or diurnal variation.

The serum zinc concentration in obese patients was 22 per cent lower than in normal controls (Chen *et al.*,1988). Many factors including pregnancy and oral contraceptives, various diseases, and stress induced the lowering of the plasma zinc concentration (Halsted and Smith, 1970).

2.2.2 Hair:

The zinc content of the hair of normal human subjects ranged from 92-255 ppm with a mean of 173 ppm (Smith, 1967). This was very close to the mean 167 ± 5 ppm for healthy

males and 172 ± 9 ppm for females as reported by Schroeder and Nason (1969).

Hair zinc concentration in man was affected by zinc intake (Hambidge *et al.*,1972). Reinhold *et al.*(1968) showed in work with rats on controlled zinc intake that hair zinc levels depended upon the dietary zinc intake but did not define the state of zinc nutrition.

Raghupathy (1987) observed geographical variation in the hair zinc concentration of different populations. He further noticed marked differences in zinc content among different socio-economic groups living in the same geographical region. Strain *et al.*(1966) had obtained a lower overall mean of 119.6 ± 4.6 ppm zinc for the hair of north American males and found that oral zinc sulphate therapy increased the level of zinc in hair and alleviated the clinical signs of zinc deficiency. Chen *et al.*(1988) noticed hair zinc level in obese patients to be 34 per cent lower than in normal controls.

Deeming and Weber (1977) assumed that nutritional deficiencies that decreased the availability of minerals to the follicle resulted in decreased mineral concentration in the hair shaft. Strain *et al.*(1966) found this assumption to hold true in severe zinc deficiency. McBean *et al.*(1971) reported that zinc content of hair was not a reliable indicator of body zinc metabolism at the time of sampling.

2.2.3 Urine:

Urinary zinc excretion in normal individuals ranged between 400-600 µg/24 hr (Roman, 1969). The quantity of zinc excreted in the urine of healthy human adults was very small (0.1 - 0.7 mg/day) compared with the 10-15 mg/day normally ingested (Underwood, 1971). Steele (1973) suggested that renal handling of zinc was different from that of other divalent cations. Increased urinary zinc excretion (zincuria) was detected in diabetes by Pidduck *et al.*(1970). Spencer and Samachson (1970) noted rise in urinary zinc excretion to high levels during total starvation in extreme obesity. Hyperzincuria was also observed in hypertensive patients (Schroeder, 1957).

2.2.4 Saliva:

Henkin *et al.*(1975) suggested that zinc levels in saliva might be a superior way to assess zinc nutritional status of human subjects. Numerous reports of lesions in the mouth, tongue, and esophagus of animals with zinc deficiency suggested that the oral cavity was particularly sensitive to zinc deficiency but the changes that occurred were not clearly indicative of a lack of zinc in the saliva bathing the oral cavity (Wight and Dewar, 1978).

Excessive salivation in zinc deficient ruminants (Ho and Hidiroglou, 1977) and changes in the composition of saliva from zinc-deficient sheep (Quarterman *et al.*1973) suggested that there might be changes in other salivary components that would be indicative of zinc status.

Henkin *et al.*(1975) reported that zinc concentrations in parotid saliva of humans reflected zinc nutriture as these levels were depressed in patients exhibiting hypogeusia. They further noted values below 10 ng/ml in parotid saliva of persons with diminished gustatory acuity and suggested these levels as indicative of zinc deficiency.

Greger and Sickles (1979) collected saliva samples from adolescent females and found that the mean zinc concentrations of the whole saliva and the supernatant samples were 173±94 (SD) and 30.5±14.8 ng/ml, respectively. Significantly lower levels of zinc were found in the supernatant samples, but not in the whole saliva samples, when the same subjects were fed 11.5 mg zinc daily rather than 14.7 mg zinc daily during the metabolic study.

Zinc levels of salivary sediment significantly decreased from initial value of 126±28 µg/g to final levels of 94 ± 14 µg/g after a low zinc diet was fed to women (Freeland-Graves *et al.*,1981). The results suggested that mixed saliva was not a useful index of zinc status, however, salivary sediment might be a sensitive parameter if contamination was avoided.

Johnson *et al.*(1978) and Baer and King (1978) found that the zinc levels in whole mixed saliva and whole parotid saliva, respectively were unaffected by dietary zinc levels but observed that the zinc content of solids from whole mixed saliva was less when subjects were fed 3 mg rather than 10 mg of zinc daily. Everett and Apgar (1979) felt that

the use of saliva as a diagnostic tool was complicated by the considerable variation in the composition of saliva that occurred within the same individual at different times.

2.3INFLUENCE OF ZINC DEFICIENCY ON PHYSIOLOGICAL CONDITIONS:

2.3.1 Pregnancy:

2.3.1.1 Clinical syndrome:

Poor zinc status and changes in zinc metabolism during human pregnancy appear important since malformations in the offsprings of zinc-deficient female animals have been reported (Warkany and Petering, 1972). Sandstead *et al.* (1978) observed impairment in the behavioral development of the offspring when pregnant rhesus monkeys were deprived of zinc during the time of rapid brain growth. Marginal zinc deprivation induced at conception produced growth retardation, skeletal anomalies, delivery complications, and impaired immune function in animals (Leek *et al.*,1984). Eltohamy (1987) noticed a high rate of spontaneous abortion, excessive bleeding during parturition, reduced plasma zinc values and low zinc availability to the foetus in zinc-deficient pregnant rabbits. Low birth weight, delayed skeletal maturation and defective mineralization of offspring as well as high pregnancy loss ratio and delivery complication were also observed in pregnant rhesus monkeys when zinc-deficient diet was fed (Golub *et al.*,1984). Apgar (1968) noticed that zinc-deficient female rats delivered their litters with extreme difficulty, suffered excessive

bleeding and failed to consume afterbirths or to prepare a nest site.

2.3.1.2 Biochemical changes in plasma, serum, hair and urine zinc:

Plasma and serum zinc:

Breskin *et al.*(1983) reported correspondence between the early and sharp decline in circulating levels of maternal zinc and the period of embryogenesis and suggested a crucial role for zinc in human fetal development.

Jameson (1976) observed association of low maternal serum zinc with congenital malformations, fetal dysmaturity, prematurity, and maternal complications in otherwise healthy women. He also reported a gradual fall in serum zinc during the first and second trimester of pregnancy, variations in serum zinc in individual healthy pregnant women being few. Swanson and King (1987) observed association of low maternal serum zinc values with pregnancy-induced hypertension, abnormal parturition and congenital anomalies.

Argemi *et al.*(1988) found mean zinc concentration in the serum of normal pregnant women to be 72.48 $\pm$ 15.00 µg/dl which was significantly less than in non-pregnant women (93.71$\pm$7.34 µg/dl). The decrease in total zinc concentration during pregnancy was slow, from 75.36$\pm$17.61 to 70.46$\pm$13.28 µg/dl during the second and third trimesters, respectively. Vir *et al.*(1981) also stated that the mean serum zinc level was significantly lower in pregnant subjects compared to non-pregnant and showed a continuous significant decline

with increasing duration of pregnancy; the concentration of zinc in serum being 0.68 ± 0.13 µg/ml; 0.62 ± 0.08 µg/ml and 0.56 ± 0.23 µg/ml, respectively during the second trimester, third trimester and postpartum period.

A large prospective study of pregnant Australian women showed association of low mid pregnancy serum zinc concentration with increased risk of intrapartum haemorrhage (McMichael *et al.*,1982). Prema (1980) in a study on Indian women found extremely low serum zinc values, that is less than 6.1 µmol/l, at delivery in mothers of infants with congenital abnormalities.

Serum zinc values significantly decreased in the first trimester and further decreased in the second trimester and then remained unchanged until delivery (Higashi *et al.*, 1988). Further, mothers with serum zinc less than 65 µg/dl in the last trimester gave birth to low birth weight babies. In a study on Indian women, Mittal *et al.*(1982) showed mean serum zinc of pregnant women in the first and second trimester and non-pregnant controls to be 109.7, 83.7 and 113.6 µg/dl, respectively, being significantly lower in the second trimester.

Like serum zinc, low plasma zinc concentration was also reported in the blood of women who had given birth to a congenitally abnormal infant (Soltan and Jenkins, 1982). Mukherjee *et al.*(1984) did not find association of low plasma zinc values with premature delivery, excessive maternal bleeding, or pregnancy-induced hypertension.

Meadows *et al.*(1981) in a longitudinal study on pregnant women observed significant decline in the mean plasma concentration of zinc during the second trimester of pregnancy which remained low until delivery. A higher percentage of low birth weight mothers had plasma zinc concentration below 50 µg/dl (Yasodhara *et al.*,1991). Lazebnik *et al.*(1988) reported association of low plasma zinc during the intrapartum period with a prolonged latent phase, a protracted active phase, labour more than 20 hours and cervical and vaginal lacerations.

Hair Zinc:

In two studies of healthy pregnant women, hair zinc concentration decreased during gestation (Hambidge and Droegemueller, 1974). Kohrs *et al.*(1986) reported greater hair zinc concentration in primiparous women than those who had two or more previous pregnancies. Hair zinc concentration was found to be most in the third trimester (Kohrs *et al.*,1977).

Vir *et al.*(1981) stated that concentration of zinc in hair decreased progressively with advancing pregnancy, the decline between second and third trimesters being significant. The hair zinc concentration during the second and third trimester was 183 $\pm$ 26 µg/g and 170 $\pm$ 26 µg/g, respectively.

Urine zinc:

Urinary zinc excretion varied with stage of pregnancy and was influenced by body size and bioavailability of dietary zinc (Campbell *et al.*,1985). Measurements of urinary zinc excretion provided evidence that zinc was conserved during early pregnancy (Hambidge *et al.*,1983). Further, during the first trimester, pregnant women excreted less urinary zinc than controls with equivalent zinc intakes. Swanson and King (1982) found more excretion of zinc in urine by pregnant women in late pregnancy as compared to non-pregnant controls.

2.3.2 Lactation:

2.3.2.1 Breast milk zinc concentration and clinical changes in offspring:

Fairweather *et al.*(1985) measured the transfer of ^{65}Zn from mothers to litters during birth and first three days of lactation. There were no differences in maternal or litter ^{65}Zn just before or just after birth but within 72 hours maternal ^{65}Zn had significantly decreased and litter ^{65}Zn increased. Offspring of rats that were deprived of zinc or energy during lactation exhibited developmental delays in reflexes which appeared after the first postnatal week (auditory, startle, air righting and rope descent) and growth retardation of zinc-deprived and undernourished rats persisted long after dietary rehabilitation (Eberhardt and Halas, 1987).

Mutch and Hurley (1974) observed diminished production of milk containing less zinc than controls in zinc deprived rats which resulted in retarded growth of young, dermatitis, rough coats and abnormal posture.

Bilinski *et al.*(1987) reported hypozincaemia and clinical features of acrodermatitis enteropathica in a breast fed, premature infant. Munro *et al.*(1989) found negative zinc balance in premature infants and the additional factor of a low maternal breast milk zinc concentration provoked signs of transient symptomatic zinc deficiency in infants resulting in scaly erythema of the cheeks and napkin area 9-13 weeks after birth. Symptomatic zinc deficiency developed in an exclusively breast-fed, full term female infant as a result of low breast milk zinc concentration throughout lactation (Glover and Atherton, 1988).

Shrimpton *et al.*(1984) noticed beneficial effect of maternal zinc supplementation on growth and diarrhoeal occurrence of breast-fed infants. Krebs and Hambidge (1985) had seen advantageous effect of increase in dietary zinc intake on both maternal zinc status and milk zinc concentrations especially with prolonged lactation.

There was progressive decline in human milk zinc with duration of lactation (Krebs and Hambidge,1986). Zhou *et al.* (1984) reported zinc content of milk taken from primiparous women on days 3, 7, 14, 28 and 56 post partum in the morning before suckling as 209.3 - 1166.7, 231.6-672.7, 138.6-525.0,

76.3-408.3 and 36.8-192.2 µg/dl, respectively with corresponding median values being 633.3, 400.0, 295.0, 181.5 and 115.0 µg/dl, respectively. Vuori and Kuitunen (1979) noticed decrease in median zinc during the course of lactation from 4.0 mg/l to 0.5 mg/l. An abnormally low concentration of zinc in mother's milk was associated with clinical nutritional zinc deficiency in premature and term new born infants. Abnormality of zinc uptake by the mammary gland from the plasma was suggested as the causative factor by Alkinson et al.(1989). Casey et al.(1989) measured zinc in milk samples from healthy lactating women and found that zinc concentration declined throughout lactation from 71.9 ± 18.3 µmol/l at 7 days to 44.3 ± 10.7 µmol/l at 1 month and 7.64 ± 4.59 µmol/l at 12 months.

2.3.2.2. Biochemical changes in plasma, serum and hair zinc:

Plasma and Serum zinc:

Soltan and Jenkins (1982) reported low plasma zinc concentration in the blood of women who had given birth to a congenitally abnormal infant. Plasma zinc and albumin values 24 to 48 hours after delivery were lower than in controls (Simmer and Thompson, 1985). Mean zinc concentration in maternal serum was found to be 72.5 ± 14.1 µg/dl by Woods et al.(1988). Kapoor et al.(1988) observed inverse relation between maternal serum zinc values and gestational age. Zinc concentration in blood serum taken from women three days post partum were 27.3-91.4 µg/dl, median value being 47.2 ug/dl (Zhou et al.,1984). Prasad et al.(1974) reported 71.6

µg/dl zinc in the venous blood of women at term with infants of normal birth weight and 68.6 µg/dl zinc in those with small for date infants.

Hair Zinc:

Mothers of malformed infants had significantly higher hair zinc concentrations than mothers of normal infants and the mother/infant hair zinc values were significantly correlated (Bergmann and Bergmann, 1985).

2.3.3. Menstrual cycle:

The effect of menstrual cycle phases on the indices of zinc status in normally menstruating women was studied by Deuster *et al.*(1987). They divided the cycle into four phases: menses, follicular, ovulatory and luteal and observed that plasma zinc concentrations were higher during menses and the follicular phases and then dropped during the ovulatory and luteal phases. This study provided evidence that the plasma zinc concentrations fluctuate during the menstrual cycle in a phase-related fashion in normal menstruating women.

Umoren and Kies (1982) reported considerable variation in menstrual zinc losses from subject to subject and further from period to period for each subject. They observed that hypozincemia occurred under these circumstances if zinc intake was lowered.

2.4.INFLUENCE OF ZINC DEFICIENCY ON:

2.4.1. Glucose metabolism:

2.4.1.1. Glucose tolerance and insulin secretion:

Quarterman *et al.*(1966) reported decreased glucose tolerance in zinc-deficient rats. Studies of Mills *et al.* (1969) indicated influences of zinc in membrane transport and utilization of glucose. Underwood (1971) observed the function of zinc during the normal production and action of insulin. He further noticed increased glucose uptake by adipose tissue in the presence of zinc as well as insulin, and even more with the combination of the two.

Crystallographic studies by Harding *et al.*(1966) clearly demonstrated the presence of zinc in crystals of insulin confirming the close relationship between zinc and insulin. Huber and Gershoff (1973) found impaired glucose tolerance in decreased insulin secretion. Hendricks and Mahoney (1972) suggested that the decreased clearance of intravenous and intraperitoneal glucose in zinc-deficient rats might be due to either a decrease in the secretion of insulin from the pancreas or to an increased rate of insulin degradation.

Acute stimulation of insulin secretion was seen when concentration of zinc was low in the β-cells of the pancreas (Engelbart and Kief,1970). Deficiency of zinc occurred in diabetes (Abdulla,1983) and zinc homeostasis altered as a consequence of glucose intolerance (Canfield *et al.*,1984).

2.4.1.2 Biochemical changes in serum, plasma, urine and hair zinc:

Patients with diabetes mellitus often have an altered trace element pattern in their plasma and urine and deficiency of zinc may develop during the course of the disease (Sjogren *et al.*,1985).

Serum zinc:

Martin Mateo *et al.*(1974) measured higher content of serum zinc in diabetic patients in comparison to normal persons, the zinc value for serum in normal persons being 90 ug/dl which increased to 138 and 176 µg/dl in diabetic men and women. In 1975, they further observed that serum zinc levels were higher in female diabetics as compared to male diabetics. Pai and Prasad (1988) observed normal to increased content of serum zinc in most of the diabetic patients in comparison to controlled subjects, whereas no difference in serum zinc was observed between normal and diabetic by Mc Nair *et al.*(1981). D'oCon *et al.*(1987) noted close relationship between serum zinc and glucose in diabetic obese patients.

Kinlaw *et al.*(1983) found low serum zinc concentration in patients with stable type-II diabetes mellitus. Winterberg *et al.*(1989) also detected significantly lower serum zinc concentration in type-I or insulin-dependent diabetes mellitus (IDDM) as compared to type-II or non-insulin-dependent diabetes mellitus (NIDDM). They also

observed beneficial effect of zinc supplementation in the improvement of diabetic complications.

Plasma zinc:

Unlike serum zinc level, fluctuating level of plasma zinc was noted, that is, normal, increased or decreased in diabetic patients by Pai and Prasad (1988), whereas lower plasma zinc content was detected in diabetics by umar and Jaya Rao (1974) and Sjogren *et al.*(1986) and no alteration was noted by Sullivan *et al.*(1969). Canfield *et al.*(1984) observed significantly higher levels of fasting plasma zinc in diabetic patients than in the controls.

Urine zinc:

Martin Mateo *et al.*(1974) found 154 ug/dl of urinary zinc in control subjects while the corresponding value for diabetic men and women were 270 and 179 ug/dl, respectively. Hyperzincuria was also observed in most of the diabetic patients as compared to controls (Pai and Prasad, 1988; Sjogren *et al.*, 1986; Kumar and Jaya Rao, 1974; Khattab *et al.*,1976 and Canfield *et al.*,1984). Martin Mateo *et al.*(1975) reported higher urinary zinc values in diabetic males as compared to diabetic females. In contrast, more excretion of urinary zinc in female diabetics than male diabetics was detected by McNair *et al.*(1981).

Kinlaw *et al.*(1983) found hyperzincuria in patients with stable type-II diabetes mellitus. Zinc loss in urine was greater when proteinuria was also present and was correlated with the mean serum glucose concentration. It was

concluded that hyperzincuria, resulting from a glucose-mediated process which is not osmotic, interacts with impaired zinc absorption to produce zinc deficiency in patients with type-II diabetes mellitus.

Urinary zinc excretion in comparison to creatinine excretion was almost doubled in the diabetic patients as compared to the normal subjects (McNair *et al.*,1981).

Hair zinc:

Canfield *et al.*(1984) noted significantly lower hair zinc concentration in male subjects with diabetes as compared to female subjects with diabetes whereas Hagglof *et al.*(1983) did not find zinc in hair to differ significantly between the diabetic and controls.

Others:

Zinc concentrations in plasma, hair and urine taken from young adults with insulin- requiring or type I diabetes mellitus were significantly correlated with their height, weight and age (Canfield *et al.*,1984). Hagglof *et al.*(1983) found no correlation between serum, urine and hair zinc content and the concentration of albumin and glucose in serum. Lower zinc values were associated with decreased plasma albumin in diabetic nephropathy patients as compared with patients without complications.

2.4.2.Protein metabolism:

Zinc was related to protein synthesis in micro-organisms, animals and animal tissues (Halsted *et al.*,1974).

Williams *et al.*(1965) and Reeves and O'Dell (1981) observed reduced or altered protein synthesis in zinc-deficient rats.

The synthesis of both DNA (Fujioka and Lieberman,1964) and RNA (Wegener and Romano, 1963) was inhibited when zinc was lacking. Somers and Underwood (1969) reported that a primary defect was an increase in ribonuclease activity resulting in increased protein catabolism. Dreosti *et al.* (1972) noted that DNA synthesis as estimated by the incorporation of thymidine was rapidly reduced in zinc-deficient rats. Buchanan and Hsu (1968) also showed suppressed liver DNA and RNA synthesis during zinc deficiency.

Zinc deprivation in rats increased total nitrogen and uric acid in urine while there was no difference in creatinine excretion between groups which suggested that zinc deprivation resulted in increased catabolism of proteins (Hsu and Anthony, 1975). Somers and Underwood (1969) found significantly higher output of urinary nitrogen and sulphur in zinc-deficient lambs than in control animals. This observation suggested impaired protein or amino acid utilization.

Animals which received high protein but zinc-deficient diet had a significant increase in non-essential free amino acids level compared to those on zinc supplemented diet (Gritfith and Alexander,1972). Anthony and Hsu (1973) noted significantly higher levels of taurine and glutamic acid in the plasma of zinc-deficient group than those of control

group. However, plasma proline and leucine levels were reduced in zinc deficiency. Hsu and Woosley (1972) showed that, in vivo, the incorporation of ^{35}S amino acids into organ and skin protein was significantly altered when metabolism of L-Methionine was studied in zinc-deficient rats.

Chesters and Will (1981) suggested albumin and transferrin as carriers of zinc in plasma and observed that albumin acted as major transport protein for zinc in plasma of most species, zinc also being present firmly bound to alpha-2 macroglobulin. Boyett and Sullivan (1970) also suggested that transferrin and alpha-2 macroglobulin had an important role in internal zinc exchange. Parisi and Vallee (1970) isolated alpha-2 macroglobulin and found that it contained 30-40 per cent of the total serum zinc which suggested that alpha-2 macroglobulin was the principal zinc metalloprotein in human serum.

Prasad and Oberleas (1970) reported that amino acid bound fraction of zinc played a significant role in biological transport. In a study on serum zinc values in healthy men, men with low serum zinc had significantly lower serum alpha-1 globulin than men with high zinc (Hartoma *et al.*,1979). Tao and Hurley (1971) observed significant decrease in the total plasma protein content of zinc-deficient rats.

2.4.3.Cholesterol metabolism:

Epidemiological studies suggested that even small differentials in high-density lipoprotein (HDL) are predictive of coronary heart disease (Castelli *et al.*,1977) and zinc therapy has been found beneficial in some cases of atherosclerosis (Pories *et al.*,1967). Hooper *et al.*(1980) reported that oral ingestion of pharmacological doses of zinc lowered HDL- cholesterol in man.

Increased levels of serum cholesterol were observed in rats fed high-zinc diets (Klevay, 1973 and Klevay, 1975). Sandstead *et al.*(1980) stated that nutritional deficiency of zinc resulted in the elevation of serum (or plasma) levels of cholesterol. Koo and Williams (1981) demonstrated that an acute depletion of zinc in adult male rats produced hypocholesterolemia which was primarily due to a selective decline in high-density lipoprotein (HDL) fraction. Further, a strong positive correlation between the concentration of serum zinc and HDL-cholesterol was observed.

In another study, Koo and Ramlet (1983) not only confirmed the positive relationship between serum zinc and HDL levels but also showed that reduction in HDL due to cholesterol intake was closely associated with the decrease in serum zinc. Serum total cholesterol was significantly less in rats deprived of zinc than in controls (Patel *et al.*,1975). Koo and Lee (1988) reported that cholesterol homeostasis was altered in zinc deficiency. Marginal zinc-deficiency in male rats significantly lowered the total

amount of circulating HDL particles with no effect on the concentration of total cholesterol.

In man, plasma zinc concentration was associated with changes in plasma cholesterol. Dietary supplementation with low doses (150 mg Zn or less daily) did not change plasma cholesterol while doses of 160 mg Zn or more daily in men resulted in decreased HDL cholesterol and increased low-density lipoprotein (LDL) cholesterol, creating risk of developing heart disease (Samman and Roberts, 1988). Lukaski et al.(1982) provided evidence that the amounts and types of dietary lipids, triglyceride and cholesterol, could significantly alter the metabolism of zinc.

2.5.ZINC CONTENT OF FOOD PRODUCTS:

Prasad (1989) observed prevalence of zinc deficiency to accompany inadequate protein intake in populations subsisting on low incomes and geriatric cases. He further observed predominant use of cereal proteins by the majority ot the world population as an important predisposing factor for zinc deficiency as high phosphate and phytate content of such diets resulted in poor availability of zinc. Underwood (1971) also reported high dietary intakes of whole wheat bread and legumes, both high in phytates, as factors which contributed to the zinc deficiency syndrome in Middle Eastern dwarfs. Oberleas and Prasad (1969) suggested zinc supplementation of cereal diets for the improvement of growth and well being of human population in many areas.

There was ample evidence that the zinc content of plants was influenced by the soil type and the fertilizer treatment (Underwood, 1971). Halsted *et al.*(1974) reported a net loss of elemental zinc from the soil due to natural leaching and erosion and constant removal of crops without repletion resulted in deficiency of zinc in the soil. Mitchell (1964) calculated that the uptake of zinc by plants was relatively high compared with the soil concentration which further added to zinc depletion in the soil.

Schroeder *et al.*(1967) found considerable variation in zinc content of common foods. While white sugar, pome and citrus fruits were among the lowest in zinc (less than 1 ppm of fresh edible portion), wheat germ and bran (40-120 ppm) and oysters which contain over 1000 ppm zinc were among the richest sources of this trace element. Between these extremes in ascending order were roots and tubers, white flour and bread, milk, leafy vegetables, meat, fish, eggs, whole cereals, nuts and leguminous seeds.

Halsted *et al.*(1974) stated that the refining of foods usually resulted in decrease in the zinc content. Czerniejewski *et al.*(1964) and Schroeder (1971) noted upto 80 per cent loss in zinc content during the milling process of wheat for flour. Bread made from white flour had a lower zinc content than whole wheat bread (Osis *et al.*,1972) and corn starch contained much less zinc than the whole corn kernel (Miller and Miller, 1963).

Zinc concentration of foods was affected by food preparation methods and the variation in zinc content of cooking water from different regions also changed the zinc level of the prepared food (Halsted et al,1974). They further reported that zinc content of foods was also affected by the equipment and utensils used to prepare and store the food.

MATERIALS AND METHODS

MATERIALS AND METHODS

The present study was conducted on the subjects (in normal (control), physiological and diseased conditions) selected from Palampur and nearby rural areas situated in Kangra district of Himachal Pradesh. Kangra valley is situated between 30° 21' to 32°59' N latitude and 75° to 75°45' E longitude. The places under study are located about 1200-1500 metres above mean sea level and mean ambient temperature ranges as low as 2° celcius during winter to 34° celcius during summer.

The details of methodology to determine zinc status of local human population in health and diseases are described below:

3.1 SELECTION OF SUBJECTS:

Healthy and diseased subjects were chosen after obtaining their willingness to become experimental subjects for the study. The details are as follows:

3.1.1 Normal (control) conditions:

Ten male and ten female adult subjects between the ages of 25-36 and 22-27, respectively were selected. All of them

were daily commuting from nearby rural areas to Palampur for work in the capacity of either laboratory assistant, technicians or clerks. Educational level ranged from matriculation to graduation and there was no eccentricity in their dietary habits.

3.1.2 Physiological conditions:

a) Pregnancy: Ten subjects each from first, second and third trimester of pregnancy belonging to nearby rural areas of Palampur were selected from the local civil hospital. All of them were coming for regular check up and were housewives belonging to low middle income group. Their ages ranged between 19-28 and dietary patterns were similar.

b) Lactation: Ten lactating women again belonging to rural areas adjacent to Palampur were selected from the local civil hospital. They were coming to the hospital for regular check ups of themselves and their infants and were housewives having similar socio-economic and dietary patterns. Their ages ranged between 23-28.

c) Menstruation: Ten normal menstruating girls belonging to nearby areas of Kangra valley were selected from the girls hostel of HPKV. Their ages ranged between 18-21 and dietary patterns were same. The socio-economic status of their parents, in general, was similar.

3.1.3. Diseased conditions:

a) Diabetes: Ten male and female diabetic patients between the ages of 20-55 and 35-46, respectively, were selected from the local civil hospital and university health centre.

All of them belonged to rural areas near to Palampur and had similar socio-economic and dietary patterns.

b) Hypertension: Ten male and female hypertensive subjects between the ages of 42-52 and 39-46, respectively, were selected from the local civil hospital and university health centre. All of them belonged to nearby villages and had similarity in income level as well as dietary habits.

3.2. ANTHROPOMETRIC MEASUREMENTS:

Height and weights of the subjects were measured by the method of Jeliffe (1966) as detailed below:

3.2.1. Height:

The subjects were made to stand on the flat floor bare-footed, by the scale with their feet parallel and the heels, buttocks, shoulders and back of the head touching the upright. The head was set comfortably erect. The arms were kept hanging by the sides in natural manner. The head piece of the scale was gently lowered, crushing the hair and making contact with the top of the head, the centimeter scale reading thus obtained was recorded.

3.2.2. Weight:

Flat base standard weighing machine was used for measuring the body weight of the subjects. Before taking the weight every time, the zero reading was observed and if needed it was reset. Weighing was done in the morning before any meal and in standard clothing, after removing the footwear. The subjects were made to stand on the centre of the platform without touching anything around.

3.3. DETERMINATION OF BODY MASS INDEX (BMI):

Body mass index of the subjects was calculated by the expression suggested by Forbes (1988):

BMI= Body weight (kg)/Height^2 (m)

3.4. DETERMINATION OF BASAL METABOLIC RATE (BMR):

BMR was calculated using the method proposed by ICMR (1990) as given below:

Equations for predicting BMR (Kcal/24hr)

Sex	Age (years)	Prediction equation proposed by ICMR expert group for Indians
Male	18-30	14.5 x B.W. (kg) + 645
	30-60	10.9 x B.W. (kg) + 833
	>60	12.8 x B.W. (kg) + 463
Female	18-30	14.0 x B.W. (kg) + 471
	30-60	8.3 x B.W. (kg) + 788
	>60	10.0 x B.W. (kg) + 565

3.5. DETERMINATION OF HAEMOGLOBIN (Hb):

Hb level of the subjects was determined by Sahli's acid hematin method as described by Hunter and Bomford (1968).

Principle: Blood when mixed with hydrochloric acid forms acid hematin which is a brown coloured pigment.

Requirements: Kit containing hemometer, pipette. hemometer tube, N/10 HCl and dropper.

Method: Index finger of each subject was pricked asceptically and first few drops were discarded. Then 0.02 ml of blood was sucked in the pipette and then transferred into the tube and three drops of N/10 HCl were mixed with

blood in the tube and allowed to stand for about five minutes. It was then diluted drop by drop with distilled water till the colour matched with that of the standard. The total amount was proportionate to the concentration of haemoglobin in the blood and was noted down.

3.6. DETERMINATION OF BLOOD PRESSURE:

Systolic and diastolic blood pressures of the subjects wre determined using a mercury sphygmomanometer.

3.7. DETERMINATION OF PROTEIN,GLUCOSE AND CHOLESTEROL IN PLASMA AND CREATININE IN URINE:

3.7.1. Collection and storage of human plasma and urine samples:

Venous blood of the experimental subjects was collected into a disposable syringe and immediately transferred into a centrifuge tube containing sufficient anticoagulant (heparin). The blood was centrifuged for 15-20 minutes at 2500 rpm and supernatant plasma was separated using a rubber-bulb pipette.

For urine samples, the total amount of urine excreted by each subject in 24 hours was collected in bottles containing little amount of toluene. Quantity of the total urine was measured and after shaking thoroughly a sufficient aliquot was kept for analysis.

The samples were stored in marked tubes at -10°C in a deep freezer.

3.7.2. Plasma Protein:

Protein level of the subjects was determined by the method of Reinhold (1953). The detailed procedure is as follows:

Principle: The -CONH groups in the protein molecule react with copper sulphate in alkaline medium to give purple colour which is then read at 540 nm.

Requirements:

1. Working biuret reagent: Firstly stock biuret solution was prepared by dissolving 45g sodium-potassium tartrate, 15g copper sulphate and 5g of potassium iodide in 0.2 N NaOH and volume was made one litre. For preparing working biuret reagent, 5g of KI was added in 200 ml of above stock biuret reagent and the volume was raised to 1 litre with 0.2 N NaOH.

2.Stock albumin solution: For preparing working standards stock albumin solution was prepared by dissolving 1g of bovine serum albumin in distilled water and volume was made to 100 ml. The final solution contained 10 mg protein/ml.

3. 0.75 N NaoH

4. Plasma samples

Preparation of working standards:

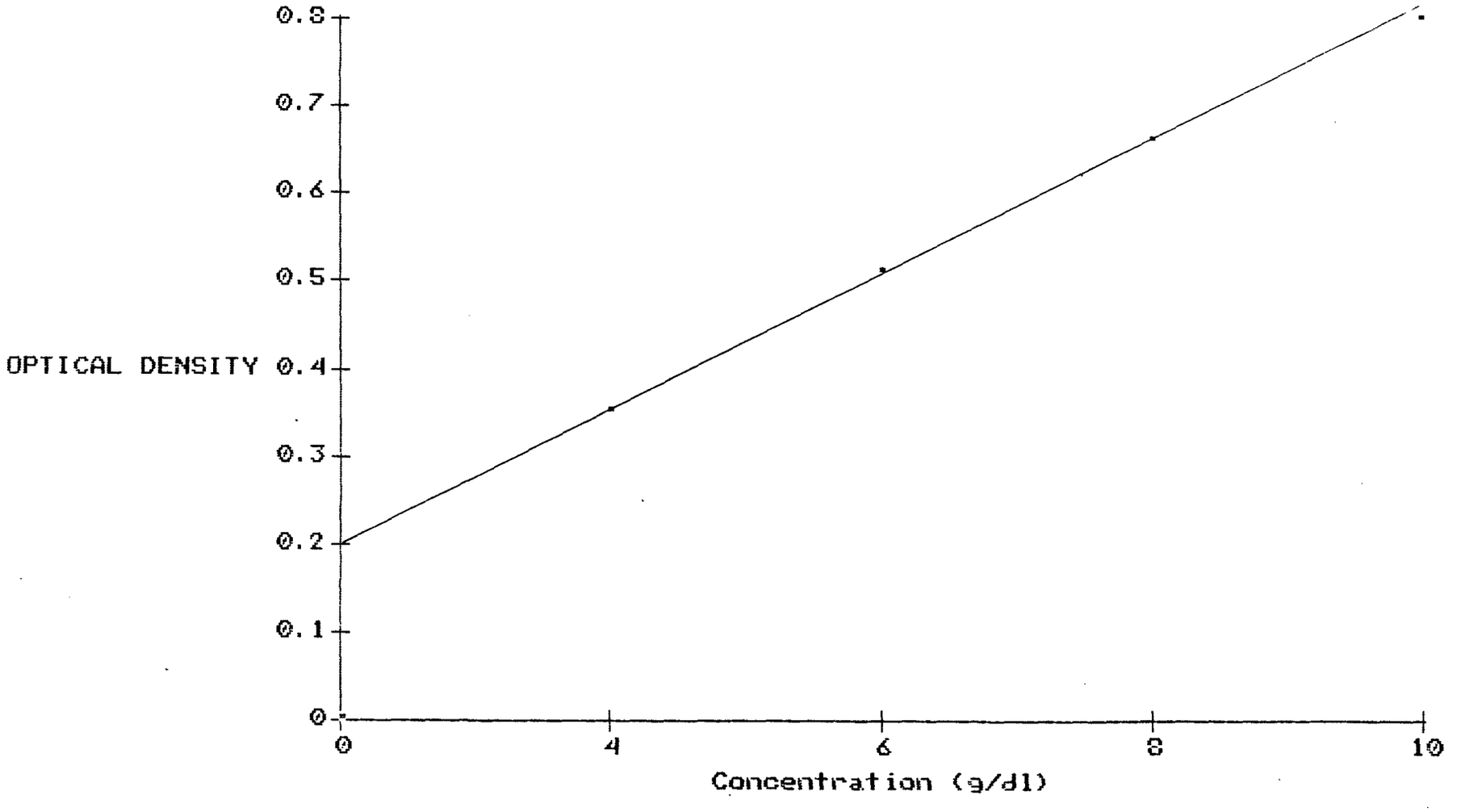
Standards	Stock albumin solution	Distilled water	Final volume	Protein concent-ration /ml	* which is equal to (g protein /dl)
S_1	1 ml	-	1 ml	10 mg	10
S_2	0.8 ml	0.2 ml	1 ml	8 mg	8
S_3	0.6 ml	0.4 ml	1 ml	6 mg	6
S_4	0.4 ml	0.6 ml	1 ml	4 mg	4

* When standard volume is 1 ml amd sample volume is 0.1 ml

Method: 1 ml of all the standards were added in their respective tubes and 0.1 ml of plasma samples were added in another set of marked test tubes. To make the total volume 2 ml; 2 ml, 1 ml and 1.9 ml of 0.75 N NaOH was added in the blank, standards and test samples, respectively. 2 ml of working biuret reagent was added to all the tubes and the contents were mixed well. Tubes were kept at room temperature for 30 minutes and the absorption was read at 540 nm.

Preparation of standard curve: After setting zero optical density (O.D.) for blank, O.D. for each concentration of the standard was noted down. It was plotted on graph paper by taking the absorbance on vertical axis versus protein standard concentrations on horizontal axis. Using a set of points, a best fit curve was drawn (Fig 3.1). The protein concentration in the sample was read directly from the standard curve and was expressed as g protein/dl plasma.

Fig.3.1 STANDARD CURVE FOR PLASMA PROTEIN



3.7.3 Plasma glucose:

Plasma glucose level of the subjects was determined by the method of Folin and Wu (1965).

Principle: The protein-free blood filtrate is heated with alkaline copper solution, using a special tube to prevent reoxidation. The cuprous oxide formed is treated with a phosphomolybdic acid solution, a blue colour being obtained which is compared with that of a standard.

Requirements:

1. Stock solution: Stock standard (1 per cent) i.e. 10 mg/ml glucose in saturated benzoic acid.
2. Working standard: A working standard of strength 0.1 mg/ml equivalent to 200 mg/100 ml of blood was prepared by taking 2 ml of stock solution and making the volume to 100 ml with distilled water.
3. 10 per cent sodium tungstate
4. N/10 H_2SO_4
5. Alkaline copper solution
6. Phosphomolybdic acid
7. Protein-free filtrates.

Preparation of working standards:

Standards	Working standard 0.1 mg/ml	Distilled water	Final volume	Glucose concentration mg/dl
S_1	2.0 ml	-	2 ml	200
S_2	1.5 ml	0.5 ml	2 ml	150
S_3	1.0 ml	1.0 ml	2 ml	100
S_4	0.5 ml	1.5 ml	2 ml	50

Preparation of protein-free filtrates (PFF): Took 8 ml of N/10 H_2SO_4 and to it added 1 ml of 10 per cent sodium tungstate and 1 ml plasma. Then mixed it properly and filtered through whatman filter paper No. 1 to obtain protein-free filtrate.

Method: Took Folin and Wu sugar tubes and marked them for blank, standards and unknown samples.

Proceeded as below:

	B	S1	S2	S3	S4	U
Working standard (0.1 mg/ml)	-	2.0 ml	1.5 ml	1.0 ml	0.5 ml	-
Distilled water	2.0 ml	-	0.5 ml	1.0 ml	1.5 ml	-
PFF	-	-	-	-	-	2.0 ml
Alkaline copper solution	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml

Heated all the tubes in boiling water bath for 10 minutes. Allowed the tubes to cool in running water and then added 2 ml of phosphomolybdic acid in all the tubes. Waited for 1 minute and then made the volume of all tubes 25 ml by adding distilled water. Mixed well and read optical density of standards and unknown samples against reagent blank at 420 nm.

Preparation of standard curve: After setting zero optical density for blank, O.D. for each concentration of the standard was noted down. It was plotted on graph paper by taking the absorbance on vertical axis and glucose standard concentrations on horizontal axis. Using a set of points a

best fit curve was drawn (Fig 3.2). The glucose concentration in the sample was read directly from the standard curve and was expressed as mg glucose/100 ml plasma.

3.7.4 Plasma cholesterol:

Cholesterol level of all the subjects in plasma was determined by the spectrophotometric method of Liebermann-Burchard given by Henry (1968).

Principle: Cholesterol forms with acetic acid, acetic anhydride and conc. H_2SO_4 a blue-green compound. The intensity of the colour is proportional to the concentration of cholesterol in the sample and is measured at 620 nm (605-635 nm).

Reagents:

1 Acetic anhydride, glacial acetic acid

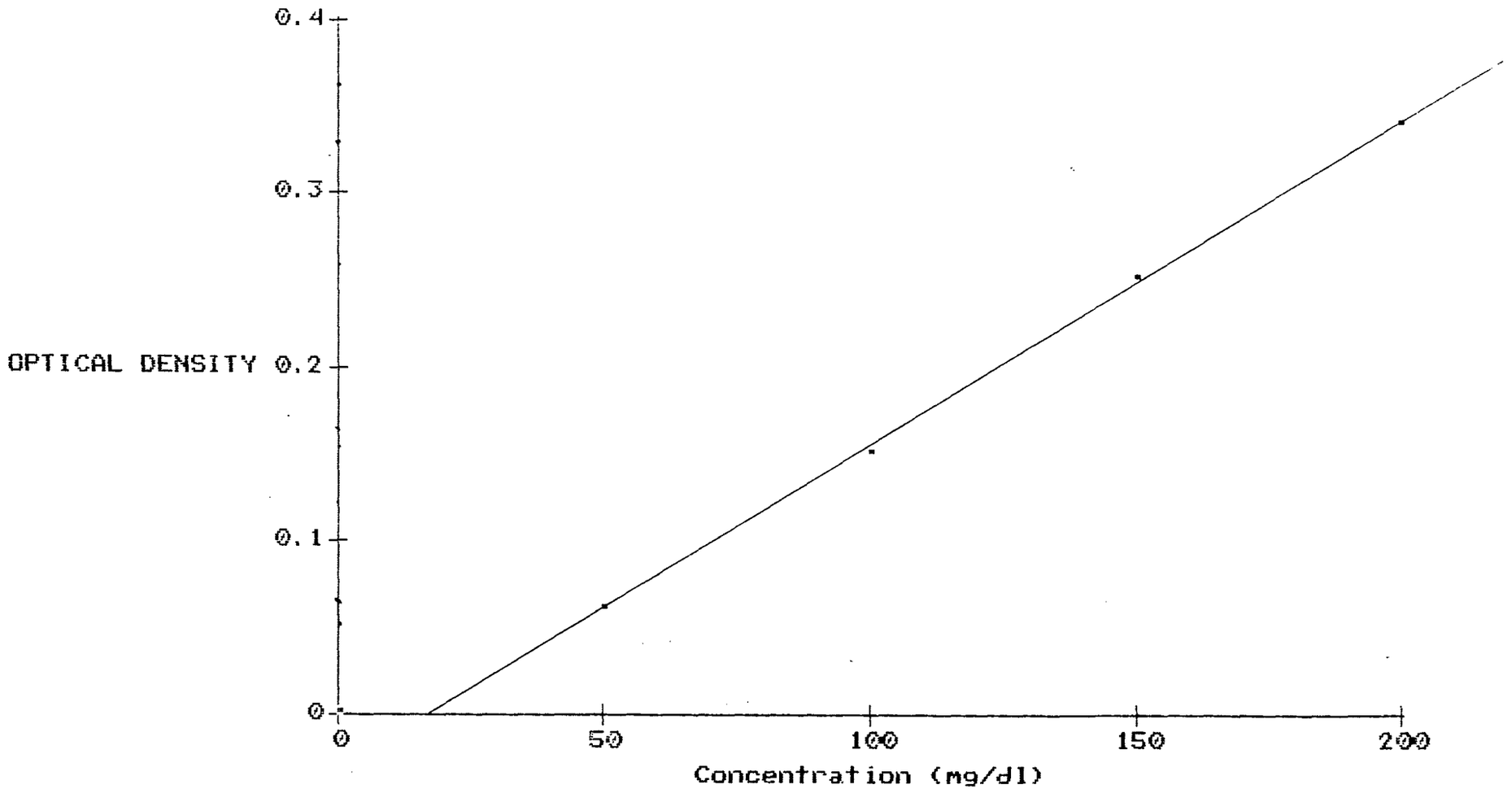
1A Glacial acetic acid, sulphuric acid.

Stock standard: 400 mg cholesterol/dl in glacial acetic acid.

Preparation of working solution:

Solution (1): Reagents 1 and 1A required to be cooled before mixing. Carefully poured the contents of one bottle 1A into one bottle 1, mixed well and stored in refrigerator for at least four hours before they were used.

Fig.3.2 STANDARD CURVE FOR PLASMA GLUCOSE



Preparation of working standards:

Standards	Stock	Glacial acetic acid	Concentration mg/dl
S_1	1 ml	3 ml	100
S_2	2 ml	2 ml	200
S_3	3 ml	1 ml	300
S_4	4 ml	-	400

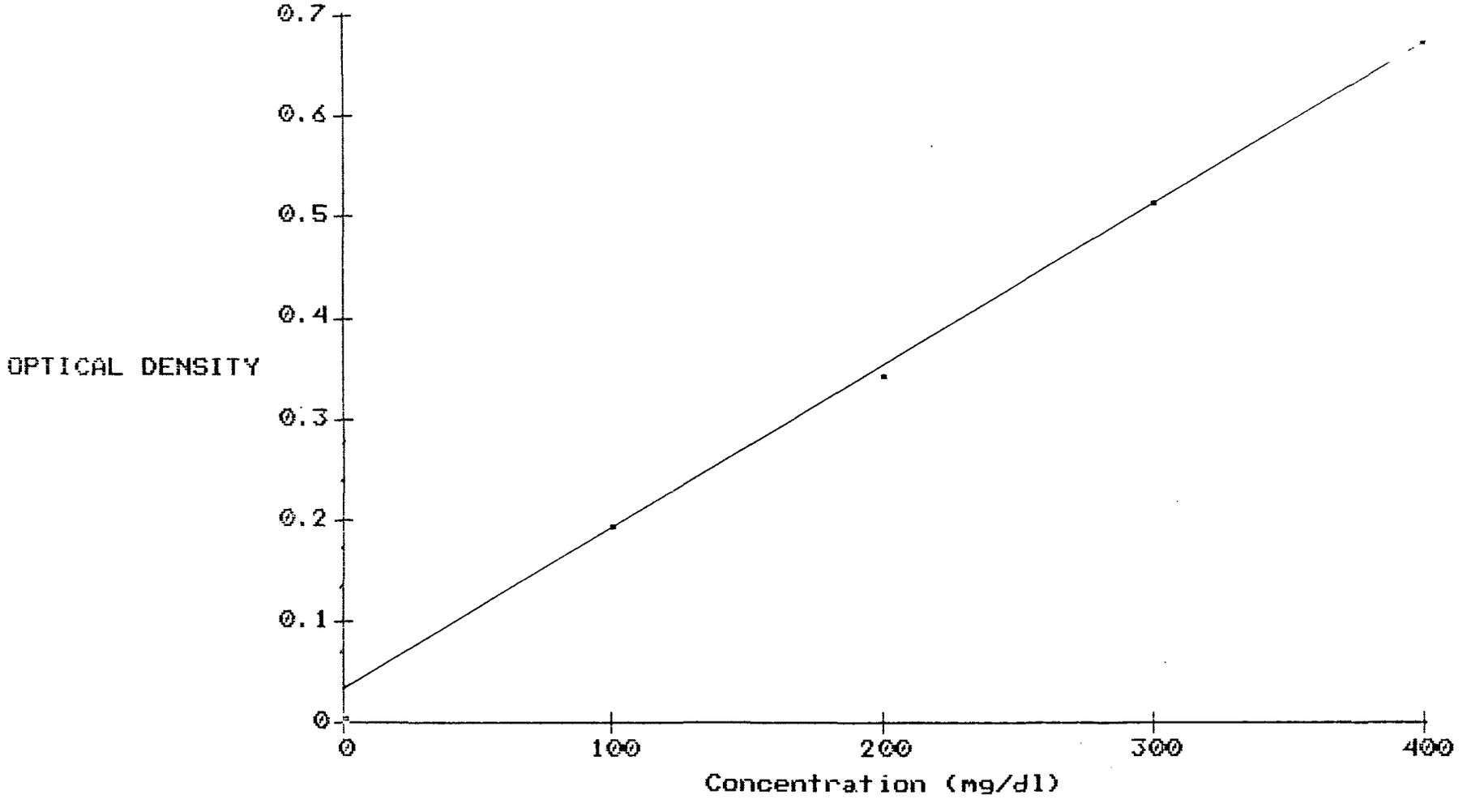
Procedure for spectrophotometer: Took test tubes and marked for blank, standard and test. Proceeded as below:

	Blank	Standard	Test
Solution (1)	2.5 ml	2.5 ml	2.5 ml
Standard	-	0.1 ml	-
Sample	-	-	0.1 ml

Added sample and standard along the walls of the test tubes to obtain a layer over the solution. Mixed immediately after layering. Then allowed the tubes to stand in a water bath at room temperature (25 $\pm$ 5°C) for 12 minutes in order to achieve adequate heat dispersion which is essential for the reaction. Then read the absorbance of the test and of the standard against the blank within 10 minutes at 620 nm (605-635 nm).

Preparation of standard curve: After setting zero optical density for blank, standard curve was constructed by plotting the O.D. values for the cholesterol standards on vertical axis and cholesterol standard concentrations on the horizontal axis, on a graph paper. Using a set of points, a best fit curve was drawn (Fig 3.3). The cholesterol

Fig.3.3 STANDARD CURVE FOR PLASMA CHOLESTEROL



concentration in the sample was read directly from the standard curve and was expressed as mg cholesterol/100 ml plasma.

3.7.5 Urinary creatinine:

Urinary creatinine was determined by the method of Wootton (1974). The details of this method are as follows:

Principle: Creatinine reacts with alkaline picrate to form a yellow red colour (Jaffe reaction).

Reagents:

1. Saturated picric acid (12 per cent solution of picric acid was prepared and then filtered using whatman filter paper No. 42).
2. Sodium hydroxide (10 per cent)
3. Standard solution of creatinine (25 mg creatinine per 100 ml of 0.1N HCl)
4. Urine samples

Method

1. For samples: Urine was centrifuged at 3000 rpm for 10 minutes. Supernatant was collected and sediment was discarded. To 1 ml of the supernatant, 3 ml of distilled water was added. TO 200 µl of this diluted urine, 800 µl of water was added. To all the tubes then, firstly, 4 ml of saturated picric acid and then 0.3 ml of 10 per cent NaOH were also added and shook well. Then added 0.7 ml of distilled water to all the tubes after which the tubes were allowed to stand for 10 minutes. Absorption was read at 520 nm.

2. For blank: In the fourth step, instead of 200 µl urine + 800 µl water, 1 ml of distilled water was taken. Rest of the procedure was the same.

3. For the standards: Five creatinine standards were taken, that is, 40, 80, 120, 160 and 200 µl from the standard stock solution of creatinine containing 10, 20, 30, 40 and 50 µg creatinine, respectively.

Preparation of standard curve: Standard curve was constructed by plotting the O.D. values for the creatinine standards on vertical axis versus creatinine standard concentrations on horizontal axis, on a graph paper. Using a set of points, the best fit curve was plotted (Fig 3.4).

Total creatinine in urine: Using the O.D. for each sample, the values for creatinine in urine samples were read from the standard curve.

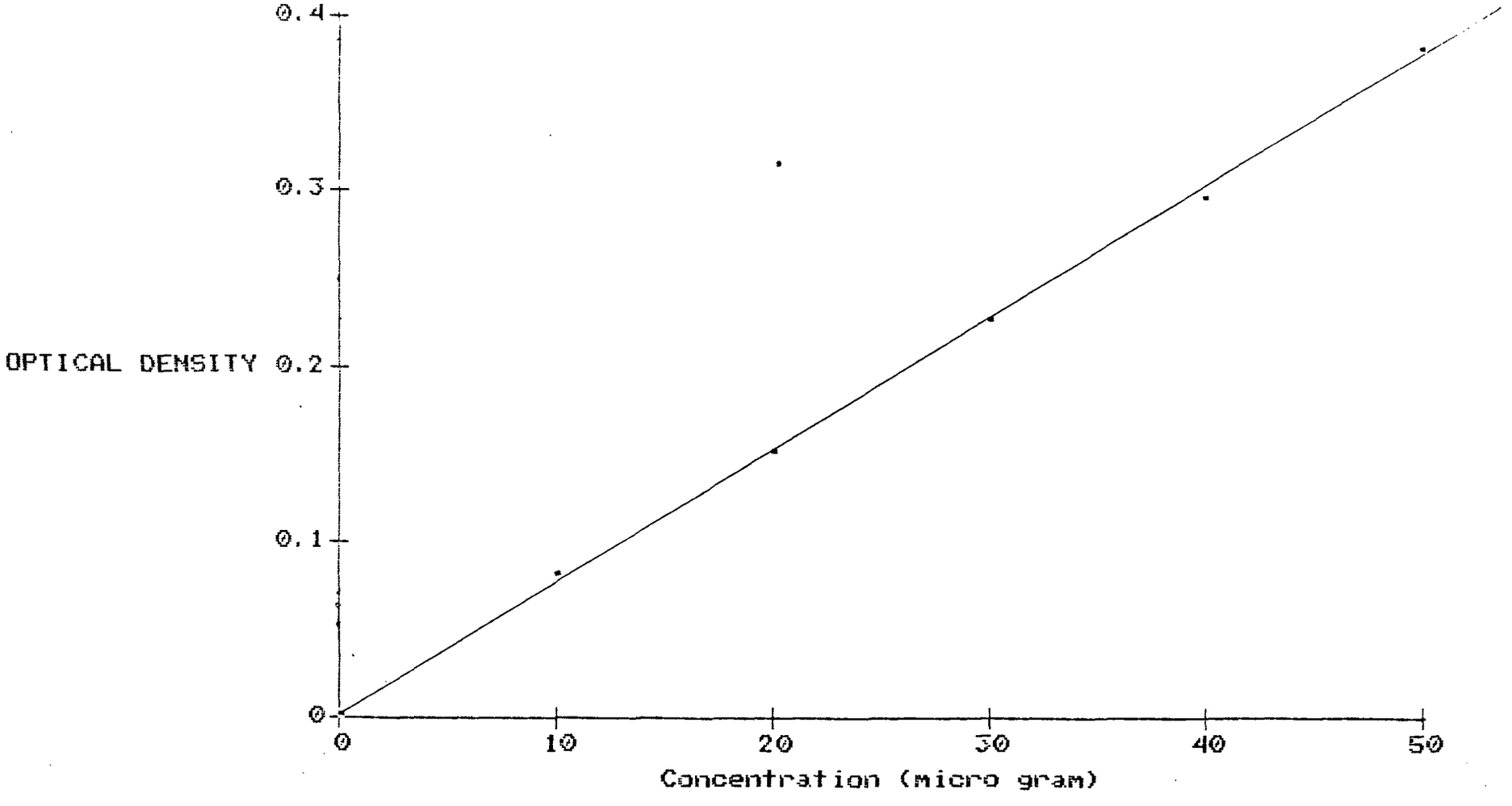
3.8 DIGESTION OF FOOD, URINE, SERUM, SALIVA, MILK AND HAIR SAMPLES FOR DETERMINATION OF ZINC.

3.8.1. Collection and storage of food, serum, saliva and milk samples:

Food samples: In case of vegetables, fresh weight of edible portion was noted down and then the vegetables were cut into small pieces and kept in the oven for drying. Upon drying, again weight was taken and the material was then finely grounded. It was then kept in tightly closed plastic bottles in order to prevent moisture gain.

Serum samples: Venous blood of the subjects was drawn into a disposable syringe. It was then transferred as quickly as

Fig.3.4 STANDARD CURVE FOR URINARY CREATININE



possible into a centrifuge tube and the blood was allowed to clot. As soon as sufficient serum was formed, it was transferred into a suitable container with the help of a rubber bulb pipette. The samples were stored at -10°C in a deep freezer.

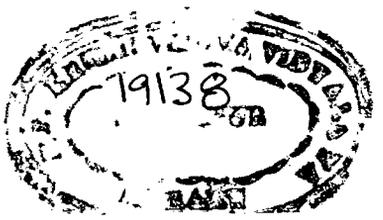
Saliva and milk samples: Whole mixed saliva samples were collected and stored in marked containers after filtering through whatman filter paper. Milk samples were collected from the lactating subjects. Both type of samples were stored at -10°C in a deep freezer.

Digestion of samples: Samples were digested in di-acid mixture (4 parts nitric acid + 1 part perchloric acid) according to the procedure of Johnson and Ulrich (1959).

Procedure: A known amount of sample (0.5 gm food sample, 2 ml each of serum, saliva, urine and milk samples and 100 mg of hair sample) was taken in a 250 ml conical flask. 10 ml of di-acid mixture was added and the flask was covered with water glass to prevent contamination and was kept overnight.

The samples were digested at low temperature of hot plate. Before heating, the watch glass was removed and a glass funnel was placed on the flask to avoid bumping and contamination. Copious red fumes were produced as reaction took place. After 40-50 minutes, the fumes of nitric acid were over.

The digestion was continued till the liquid was yellow green and became colourless on cooling. The flask was



removed from the hot plate when approximately 1 ml of digested material was left.

If there was carbonization, the solution became black. 1-2 ml of nitric acid was added and digested again as usually. The solution became clear in about 2-3 minutes. If it persisted, 2-3 ml of nitric acid were added and digestion was continued until solution became clear.

After cooling, the digested material was transferred alongwith some glass distilled water to volumetric flask. The volume was made to 50 ml in case of food samples and 25 ml in case of serum, urine, saliva, milk and hair samples.

3.9 DATA ANALYSIS:

Data analysis was done following the standardized statistical method by Gupta (1988). The statistical analysis was viewed through to the mean and its standard deviation for analysing the general trend in data. For comparing the treatments, the analysis of variance test was undertaken following the completely randomized design. Difference within and between each group were tested through least significant difference value. In all these tests, the significance level was kept constant at 5 per cent ($P < 0.05$). Relationship between various parameters was also worked out through cause and effect analysis (correlations). For a given parameter, difference between sexes was tested using 'student's t' test.

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

The results of the study to determine the zinc status of local human population in health and diseases are presented and discussed in this chapter under the following headings:

1) During diseased conditions
2) During different physiological stages (Pregnancy, lactation)
3) During menstrual cycle phases.

4.1. DURING DISEASED CONDITIONS:

4.1.1. Age, Height and Weight:

Age: The average age (years) of normal, diabetic and hypertensive male subjects were 28.0 $\pm$ 3.26, 43.0 $\pm$ 9.93, 47.1 $\pm$ 2.73, respectively (Table 4.1). The mean age in case of female subjects (Table 4.2) was highest for hypertensives (42.7 $\pm$ 2.40), followed by diabetics (40.5 $\pm$ 4.11) and normals (24.6 $\pm$ 1.50).

There was almost twenty years difference in the age of normal and diseased subjects which implied that age was an important criteria for the development of diseases such as diabetes and hypertension. This was in concurrence with the

report of Shils and Young (1988) who stated that diabetes and hypertension usually develop after the age of 40 and 45 years, respectively, in majority of human population.

Height and weight: Non-significant difference was observed in height and weight of normal and diseased male subjects (Table 4.1). The mean heights (cm) of normal, diabetic and hypertensive subjects were 167.8 $\pm$ 2.69, 166.3 $\pm$ 5.47 and 167.9 $\pm$ 2.44, respectively. The values for weight (kg) of respective subjects were 63.0 $\pm$ 4.39, 58.1 $\pm$ 8.6 and 59.5 $\pm$ 3.02 kg (Table 4.1). The mean weight for height observed in our study was slightly lower than the reference man's weight of 60 kg for 163 cm height reported by ICMR (1990).

Like male subjects, in female subjects too, non-significant difference was observed in the heights and weights during normal and diseased conditions. The mean values for height (cm) were 152.6 $\pm$ 2.46, 153.5 $\pm$ 2.13 and 152.9 $\pm$ 1.97, respectively for normal, diabetic and hypertensive subjects (Table 4.2). The respective values for weight (kg) in the same order were 46.2 $\pm$ 1.75, 46.7 $\pm$ 6.05 and 47.6 $\pm$ 3.83. The mean weight for height was found to be lower than the reference woman's weight of 50 kg for 151 cm height (ICMR, 1990).

The difference between sexes for both height and weight was found to be statistically significant ($P<0.05$) in all the three conditions. ICMR (1990) also reported lower height and weight values in the females as compared to their male counterparts.

Table 4.1 Age (years), height (cm) and weight (kg) of normal (control) and diseased male subjects.

	Age			Height			Weigh	
S.No	N	D	H	N	D	H	N	D
1	36	20	48	170.0	152.5	167.5	65	37
2	29	55	52	170.0	172.5	165.0	68	70
3	28	42	47	165.0	167.5	170.0	64	58
4	26	52	42	172.5	170.0	170.0	70	64
5	30	36	47	167.5	165.0	165.0	64	54
6	28	40	46	170.0	170.5	167.0	65	62
7	26	42	46	165.5	167.5	172.0	58	60
8	25	46	45	167.5	165.0	165.5	61	58
9	25	50	48	165.0	165.0	170.0	57	57
10	27	47	50	165.0	167.5	167.5	58	61
Mean	28.0	43.0	47.1	167.8	166.3	167.9	63.0	58.1
S.D.(±)	3.26	9.93	2.73	2.69	5.47	2.44	4.39	8.6
C.V. (%)	11.7	23.1	5.8	1.6	3.3	1.4	6.9	14.8
Overall Mean		39.4			167.4			60.2
Overall SD (±)		10.29			3.74			6.02
Overall CV (%)		26.2			2.2			10.0
CD (5%)					N.S			N.S

N = Normal D = Diabetic H = Hypertensive NS = Non-signific

Table 4.2 Age (years), height (cm) and weight (kg) of normal (contr
and diseased female subjects.

	Age			Height			Weight		
S.No	N	D	H	N	D	H	N	D	
1	25	36	45	152.0	157.5	152.5	48	40	40
2	26	46	42	155.0	152.5	155.0	49	60	47
3	23	37	42	150.0	155.0	155.0	45	43	49
4	24	40	46	150.0	150.0	150.0	44	40	43
5	24	37	45	152.5	152.5	152.5	47	45	45
6	27	35	40	152.5	153.0	154.0	48	46	51
7	26	42	45	157.0	154.5	152.0	46	52	49
8	24	44	42	150.0	152.0	152.5	46	44	50
9	22	46	39	155.5	152.5	150.0	45	47	50
10	25	42	41	152.0	155.5	155.5	44	50	52
Mean	24.6	40.5	42.7	152.6	153.5	152.9	46.2	46.7	47.
SD (±)	1.50	4.11	2.40	2.46	2.13	1.97	1.75	6.05	3.
CV (%)	6.1	10.2	5.6	1.6	1.4	1.3	3.8	12.9	8.
Overall Mean		35.9			153.0			46.8	
Overall SD (±)		8.66			2.15			4.15	
Overall CV (%)		24.1			1.4			8.8	
CD (5%)					N.S			N.S	

N = Normal D = Diabetic H = Hypertensive NS = Non-significa

Table 4.3 Analysis of variance for height of the subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Male				
Treatment	2	16.650	8.325	0.578
Error	27	388.925	14.405	
Total	29	405.575	13.985	
Female				
Treatment	2	3.817	1.908	0.395
Error	27	130.425	4.831	
Total	29	134.242	4.630	

* Significant at 5% level

Table 4.4 Analysis of variance for weight of the subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Male				
Treatment	2	127.400	63.700	1.863
Error	27	923.400	34.200	
Total	29	1050.800	36.234	
Female				
Treatment	2	10.067	5.033	0.277
Error	27	490.100	18.152	
Total	29	500.167	17.247	

* Significant at 5% level

Shils and Young (1988) and Kannel and Gordon (1979) reported that overweight was a major cause of insulin resistance leading to diabetic state and hypertension, respectively. However, in the present study this cause was ruled out as none of the diabetic and hypertensive patients were overweight.

4.1.2 Body Mass Index (BMI): The mean BMI values (kg/m^2) of normal, diabetic and hypertensive male subjects were 22.35 $\pm$ 1.01, 20.87 $\pm$ 1.99 and 21.08 $\pm$ 0.66, respectively (Table 4.5) and the difference was found to be statistically significant ($P<0.05$).

Unlike males, the statistical analysis showed non-significant difference in the BMI values of female subjects and corresponding values for normal (control), diabetic and hypertensive subjects were 19.84 $\pm$ 0.79, 19.83 $\pm$ 2.64 and 20.35 $\pm$ 1.53 kg/m^2, respectively.

Further analysis revealed that the BMI values of male subjects were significantly higher than those of female subjects under normal conditions.

Both male and female subjects had lower BMI values than the standards 22.64 kg/m^2 and 21.93 kg/m^2 proposed by ICMR (1990) for reference men and women. The results were, however, within the range of 19-27 kg/m^2 (Shils and Young, 1988) and 20-25 kg/m^2 (Nutrition News, 1991) reported for normal men and women.

Further investigation revealed that there was no influence of diseased conditions on the BMI of the subjects.

Cronk and Roche (1982) had also reported that BMI was not always an accurate index of body composition as the average index was about the same for both sexes during the adolescent and young adult years despite the difference in body fat. This might be the possible reason for nearly similar BMI values in normal and diseased conditons.

4.1.3 Basal Metabolic Rate (BMR): Mean BMR (Kcal/24 hr) was significantly higher in normal male subjects(1553.9 $\pm$ 63.08) than both hypertensives (1481.5 $\pm$ 33.0) and diabetics (1460.8 $\pm$ 109.11) (Table 4.5).

In case of female subjects, highest mean BMR value (kcal/24 hr) was observed in hypertensives (1183.1 $\pm$ 31.83), followed by diabetics (1175.6 $\pm$ 50.26) and normals (1117.8 $\pm$ 24.51). Statistical analysis revealed that the BMR values for diabetics and hypertensives were significantly ($P<0.05$) higher than those for normals while the difference between diabetics and hypertensives was non-significant.

Difference between sexes was found to be statistically significant in all the three conditions.

The higher BMR values obtained for male subjects in the present study were probably due to the positive relation of BMR with body surface area which in turn is directly related to height and weight of an individual. Since, in the present study, both these anthropometric measurements were found to be more in males, higher surface area might have contributed to more value of BMR in male subjects. Moreover, it is well

Table 4.5 Body Mass Index (BMI) and Basal Metabolic Rate (BMR) of normal (control) and diseased male and female subjects

	Male						Female					
	BMI (kg/m²)			BMR (Kcal/24 hr)			BMI (kg/m²)			BMR (Kcal/24 hr)		
S.No	N	D	H	N	D	H	N	D	H	N	D	H
1	22.49	15.91	20.31	1541.5	1181.5	1454.3	20.77	16.12	17.20	1143.0	1120.0	1120.0
2	23.53	23.52	21.67	1631.0	1596.0	1476.1	20.39	25.80	19.56	1157.0	1286.0	1178.1
3	23.50	20.67	21.45	1573.0	1465.2	1508.8	20.00	17.89	20.39	1101.0	1144.9	1194.7
4	23.52	22.14	22.14	1660.0	1530.6	1530.6	19.55	17.77	19.11	1087.0	1120.0	1144.9
5	22.81	19.83	21.30	1573.0	1421.6	1465.2	20.21	19.35	19.35	1129.0	1161.5	1161.5
6	22.49	21.32	20.44	1587.5	1508.8	1454.3	20.64	19.65	21.50	1143.0	1169.8	1211.3
7	21.17	21.38	21.63	1486.0	1487.0	1530.6	18.66	21.78	21.21	1115.0	1219.6	1194.7
8	21.74	21.30	20.44	1529.5	1465.2	1443.4	20.44	19.04	21.50	1115.0	1153.2	1203.0
9	20.94	20.94	21.11	1471.5	1454.3	1497.9	18.73	20.21	22.22	1101.0	1178.1	1203.0
10	21.30	21.74	20.31	1486.0	1497.9	1454.3	19.04	20.68	21.50	1087.0	1203.0	1219.6
Mean	22.35	20.87	21.08	1553.9	1460.8	1481.5	19.84	19.83	20.35	1117.8	1175.6	1183.1
SD (±)	1.01	1.99	0.66	63.08	109.11	33.00	0.79	2.64	1.53	24.51	50.26	31.83
CV (%)	4.5	9.6	3.2	4.1	7.5	2.2	4.0	13.3	7.6	2.2	4.3	2.7
Overall Mean		21.43			1498.75			20.01			1158.83	
Overall SD (±)		1.46			83.15			1.77			46.53	
Overall CV (%)		6.8			5.5			8.8			4.0	
CD (5%)		0.87			48.81			N.S			24.11	

N = Normal D = Diabetic H = Hypertensive NS = Non-significant

Table 4.6 Analysis of variance for body mass index of the subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Male				
Treatment	2	12.750	6.375	3.518*
Error	27	48.923	1.812	
Total	29	61.673	2.126	
Female				
Treatment	2	1.790	0.895	0.269
Error	27	89.937	3.331	
Total	29	91.726	3.163	

* Significant at 5% level

Table 4.7 Analysis of variance for basal metabolic rate of the subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Male				
Treatment	2	47768.061	23884.03	4.221*
Error	27	152765.754	5657.991	
Total	29	200533.815	6914.959	
Female				
Treatment	2	25530.918	12765.459	9.248*
Error	27	37271.225	1380.416	
Total	29	62802.143	2165.59	

* Significant at 5% level

established that women have 6 to 10 per cent lower BMR than that of the men (Robinson and Lawler, 1982).

4.1.4 Blood Pressure (Bp): The mean values for systolic and diastolic Bp of the subjects in the three conditions are given in table 4.8.

Systolic and diastolic Bp: Mean systolic Bp value (mm Hg) for male subjects was recorded highest in hypertensives (141.5 ± 8.51), followed by diabetics (136.5 ± 9.77) and normals (118.4 ± 4.92) and the difference was statistically significant ($P<0.05$).

Similar trend was observed in systolic Bp values of female subjects. Hypertensives had highest value (141.5 ± 6.68 mm Hg), diabetics intermediate (138.1 ± 7.5 mm Hg) and normals lowest (120.1 ± 7.31 mm Hg). Statistically the difference was again significant ($P<0.05$).

Mean diastolic Bp value (mm Hg) was 113.5 ± 9.44 for hypertensives, 89.3 ± 7.63 for diabetics and 76.8 ± 3.67 for normal male subjects and statistically the difference was significant ($P<0.05$). Similar pattern was observed in diastolic Bp values of female subjects and the respective values for hypertensives, diabetics and normals were 114.0 ± 6.58, 91.2 ± 8.49 and 77.6 ± 3.37 mm Hg. Difference was again significant ($P<0.05$).

In the present study, the mean systolic and diastolic Bp values of normal subjects were within the range specified by Jackson (1981). Higher values observed for diabetics and hypertensive could be related to the age of the subjects.

Table 4.8 Systolic and diastolic blood pressures (mm Hg) of normal (control) and diseased male and female subjects.

	Male						Female					
	Systolic			Diastolic			Systolic			Diastolic		
S.No	N	D	H	N	D	H	N	D	H	N	D	H
1	125	120	140	76	80	105	110	135	150	76	80	120
2	110	145	155	78	90	120	125	148	135	80	90	105
3	115	138	130	72	85	105	120	130	145	80	82	110
4	114	150	130	80	95	110	130	135	150	80	85	125
5	120	125	150	80	80	125	114	130	140	70	100	110
6	120	130	140	78	90	115	110	140	130	74	95	115
7	115	130	145	70	85	110	118	150	145	78	105	120
8	122	142	135	80	88	100	124	138	140	80	90	105
9	125	145	140	80	95	115	120	145	135	78	100	115
10	118	140	150	74	105	130	130	130	145	80	85	115
Mean	118.4	136.5	141.5	76.8	89.3	113.5	120.1	138.1	141.5	77.6	91.2	114.0
S.D.(±)	4.92	9.77	8.51	3.67	7.63	9.44	7.31	7.50	6.68	3.37	8.49	6.58
C.V. (%)	4.2	7.2	6.0	4.8	8.5	8.3	6.1	5.4	4.7	4.3	9.3	5.8
Overall Mean		132.1			93.2			133.2			94.3	
Overall SD (±)		12.71			17.03			11.79			16.51	
Overall CV (%)		9.6			18.3			8.9			17.5	
CD (5%)		5.19			4.75			4.65			4.22	

N = Normal D = Diabetic H = Hypertensive

Table 4.9 Analysis of variance for systolic blood pressure of the subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Male				
Treatment	2	2954.067	1477.033	23.033*
Error	27	1731.400	64.126	
Total	29	4685.467	161.567	
Female				
Treatment	2	2645.067	1322.533	25.684*
Error	27	1390.300	51.493	
Total	29	4035.367	139.15	

* Significant at 5% level

Table 4.10 Analysis of variance for diastolic blood pressure of the subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Male				
Treatment	2	6962.600	3481.300	64.905*
Error	27	1448.200	53.637	
Total	29	8410.800	290.030	
Female				
Treatment	2	6765.867	3382.933	79.982*
Error	27	1142.000	42.296	
Total	29	7907.867	272.680	

* Significant at 5% level

Passmore and Eastwood (1986) have stated that blood pressure increases with age and the mean figure is 160/90 mm of Hg at 65 years. Shils and young (1988) also reported that the generally accepted criteria for hypertension are a systolic blood pressure greater than 130 mm Hg in people less than age 45, and systolic blood pressure greater than 150 mm Hg in people above age 45 years. The diastolic Bp values obtained in the present finding for both male and female subjects revealed that barring 2 male and 1 female subject, all were suffering from moderate hypertension while the former were suffering from severe hypertension. Passmore and Eastwood (1986) have also stated that unless diastolic blood pressure is above 105, hypertension may be said to be mild or moderate and when it exceeds 120, to be severe.

4.1.5 Haemoglobin (Hb): Mean Hb concentration (g/dl) of male subjects ranged from 12.8 $\pm$ 0.26 in hypertensives to 12.2 $\pm$ 0.79 in diabetics (Table 4.11). The difference was found to be statistically significant ($P<0.05$).

In case of female subjects, mean Hb concentration (g/dl) was highest in normals (11.4 $\pm$ 0.23), followed by hypertensives (10.7 $\pm$ 0.45) and diabetics (10.4 $\pm$ 0.76) and the difference was again statistically significant ($P<0.05$).

The difference between the sexes was statistically significant ($P<0.05$) in all the three conditions.

The values for Hb obtained in the present study were much lower than those given by Oser (1976) i.e. 14-16 g/dl for adult men and 13.5-15.0 g/dl for adult women. Following

Table 4.11 Haemoglobin (Hb) level of normal (control) and diseased male and female subjects.

	Hb (g/dl)					
	Male			Female		
S.No	N	D	H	N	D	H
1	12.5	10.0	12.3	11.2	9.0	9.
2	12.8	12.6	12.8	11.6	11.3	10.
3	13.0	12.2	12.8	11.0	10.2	10.
4	13.2	12.3	13.0	11.6	9.8	11.
5	12.5	12.0	12.7	11.4	11.0	10.
6	12.5	12.8	12.7	11.2	9.8	11.
7	12.4	12.2	13.1	11.4	11.4	10.
8	12.8	12.4	12.6	11.5	10.3	10.
9	13.0	12.4	13.2	11.4	10.6	10.
10	13.1	12.6	13.0	11.8	11.0	11.
Mean	12.8	12.2	12.8	11.4	10.4	10.
S.D.(±)	0.29	0.79	0.26	0.23	0.76	0.
C.V. (%)	2.3	6.5	2.1	2.0	7.3	4.
Overall Mean		12.6			10.9	
Overall SD (±)		0.58			0.66	
Overall CV (%)		4.6			6.1	
CD (5%)		0.33			0.34	

N = Normal D = Diabetic H = Hypertensive

Table 4.12 Analysis of variance for blood haemoglobin level of the subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Male				
Treatment	2	2.825	1.412	5.434*
Error	27	7.017	0.260	
Total	29	9.842	0.339	
Female				
Treatment	2	5.042	2.521	8.941*
Error	27	7.613	0.282	
Total	29	12.655	0.436	

* Significant at 5% level

this criteria, all the subjects could be classified as anaemic. Szewczynski (1979) stressed that eating practices could influence the Hb concentration. Hence poor eating practices alongwith nutritional ignorance and negligence could be the explanation for lower Hb values recorded in the present investigation. Shils and Young (1988) found a high percentage of below-normal haemoglobin concentration with the increase in age of the subjects. This could also be the possible reason for lower than normal Hb level of the diseased subjects in the present study.

4.1.6 Plasma protein: Mean plasma protein concentration (g/dl) was highest in diabetics (8.23 $\pm$ 0.62), followed by hypertensives (7.85 $\pm$ 0.57) and normals (7.45 $\pm$ 0.51) and analysis of variance revealed statistically significant difference ($P<0.05$) in the plasma protein concentrations of male subjects.

Similar trend was seen in the plasma protein concentrations of the female subjects. The values in descending order were 8.09 $\pm$ 0.80 g/dl for diabetics, 7.92 $\pm$ 0.22 g/dl for hypertensives and 7.16 $\pm$ 0.53 g/dl for normals (Table 4.13).

Most of the values for plasma protein obtained in the present investigation were within the normal range of 6-8 g/dl specified by Oser (1976) barring three male and female diabetics and one hypertensive male subject.

4.1.7 Plasma glucose: Mean plasma glucose was significantly higher in case of diabetic male subjects as compared to the

Table 4.13 Plasma protein and glucose level of normal (control) and diseased male and female subjects.

	Male						Female					
	Plasma protein (g/dl)			Plasma glucose (mg/dl)			Plasma protein (g/dl)			Plasma glucose (mg/dl)		
S.No	N	D	H	N	D	H	N	D	H	N	D	H
1	7.80	9.25	7.95	88	356	122	6.70	6.90	8.05	96	549	146
2	7.95	8.00	7.30	96	172	115	6.80	9.15	8.05	86	324	130
3	8.00	7.85	7.55	94	197	100	8.20	8.05	8.15	105	322	121
4	6.60	7.55	9.05	100	328	130	7.50	9.00	8.25	94	243	120
5	7.10	9.00	7.55	91	258	132	7.40	7.30	7.80	88	427	118
6	7.30	7.90	8.20	110	342	110	6.85	8.40	7.95	92	332	120
7	7.70	8.00	7.80	89	234	130	7.20	8.25	7.50	96	316	124
8	8.05	8.15	6.95	93	228	124	7.70	7.60	8.00	89	266	130
9	6.90	9.05	8.00	96	326	127	6.50	7.30	7.80	96	243	132
10	7.10	7.60	8.20	87	315	115	6.80	9.00	7.70	94	324	120
Mean	7.45	8.23	7.85	94.40	275.60	120.50	7.16	8.09	7.92	93.60	334.60	126.10
S.D.(±)	0.51	0.62	0.57	6.81	65.76	10.33	0.53	0.80	0.22	5.37	92.33	8.62
C.V. (%)	6.9	7.6	7.4	7.2	23.9	8.6	7.4	9.9	2.8	5.7	27.6	6.8
Overall Mean		7.84			163.50			7.72			184.76	
Overall SD (±)		0.64			89.48			0.68			120.29	
Overall CV (%)		8.2			54.7			8.9			65.1	
CD (5%)		0.37			25.07			0.37			34.80	

N = Normal D = Diabetic H = Hypertensive

Table 4.14 Analysis of variance for plasma protein level of the subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Male				
Treatment	2	3.082	1.541	4.658*
Error	27	8.932	0.331	
Total	29	12.015	0.414	
Female				
Treatment	2	4.905	2.452	7.512*
Error	27	8.814	0.326	
Total	29	13.718	0.473	

* Significant at 5% level

Table 4.15 Analysis of variance for plasma glucose level of the subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Male				
Treatment	2	191902.20	95951.100	54.273*
Error	27	40307.300	1492.863	
Total	29	232209.500	8007.220	
Female				
Treatment	2	342031.667	171015.833	59.459*
Error	27	77657.700	2876.211	
Total	29	419689.367	14472.040	

* Significant at 5% level

hypertensive and normal subjects. The corresponding values were 275.6 ± 65.76 mg/dl, 120.5 ± 10.33 mg/dl and 94.4 ± 6.81 mg/dl (Table 4.13).

Similar pattern was also observed in female subjects and the respective values for diabetic, hypertensive and normal subjects were 334.6 ± 92.33 mg/dl, 126.1 ± 8.62 mg/dl and 93.6 ± 5.37 mg/dl and difference was statistically significant ($P<0.05$).

In the present study, the plasma glucose level of all the normal subjects and some of the hypertensive subjects were within the normal range of 90-120 mg/dl reported by Oser (1976). However, since most of the hypertensive subjects had blood sugar level above the normal, they could also be classified as mildly diabetic. Further analysis revealed that female diabetics had significantly higher plasma glucose level than male diabetics in the present finding.

4.1.8 Plasma cholesterol: Mean plasma cholesterol level (mg/dl) of the male subjects was significantly higher for both diabetics (222.3 ± 24.92) and hypertensives (210.1 ± 16.31) than that of normals (170.4 ± 20.86) but the difference between diabetics and hypertensives was non-significant.

The mean plasma cholesterol level (mg/dl) of the female subjects (Table 4.16) was also highest for diabetics (224.3 ± 18.73), followed by hypertensives (183.4 ± 19.46) and

normals (171.3 ± 11.53). The difference was found to be statistically significant (P<0.05).

Further analysis showed that difference between male and female values was statistically significant (P<0.05) in hypertensive subjects.

All the values for plasma cholesterol obtained in the present investigation were within the normal range of 150-250 mg/dl (Oser, 1976). However, the values obtained for diabetics were more towards the higher side of this range which was in agreement with the findings of Saudek and Young (1981) who stated that diabetic individuals have higher serum cholesterol values than matched non-diabetic controls. However, Passmore and Eastwood (1986) found little association of hypertension with hypercholesterolaemia which also concurred with the findings of the present study.

4.1.9 Urinary creatinine: Maximum mean urinary creatinine excretion (g/day) was observed in diabetics (0.660 ± 7.32) followed by normals (0.566 ± 7.83) and hypertensives (0.547 ± 6.20) and difference was statistically significant (P<0.05) in case of male subjects (Table 4.16).

In case of female subjects, the mean urinary creatinine excretion (g/day) for diabetics, hypertensives and normals was 0.656 ± 0.06,0.528 ± 0.05 and 0.523 ± 6.38 respectively. Difference was statistically significant (P<0.05)

The normal range of urinary output of creatinine in case of women is reported as 0.8-1.5 g/day (Best and Taylor, 1969) and in case of adults the specified range is 1-1.5

Table 4.16 Plasma cholesterol and urinary creatinine level of normal (control) and diseased male and female subjects.

	Male						Female					
	Plasma cholesterol (mg/dl)			Urinary creatinine (g/day)			Plasma cholesterol (mg/dl)			Urinary creatinine (g/day)		
S.No	N	D	H	N	D	H	N	D	H	N	D	H
1	142	249	234	0.672	0.767	0.532	188	230	174	0.542	0.668	0.435
2	174	194	192	0.554	0.563	0.465	172	222	152	0.612	0.755	0.514
3	154	188	194	0.608	0.590	0.627	180	236	203	0.593	0.560	0.580
4	157	204	228	0.486	0.742	0.469	160	246	167	0.466	0.742	0.495
5	146	233	207	0.703	0.574	0.625	176	246	188	0.495	0.608	0.532
6	178	225	221	0.498	0.620	0.582	155	199	175	0.515	0.625	0.605
7	169	194	199	0.505	0.714	0.615	177	206	169	0.525	0.598	0.585
8	204	240	192	0.543	0.721	0.495	184	236	214	0.602	0.630	0.580
9	183	246	206	0.488	0.648	0.523	161	194	188	0.446	0.710	0.478
10	197	250	228	0.611	0.668	0.546	160	228	204	0.438	0.668	0.480
Mean	170.4	222.3	210.1	0.566	0.660	0.547	171.3	224.3	183.4	0.523	0.656	0.528
S.D.(±)	20.86	24.92	16.31	7.83	7.32	6.20	11.53	18.73	19.46	6.38	0.06	0.05
C.V. (%)	12.2	11.2	7.8	13.8	11.1	11.3	6.7	8.4	10.6	12.2	9.8	10.8
Overall Mean		200.9			0.592			193.0			0.569	
Overall SD (±)		30.30			0.08			28.28			0.08	
Overall CV (%)		15.1			14.4			14.6			15.2	
CD (5%)		13.62			0.04			11.00			0.04	

N = Normal D = Diabetic H = Hypertensive

Table 4.17 Analysis of variance for plasma cholesterol level of the subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Male				
Treatment	2	14728.467	7364.233	16.704*
Error	27	11903.400	440.867	
Total	29	26631.867	918.340	
Female				
Treatment	2	15427.400	7713.700	26.816*
Error	27	7766.600	287.652	
Total	29	23194.000	799.790	

* Significant at 5% level

Table 4.18 Analysis of variance for urinary creatinine level of the subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Male				
Treatment	2	0.073	0.036	7.128*
Error	27	0.138	5.120×10^{-3}	
Total	29	0.211	7.270×10^{-3}	
Female				
Treatment	2	0.114	0.057	14.907*
Error	27	0.103	3.812×10^{-3}	
Total	29	0.217	7.480×10^{-3}	

* Significant at 5% level

g/day (Oser, 1976). The results of the creatinine excretion in male and female subjects of the present study showed values well below the normal ranges specified. Mariyama *et al.*(1987) correlated urinary creatinine with age, height and weight. They further disclosed that weight alone or weight and height accounted for most of the variation in 24 hr. creatinine excretion. Bertrand *et al.*(1987) observed increased and decreased creatinine concentration with weight and urine volume, respectively. Michell and Lipschitz (1982) stated that creatinine excretion decreases with normal aging owing to the loss of lean body mass. This could be the possible reason for low creatinine excretion in case of diseased subjects in the present investigation. Oser (1976) also stated that in conditions of starvation and disorders associated with muscular atrophy and muscular weakness, the excretion of creatinine in urine decreased.

4.1.10 Serum zinc: In case of male subjects, significantly higher serum zinc concentration (µg/dl) was observed in diabetics (139.1 $\pm$ 6.08) as compared to the hypertensives (93.2 $\pm$ 10.99) and normals (87.8 $\pm$ 11.99).

In case of female subjects too, significant difference ($P< 0.05$) was found in the serum zinc concentration during the three conditions and the respective values for diabetics, normals and hypertensives were 168.5 $\pm$ 6.47, 97.1 $\pm$ 18.38 and 88.7 $\pm$ 6.41 µg/dl (Table 4.19).

Further analysis revealed statistically significant difference ($P < 0.05$) in male and female serum zinc levels in case of diabetics.

Serum zinc values obtained in case of normal and hypertensive male and female subjects resembled with the values reported by Monceda *et al.*(1989) and Meret and Henkin (1971). They reported the values of 84 $\pm$ 20 µg/dl and 92 $\pm$ 3 µg/dl, respectively for normal male and female subjects. Further, the serum zinc concentration was found to be higher in case of normal female subjects as compared to the male subjects in the present investigation. This was in contrast to the findings of Lindeman *et al.*(1971) and Rose *et al.*(1972) who reported higher serum zinc concentration in normal male subjects as compared to the female subjects.

Halsted *et al.*(1974) reported that age, stress and a variety of disease states affected the serum zinc level. In the present study, considerable difference in serum zinc level was found between diabetic and normal subjects. Martin Mateo *et al.*(1974) had also recorded higher content of serum zinc in diabetic patients in comparison to normal persons. They obtained the value of 138 and 176 µg/dl for diabetic men and women. The results obtained for diabetic men in the present finding agreed with this value while those for diabetic women were slightly lower than the above reported value. Martin Mateo *et al.*(1975) further observed higher serum zinc in female diabetics as compared to the male diabetics and our results are in line with their

observations. However, the results were in contrast to the observation of McNair et al.(1981) who found no difference in serum zinc between normal and diabetic and Kumar and Jaya Rao (1974) who reported significant reduction in serum zinc concentration during diabetes.

4.1.11 Saliva zinc: Mean saliva zinc level (ng/ml) in male subjects was highest for hypertensives (225.8 ± 51.67), intermediate for normals (208.8 ± 26.88) and lowest for diabetics (205.4 ± 33.61) but the difference was found to be non- significant.

Similar trend was seen in case of female subjects (Table 4.19) and the mean saliva zinc concentration (ng/ml) was higher in hypertensives (252.9 ± 51.76) and lower in diabetics (189.7 ± 40.66) as compared to normals (200.0 ± 19.16). However, non-significant difference in saliva zinc concentration was observed between normal and diabetic subjects.

The results of the present study for normal and diabetic subjects are comparable to the saliva zinc concentration of 190 ng/ml reported by Freeland-Graves et al.(1981) for adults. They also obtained the value of 266 ± 89 ng/ml for females which is in concurrence with the value obtained for hypertensive female subjects in the present finding. The saliva zinc level of hypertensive male subjects was much higher than the value reported by Freeland-Graves et al.(1981) of 190 ng/ml. They further suggested that mixed saliva was not a useful index of zinc status. Everett and

Table 4.19 Serum and saliva zinc level of normal (control) and diseased male and female subjects.

	Male						Female					
	Serum zinc (µg/dl)			Saliva zinc (ng/ml)			Serum zinc (µg/dl)			Saliva zinc (ng/ml)		
S.No	N	D	H	N	D	H	N	D	H	N	D	H
1	72.0	147.0	110.0	233.0	172.4	181.0	81.0	166.0	98.0	172.0	156.0	327.0
2	76.5	137.5	87.5	181.0	246.0	214.0	77.5	172.0	87.5	229.0	145.0	246.4
3	84.0	145.5	79.5	180.5	170.0	177.5	122.0	178.5	93.0	229.0	246.0	190.0
4	82.0	138.0	93.0	260.5	216.6	323.6	87.5	163.5	88.5	187.5	138.8	264.6
5	93.0	130.0	87.5	199.0	148.8	190.0	116.5	159.0	79.0	191.5	173.0	189.0
6	103.0	130.0	104.0	204.0	228.0	242.4	92.0	163.5	93.0	200.0	166.0	314.4
7	85.0	142.5	89.5	228.0	187.8	198.0	104.0	169.0	86.0	216.0	217.6	240.0
8	76.5	138.0	93.0	196.0	246.0	236.0	78.0	172.0	88.5	186.0	246.6	246.4
9	99.0	136.5	108.5	224.5	220.2	190.0	87.5	177.5	78.5	187.5	223.0	198.8
10	106.5	145.5	79.5	181.0	218.0	305.0	125.0	163.5	95.0	201.5	185.0	312.0
Mean	87.8	139.1	93.2	208.8	205.4	225.8	97.1	168.5	88.7	200.0	189.7	252.9
S.D.(±)	11.99	6.08	10.99	26.88	33.61	51.67	18.38	6.47	6.41	19.16	40.66	51.76
C.V. (%)	13.7	4.4	11.8	12.9	16.4	22.9	18.9	3.8	7.2	9.6	21.4	20.5
Overall Mean		106.7			213.3			118.1			214.2	
Overall SD (±)		25.32			38.54			38.14			47.44	
Overall CV (%)		23.7			18.1			32.3			22.2	
CD (5%)		6.5			N.S			7.68			25.68	

N = Normal D = Diabetic H = Hypertensive NS = Non-significant

Table 4.20 Analysis of variance for serum zinc level of the subjects

Source of variation	df	Sum of squares	Mean Squares	F value
Male				
Treatment	2	15878.717	7939.358	78.971*
Error	27	2714.450	100.535	
Total	29	18593.167	641.140	
Female				
Treatment	2	38404.817	19202.408	136.862*
Error	27	3788.225	140.305	
Total	29	42193.042	1454.930	

* Significant at 5% level

Table 4.21 Analysis of variance for saliva zinc level of the subjects

Source of variation	df	Sum of squares	Mean Squares	F value
Male				
Treatment	2	2384.313	1192.156	0.791
Error	27	40701.666	1507.469	
Total	29	43085.979	1485.720	
Female				
Treatment	2	22964.851	11482.425	7.328*
Error	27	42306.944	1566.924	
Total	29	65271.795	2250.750	

* Significant at 5% level

Apgar (1979) felt that the use of saliva as a diagnostic tool was complicated by the considerable variation in the composition of saliva that occurred within the same individual at different times. Dawes (1970) also stated that a number of factors such as the flow rate of saliva, the nature and duration of stimulation, the time of day, the degree of hydration of the subjects, and plasma composition could potentially influence the zinc content of saliva. The disparity observed in the saliva zinc concentration of the subjects in this study could be the result of any such factor.

4.1.12 Urinary zinc: Mean urinary zinc excretion (µg/24 hr) in case of male subjects was significantly higher in diabetics (944.3 ± 67.99) as compared to hypertensives (540.8 ± 51.89) and normals (519.8 ± 49.33). The difference between normal and hypertensive subjects was however non-significant.

Urinary zinc excretion (µg/24 hr) was similarly patterned in case of female subjects and the respective values for diabetic, hypertensive and normal subjects were 1034.8 ± 115.39, 541.1 ± 53.44 and 501.1 ± 47.27 (Table 4.22). Statistically, the difference ($P<0.05$) was again significant.

Difference between sexes was found to be statistically significant ($P<0.05$) in diabetics only.

The urinary zinc excretion in normal and hypertensive subjects was within the normal range of 400-600 µg/24 hr

given by Roman (1969). However, greater than normal urinary zinc excretion was seen in case of diabetics in the present study which is in agreement with the similar observation of Pai and Prasad (1988). The present study further revealed that urinary zinc excretion was much higher in case of female diabetics as compared to the male diabetics. This finding agreed with that of McNair *et al.* (1981) but was in contrast to the report of Martin Mateo *et al.*(1975). Schroeder (1957) had observed hyperzincuria in hypertensive patients too. But our results differed from the above observation as urinary zinc excretion in hypertensive patients was within the normal range specified by Roman (1969).

4.1.13 Hair Zinc: Mean hair zinc concentration (µg/g) was higher in hypertensive male subjects (136.2 $\pm$ 20.76) and lower in diabetics (116.4 $\pm$ 9.07) than the values for normals (135.9 $\pm$ 15.86) while the difference between hypertensive and normal subjects was non-significant.

Unlike males, in female subjects mean hair zinc concentration (µg/g) was significantly higher in normal subjects (145.5 $\pm$ 17.38) than both diabetics (126.2 $\pm$ 9.14) and hypertensives (128.1 $\pm$ 7.50). However, the difference betweeen diabetic and hypertensive subjects was non-significant (Table 4.22).

Further analysis revealed statistically significant difference ($P<0.05$) between male and female values in case of diabetic subjects.

Table 4.22 Urinary and hair zinc level of normal (control) and diseased male and female subjects.

	Male						Female					
	Urinary zinc (µg/24hr)			Hair zinc (µg/g)			Urinary zinc (µg/24hr)			Hair zinc (µg/g)		
S.No	N	D	H	N	D	H	N	D	H	N	D	H
1	425.0	913.5	549.4	112.0	121.6	106.0	428.0	1225.0	465.4	139.0	132.0	127.0
2	482.5	853.0	436.2	134.0	105.0	121.0	540.5	957.0	522.0	165.5	132.4	136.4
3	591.0	896.0	590.0	153.6	127.0	155.0	499.0	989.0	549.4	119.6	119.0	119.6
4	529.0	1036.5	495.0	158.0	103.6	162.4	480.0	887.0	636.8	165.0	105.0	136.4
5	567.0	868.0	555.0	134.0	125.5	160.0	512.0	1142.5	523.6	123.6	134.0	139.2
6	552.0	1015.0	580.0	155.0	117.0	116.2	438.0	970.0	482.2	133.4	127.0	120.0
7	535.5	992.0	486.6	131.6	121.6	123.0	469.0	1104.0	515.0	165.0	125.5	119.6
8	490.0	886.0	590.5	118.8	110.5	145.0	522.5	896.0	547.0	158.8	132.0	132.2
9	482.5	1008.2	545.5	122.0	125.0	121.0	560.0	1025.5	617.0	139.0	121.5	124.0
10	544.0	975.0	580.0	140.0	107.0	152.0	562.0	1152.0	552.6	146.0	134.0	127.0
Mean	519.8	944.3	540.8	135.9	116.4	136.2	501.1	1034.8	541.1	145.5	126.2	128.1
S.D.(±)	49.33	67.99	51.89	15.86	9.07	20.76	47.27	115.39	53.44	17.38	9.14	7.50
C.V. (%)	9.5	7.2	9.6	11.7	7.8	15.3	9.4	11.2	9.90	11.9	7.2	5.9
Overall Mean		668.3			129.5			692.3			133.3	
Overall SD (±)		206.14			18.06			258.17			14.65	
Overall CV (%)		30.8			13.9			37.3			10.9	
CD (5%)		36.39			10.36			50.83			7.87	

N = Normal D = Diabetic H = Hypertensive

Table 4.23 Analysis of variance for urine zinc level of the subjects

Source of variation	df	Sum of squares	Mean Squares	F value
Male				
Treatment	2	1144645.513	572322.756	176.081*
Error	27	87759.261	3250.343	
Total	29	1232404.774	42496.710	
Female				
Treatment	2	1767251.267	883625.633	144.012*
Error	27	165665.720	6135.767	
Total	29	1932916.987	66652.300	

* Significant at 5% level

Table 4.24 Analysis of variance for hair zinc level of the subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Male				
Treatment	2	2574.488	1287.244	5.046*
Error	27	6888.140	255.116	
Total	29	9462.628	326.297	
Female				
Treatment	2	2250.650	1125.325	7.638*
Error	27	3977.977	147.332	
Total	29	6228.627	214.780	

* Significant at 5% level

The hair zinc levels obtained in the present study were within the range of 92-255 ug/g as specified by Smith (1967) for normal subjects. However, the values obtained for both male and female subjects were much lower than the mean 167 ± 5 ug/g and 172 ± 9 ug/g for healthy males and females reported by Schroeder and Nason (1969).

Further observation disclosed that the hair zinc concentration in female diabetics was higher than that in male diabetics in the present study. This is in line with the findings of Canfield *et al.*(1984). Significant difference was also seen in the hair zinc concentration of normal and diabetic subjects in the present investigation. This finding was in contrast to that of Hagglof *et al.*(1983) who did not find zinc in hair to differ significantly between the diabetic and controls.

4.2 DURING DIFFERENT PHYSIOLOGICAL STAGES (Pregnancy and lactation):

4.2.1 Age, height and weight: The mean age (years) of the female subjects during first, second and third trimester of pregnancy and lactation were 22.7 ± 1.76, 21.7 ± 1.94, 23.3 ± 2.16 and 25.5 ± 1.43, respectively. The overall coefficient of variation emerged out to be 6.41 per cent indicating only small disparity in the ages of the subjects (Table 4.25).

Height and weight: Non-significant difference was observed in the heights of the subjects and the corresponding values for normal (control) women, pregnant women in first, second

Table 4.25 Age (years) of the experimental female subjects during physiological conditions.

S.No	Control	Pregnancy (in trimester) Ist	2nd	3rd	Lactation
1	25	20	23	28	24
2	26	22	19	23	25
3	23	24	19	26	25
4	24	22	21	24	23
5	24	21	25	23	26
6	27	25	23	22	28
7	26	24	22	22	26
8	24	25	22	22	27
9	22	23	20	21	26
10	25	21	23	22	25
Mean	24.6	22.7	21.7	23.3	25.5
S.D.(±)	1.50	1.76	1.94	2.16	1.43
C.V. (%)	6.1	7.8	8.9	9.3	5.6
Overall Mean			23.6		
Overall SD (±)			1.51		
Overall CV (%)			6.4		

and third trimester of pregnancy and lactating women were 152.7 ± 2.46, 153.1 ± 2.66, 151.8 ± 1.76, 151.5 ± 2.09 and 153.9 ± 3.22 cms, respectively (Table 4.26).

However, significant difference ($P < 0.05$) was seen in the weights of the subjects and the respective values for control, pregnant women in three trimesters of pregnancy and lactating women were 46.2 ± 1.75, 48.0 ± 2.66, 46.9 ± 2.58, 56.6 ± 3.62 and 54.8 ± 2.89 kg, respectively.

The findings of the present study revealed lower weight for height in the female subjects in comparison to the standard of 50 kg weight for 151 cm height reported by ICMR (1990). Kochar and Renu (1991) also observed lower weights for heights in rural females of Kangra valley. National Nutrition Monitoring Bureau (1980) in its report stated that the inhabiting environment affected height and weight of the population. The low body weights for height recorded in the present study could be due to the genetics of the population, coupled with their eating practices and effect of environment as well as topography, and malnutrition.

4.2.2 Body Mass Index (BMI): Mean value of BMI (Kg/m^2) was significantly ($P < 0.05$) higher during third trimester of pregnancy and lactation in comparison to those recorded for control and pregnant women in first and second trimester of pregnancy. The values for normal, three trimesters of pregnancy and lactation were 19.84 ± 0.79, 20.46 ± 0.72, 20.36 ± 1.0, 24.62 ± 1.38, 23.13 ± 0.89, respectively (Table 4.29).

Table 4.26 Height (cm) and weight (kg) of the experimental female subjects during physiological conditions.

	Height					Weight				
		Pregnancy (in trimester)					Pregnancy (in trimester)			
S.No	Control	Ist	2nd	3rd	Lactation	Control	Ist	2nd	3rd	Lactation
1	152.0	150.0	150.0	147.5	152.5	48	43	48	57	55
2	155.0	156.2	152.5	152.0	155.0	49	48	44	58	52
3	150.0	152.5	152.0	150.0	155.0	45	47	45	57	52
4	150.0	150.5	150.0	153.5	150.0	44	48	49	60	53
5	152.5	155.0	155.0	152.5	157.5	47	50	52.5	60	58
6	152.5	152.0	150.0	155.0	155.0	48	46	46	62	58
7	157.0	150.0	153.0	150.0	150.0	46	46	48	54	54
8	150.0	157.5	152.0	152.0	152.0	46	52	46	54.5	52
9	155.5	155.0	150.0	152.0	160.0	45	51	44	52	60
10	152.0	152.5	153.5	150.5	152.0	44	49	47	51	54
Mean	152.7	153.1	151.8	151.5	153.9	46.2	48.0	46.9	56.6	54.8
S.D.(±)	2.46	2.66	1.76	2.09	3.22	1.75	2.66	2.58	3.62	2.89
C.V. (%)	1.6	1.7	1.2	1.4	2.1	3.8	5.6	5.5	6.4	5.3
Overall Mean			152.5					50.5		
Overall SD (±)			2.54					5.09		
Overall CV (%)			1.7					10.1		
CD (5%)			N.S					1.76		

NS = Non-significant

Table 4.27 Analysis of variance for height of the female subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Treatment	4	38.127	9.532	1.534
Error	45	279.621	6.214	
Total	49	317.748	6.484	

* Significant at 5% level

Table 4.28 Analysis of variance for weight of the female subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Treatment	4	924.350	231.088	30.085*
Error	45	345.650	7.681	
Total	49	1270.000	25.910	

* Significant at 5% level

The present investigation revealed that the BMI values obtained for control and pregnant women in first two trimesters of pregnancy were lower than the standard 21.93 kg/m² given by ICMR (1990) while those for lactating and pregnant women in third trimester of pregnancy higher than this standard. However all the values were within the range of 19-27 kg/m² specified by Shils and Young (1988) for adults. Cronk and Roche (1982) and Rolland-Cachera et al.(1982) had also indicated that BMI was not always an accurate index of body composition. This was because of the fact that despite differences in body fat, the average index was about the same during young adult years. The mean values obtained for BMI in the present investigation were below the lowest BMI value of 25 kg/m² above which obesity starts (Garrow, 1981). The low BMI values recorded for control and pregnant women in first and second trimester of pregnancy were probably due to the lower body weights of these subjects. Barring controls, the mean BMI values for all the other subjects were also within the Indian standards of 20-25 kg/m² for normal women (Nutrition News, 1991).

4.2.3 Basal Metabolic Rate (BMR): Significantly ($P < 0.05$) higher BMR (Kcal/24 hrs) was observed in lactating and pregnant women in third trimester of pregnancy compared to control and pregnant women in first and second trimester of pregnancy. The respective values for control, lactating and pregnant women in three trimesters of pregnancy were 1117.8

Table 4.29 Body Mass Index (BMI) and Basal Metabolic Rate (BMR) of the experimental female subjects during physiological conditions.

	BMI (kg/m^2)					BMR (kcal/24 hr)				
		Pregnancy (in trimester)					Pregnancy (in trimester)			
S.No	Control	Ist	2nd	3rd	Lactation	Control	Ist	2nd	3rd	Lactation
1	20.78	19.11	21.33	26.20	23.65	1143	1073	1143	1269	1241
2	20.39	19.68	18.92	25.10	21.64	1157	1143	1087	1283	1199
3	20.00	20.21	19.47	25.33	21.64	1101	1129	1101	1269	1199
4	19.55	21.19	21.77	25.46	23.55	1087	1143	1157	1311	1213
5	20.21	20.81	21.85	25.80	23.38	1129	1171	1206	1311	1283
6	20.64	19.91	20.44	25.80	24.14	1143	1115	1115	1339	1283
7	18.66	20.44	20.50	24.00	24.00	1115	1115	1143	1227	1227
8	20.44	20.96	19.91	23.58	22.50	1115	1199	1115	1234	1199
9	18.73	21.23	19.55	22.50	23.43	1101	1185	1087	1199	1311
10	19.04	21.07	19.95	22.51	23.37	1087	1157	1129	1185	1227
Mean	19.84	21.46	20.36	24.62	23.13	1117.8	1143.0	1128.3	1262.7	1238.2
S.D.(±)	0.79	0.72	1.00	1.38	0.89	24.51	37.33	36.21	50.74	40.57
C.V. (%)	4.0	3.6	4.9	5.6	3.9	2.2	3.3	3.2	4.0	3.3
Overall Mean			21.68					1178.0		
Overall SD (±)			2.10					71.27		
Overall CV (%)			9.7					6.1		
CD (5%)			0.63					24.72		

Table 4.30 Analysis of variance for body mass index of the female subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Treatment	4	173.686	43.421	44.576*
Error	45	43.835	0.974	
Total	49	217.52	4.439	

* Significant at 5% level

Table 4.31 Analysis of variance for basal metabolic rate of the female subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Treatment	4	181172.600	45293.150	30.085*
Error	45	67747.400	1505.498	
Total	49	248920.000	5080.000	

* Significant at 5% level

± 24.51, 1238.2 ± 40.57, 1143.0 ± 37.33, 1128.3 ± 36.21 and 1262.7 ± 50.74 kcal/24 hrs. (Table 4.29).

Robinson and Lawler (1982) stated that BMR is influenced by weight, age and sex of the individual. They further observed 15-25 per cent increase in basal metabolism during the third trimester of pregnancy as a result of increase in the body weight of the women and the high rate of metabolism of the foetus. The significantly higher BMR recorded in pregnant women in the last trimester of pregnancy in the present study agreed with the reports of Robinson and Lawler (1982). Passmore and Eastwood (1986) stated that the type of food eaten and climatic and racial differences also influenced BMR. It was also reported by Robinson and Lawler (1982) that protein when eaten alone increased BMR by 30 per cent, whereas carbohydrates and fats produced much smaller increase in metabolism.

4.2.4 Haemoglobin (Hb): Mean Hb level (g/dl) was recorded highest in control women (11.4 ± 0.23) followed by lactating (11.3 ± 0.30) and pregnant women in third trimester (10.7 ± 0.52), second trimester (10.4 ± 0.65) and first trimester (10.4 ± 0.60) of pregnancy (Table 4.32). Difference was statistically significant ($P < 0.05$).

The Hb levels of the subjects in the present study were much below the range of 13.5-15.0 g/dl given by Oser (1976). Further observation revealed that the concentration of Hb in the blood of pregnant women was much lower than in control women in the present investigation. This was in agreement

Table 4.32 Haemoglobin (Hb) level of the experimental female subjects during physiological conditions.

S.No	Control	Pregnancy (in trimester) Ist	2nd	3rd	Lactation
1	11.2	10.1	9.6	10.0	11.8
2	11.6	10.8	10.8	10.6	11.5
3	11.0	9.9	11.0	11.0	11.5
4	11.6	11.0	11.2	11.4	11.6
5	11.4	10.4	11.5	11.2	11.2
6	11.2	9.5	10.0	11.0	11.0
7	11.4	10.6	9.9	11.2	11.2
8	11.5	11.5	10.5	10.8	10.9
9	11.4	10.4	9.8	10.3	11.0
10	11.8	9.8	10.2	9.9	11.5
Mean	11.4	10.4	10.4	10.7	11.3
S.D.(±)	0.23	0.60	0.65	0.52	0.30
C.V. (%)	2.0	5.8	6.2	4.9	2.7
Overall Mean			10.9		
Overall SD (±)			0.63		
Overall CV (%)			5.9		
CD (5%)			0.31		

Table 4.33 Analysis of variance for blood haemoglobin level of the female subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Treatment	4	9.081	2.270	9.413*
Error	45	10.854	0.241	
Total	49	19.935	0.406	

* Significant at 5% level

with the observation of Swaminathan (1990). WHO study group (1959) had suggested that a haemoglobin concentration of less than 10 g/dl during pregnancy was indicative of anaemia. Following this criteria, the pregnant women in the present study could be classified as marginally anaemic. Marginally low content of Hb among the rural females of Kangra valley was also reported by Kochar and Renu (1991). Swaminathan (1988) had also reported that Hb level of blood was a reliable index of overall state of nutrition in addition to its diagnostic importance in anaemia. Szewczynski (1979) also stressed the importance of eating foods of high nutritive value on the Hb concentration. The low levels of Hb recorded in the present study could be due to poor eating practices and nutritional ignorance.

4.2.5 Plasma protein: Non-significant difference was observed in the plasma protein concentration of the subjects. The mean values recorded for controls, lactating and pregnant women in the three trimesters of pregnancy were 7.16 $\pm$ 0.53, 7.52 $\pm$ 0.78, 7.50 $\pm$ 0.72, 7.67 $\pm$ 0.83 and 7.63 $\pm$ 0.51 g/dl, respectively.(Table 4.34).

Barring one subject each in first and second trimester of pregnancy and during lactation, all the values obtained for plasma protein were within the normal range of 6-8 g/dl given by Oser (1976). Swaminathan (1990) reported that total serum protein falls during pregnancy which was not in line with the findings of the present study as normal

Table 4.34 Plasma protein and glucose level of the experimental female subjects during physiological conditions.

	Plasma protein (g/dl)					Plasma glucose (mg/dl)				
		Pregnancy (in trimester)					Pregnancy (in trimester)			
S.No	Control	Ist	2nd	3rd	Lactation	Control	Ist	2nd	3rd	Lactation
1	6.70	9.15	7.15	8.05	8.15	96	87.4	89	100	108.0
2	6.80	7.50	7.80	7.55	6.80	86	92.0	105	94	97.5
3	8.20	7.30	8.60	7.30	6.75	105	110.5	125	100	94.0
4	7.50	6.65	9.15	7.80	7.40	94	94.0	110	91	94.0
5	7.40	8.05	6.65	8.60	7.15	88	100.0	120	100	88.5
6	6.85	7.15	7.25	7.80	9.05	92	105.0	130	118	105.0
7	7.20	7.80	7.30	6.65	8.26	96	89.5	94	105	100.0
8	7.70	6.65	6.60	7.80	6.60	89	96.8	110	94	120.0
9	6.50	7.30	8.05	7.30	7.80	96	96.0	84	94	98.0
10	6.80	7.50	8.15	7.50	7.30	94	88.7	87	110	87.0
Mean	7.16	7.50	7.67	7.63	7.52	93.6	95.9	105.4	100.6	99.2
S.D.(±)	0.53	0.72	0.83	0.51	0.78	5.37	7.43	16.46	8.39	9.80
C.V. (%)	7.4	9.7	10.8	6.8	10.4	5.7	7.7	15.6	8.3	9.9
Overall Mean			7.50					98.9		
Overall SD (±)			0.68					10.61		
Overall CV (%)			9.1					10.7		
CD (5%)			N.S					N.S		

NS = Non-significant

Table 4.35 Analysis of variance for plasma protein level of the fe
subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Treatment	4	1.601	0.400	0.839
Error	45	21.455	0.477	
Total	49	23.055	0.470	

* Significant at 5% level

Table 4.36 Analysis of variance for plasma glucose level of the fem
subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Treatment	4	817.713	204.428	1.958
Error	45	4698.689	104.415	
Total	49	5516.402	112.580	

* Significant at 5% level

concentration of plasma protein was recorded in the pregnant subjects.

4.2.6 Plasma glucose: The mean plasma glucose level (mg/dl) of the control, lactating and pregnant women in three trimesters of pregnancy was 93.6 ± 5.37, 99.2 ± 9.8, 95.9 ± 7.43, 105.4 ± 16.46 and 100.6 ± 8.39, respectively (Table 4.34). Non-significant difference was observed in the plasma glucose level of the subjects.

Barring one subject in second trimester of pregnancy who was mildly diabetic, all the subjects had plasma glucose concentration within the normal range of 90-120 mg/dl (Oser, 1976).

4.2.7 Plasma cholesterol: Mean plasma cholesterol level (mg/dl) was significantly ($P<0.05$) higher in case of control, lactating and pregnant women in second and third trimester of pregnancy as compared to pregnant women in first trimester of pregnancy. The corresponding values were 171.3 ± 11.53, 183.4 ± 26.03, 181.3 ± 28.46, 180.6 ± 36.42 and 142.2 ± 11.09 mg/dl, respectively (Table 4.37).

Most of the subjects in the present study had plasma cholesterol concentration within the normal range of 150-250 mg/dl specified by Oser (1976). However, seven out of ten pregnant subjects in first trimester of pregnancy had below normal values. This was in contrast to the report of Swaminathan (1990) of an increase in plasma cholesterol level during pregnancy.

4.2.8 Urinary creatinine: Maximum urinary creatinine excretion (g/day) was recorded for lactating women (0.544 ± 6.10) followed by normal subjects (0.523 ± 6.38) and pregnant subjects in third (0.497 ± 9.18), second (0.474 ± 0.103) and first (0.455 ± 0.101) trimester of pregnancy (Table 4.37). Non-significant difference was, however, observed in the urinary creatinine excretion of the subjects in the selected conditions.

The normal range of urinary output of creatinine in case of adult women is reported as 0.8-1.5 g/day (Best and Taylor, 1969) and in case of adults, the specified range is 1-1.5 g/day (Oser, 1976). The results of the creatinine excretion in female subjects of the present study showed that all had values well below the normal ranges specified above. Renu (1990) reported similar findings of lower than normal creatinine excretion in rural females of Kangra Valley in H.P. Mariyama *et al.*(1987) and Bertrand *et al.* (1987) correlated variation in urinary creatinine excretion with weight. The lower body weights recorded in the present study for controls and during physiological conditions might be responsible for the lower than normal urinary creatinine excretion in the rural females selected for this finding.

4.2.9 Serum zinc: Significant difference ($P<0.05$) was seen in the serum zinc concentrations of the studied subjects. The mean serum zinc concentrations (µg/dl) in case of control, lactating and pregnant women in three trimesters of

Table 4.37 Plasma cholesterol and urinary creatinine level of the experimental female subjects during physiological conditions.

	Plasma cholesterol (mg/dl)					Urinary creatinine (g/day)				
		Pregnancy (in trimester)					Pregnancy (in trimester)			
S.No	Control	1st	2nd	3rd	Lactation	Control	1st	2nd	3rd	Lactation
1	188	134	149	183	185	0.542	0.621	0.502	0.452	0.605
2	172	148	178	154	185	0.612	0.447	0.376	0.415	0.627
3	180	154	153	210	230	0.593	0.408	0.353	0.512	0.574
4	160	138	204	172	192	0.466	0.518	0.485	0.367	0.495
5	176	124	184	161	155	0.495	0.579	0.612	0.615	0.485
6	155	142	234	142	174	0.515	0.360	10.625	0.580	0.488
7	177	134	216	136	215	0.525	0.314	0.354	0.495	0.640
8	184	160	156	240	149	0.602	0.439	0.548	0.605	0.521
9	161	152	172	234	157	0.446	0.514	0.505	0.378	0.495
10	160	136	167	174	192	0.438	0.354	0.386	0.556	0.514
Mean	171.3	142.2	181.3	180.6	183.4	0.523	0.455	0.474	0.497	0.544
S.D.(±)	11.53	11.09	28.46	36.42	26.03	6.380	0.101	0.103	9.180	6.100
C.V. (%)	6.7	7.8	15.7	20.2	14.2	12.2	22.2	21.7	18.5	11.2
Overall Mean			171.8					0.499		
Overall SD (±)			28.36					0.088		
Overall CV (%)			16.5					17.8		
CD (5%)			15.79					N.S		

NS = Non-significant

Table 4.38 Analysis of variance for plasma cholesterol level of the female subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Treatment	4	11786.520	2946.630	4.79*
Error	45	27632.600	614.058	
Total	49	39419.12	804.47	

* Significant at 5% level

Table 4.39 Analysis of variance for urinary creatinine levels of the female subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Treatment	4	0.052	0.013	1.737
Error	45	0.334	7.418×10^{-3}	
Total	49	0.385	7.850×10^{-3}	

* Significant at 5% level

pregnancy were 97.1 ± 18.38, 58.2 ± 7.99, 102.6 ± 9.37, 74.6 ± 6.03 and 65.5 ± 7.17, respectively (Table 4.40).

In the present study, the mean serum zinc concentration in control women was comparable to the mean values reported by Argemi *et al.*(1988) of 93.71 ± 7.34 µg/dl, Underwood (1971) of 0.96 ± 0.13 µg/ml and Gofman *et al.*(1964) of 98 ± 2 µg/dl. Further observation revealed that the mean serum zinc concentration in the first trimester of pregnancy was slightly higher than in control women. This was in contrast to the observation of Vir *et al.*(1981) who found significantly lower mean serum zinc level in pregnant subjects as compared to non-pregnant. The results obtained for the the second and third trimester of pregnancy were, however, in agreement with the above observation. It was further noticed that there was a significant decline in the mean serum zinc concentration with increasing duration of pregnancy in the present study. This finding was in concurrence with the reports of Jameson (1976), Vir *et al.* (1981) and Argemi *et al.*(1988). The values obtained for mean serum zinc in first and second trimester of pregnancy were lower than those reported by Mittal *et al.*(1982) for Indian women *i.e.* 109.7 and 83.7 µg/dl, respectively for first and second trimester. However, the values of mean serum zinc recorded during the second and third trimester of pregnancy were in close association with those reported by Argemi *et al.*(1988) of 75.36 ± 17.61 and 70.46 ± 13.28 µg/dl, respectively. Mean serum zinc level in case of lactating

Table 4.40 Serum and saliva zinc level of the experimental female subjects during physiological conditions.

	Serum zinc (µg/dl)					Saliva zinc (ng/ml)				
		Pregnancy (in trimester)					Pregnancy (in trimester)			
S.No	Control	Ist	2nd	3rd	Lactation	Control	Ist	2nd	3rd	Lactation
1	81.0	102.5	72.5	70.5	62.0	172.0	190.8	302.0	226.5	208.0
2	77.5	112.5	78.0	63.5	55.5	229.0	266.0	188.0	198.5	194.5
3	122.0	112.5	77.5	74.0	50.0	229.0	233.0	202.5	279.0	242.0
4	87.5	96.0	69.0	67.5	68.0	187.5	196.5	269.0	245.6	229.0
5	116.5	87.0	66.0	60.5	63.5	191.5	315.0	349.0	183.0	183.0
6	92.0	99.5	68.5	60.5	71.0	200.0	372.8	197.6	302.0	187.5
7	104.0	97.0	82.5	50.0	54.5	216.0	343.0	173.0	241.8	222.0
8	78.0	115.0	78.0	72.0	60.0	186.0	212.8	413.8	268.0	242.0
9	87.5	109.5	70.5	70.5	47.5	187.5	178.0	344.5	348.5	226.5
10	125.0	94.5	83.0	65.5	50.0	201.5	194.0	281.5	187.0	179.0
Mean	97.1	102.6	74.6	65.5	58.2	200.0	250.2	272.1	247.9	211.4
S.D.(±)	18.38	9.37	6.03	7.17	7.99	19.16	70.37	81.31	52.99	24.14
C.V. (%)	18.93	9.13	8.10	10.95	13.74	9.58	28.12	29.88	21.37	11.42
Overall Mean			79.6					236.3		
Overall SD (±)			20.39					59.49		
Overall CV (%)			25.6					25.2		
CD (5%)			6.85					35.27		

Table 4.41 Analysis of variance for serum zinc level of the female subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Treatment	4	15189.330	3797.333	32.87*
Error	45	5198.35	115.519	
Total	49	20387.680	416.070	

* Significant at 5% level

Table 4.42 Analysis of variance for saliva zinc level of the female subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Treatment	4	35507.019	8876.755	2.896
Error	45	137915.952	3064.799	
Total	49	173422.971	3539.240	

* Significant at 5% level

women also compared well with the value 56.0 ± 0.23 µg/ml reported by Vir *et al.*(1981) during the post partum period.

4.2.10 Saliva zinc: Significantly (P<0.05) higher saliva zinc levels were observed in pregnant women as compared to the control and lactating women while non-significant difference was present during the three trimesters of pregnancy. The mean values of saliva zinc (ng/ml) in descending order were 272.1 ± 81.31, 250.2 ± 70.37, 247.9 ± 52.99, 211.4 ± 24.14 and 200.0 ± 19.16, respectively, for pregnant women in second, first and third trimester and lactating and control women (Table 4.40).

Freeland-Graves *et al.*(1981) reported the mean saliva zinc level in adult female subjects to be 266 ± 89 ng/ml and for healthy adults to be 190 ng/ml. The mean values for saliva zinc obtained in the present study were found to range between the values given by Freeland-Graves *et al.* (1981) for healthy adults and female subjects.

4.2.11 Urinary zinc: Mean urinary zinc excretion (µg/24 hrs) was significantly (P<0.05) higher in control, lactating and pregnant women in third trimester of pregnancy than pregnant women in first and second trimester of pregnancy. The difference in urinary zinc excretion between the first and second trimester of pregnancy was also significant (P<0.05). The respective values for control, lactating and pregnant women in first, second and third trimester of pregnancy were 501.1 ± 47.28, 510.8 ± 55.08, 413.6 ± 17.14, 469.9 ± 43.89 and 519.3 ± 56.04 µg/24 hrs (Table 4.43).

The mean values for urinary zinc obtained in the present study were well within the normal range of 400-600 µg/24 hr given by Roman (1969). Underwood (1971) had also reported that quantity of zinc excreted in the urine of healthy adults is very small *i.e.* 0.1-0.7 mg/day. Campbell *et al.*(1984) reported variation in urinary zinc excretion with stage of pregnancy. This was found to be true in the present investigation as there was gradual increase in mean urinary zinc excretion with duration of pregnancy. The results of the present study also resembled those of Hambidge *et al.*(1983) who stated that during the first trimester, pregnant women excreted less urinary zinc than controls. Swanson and King (1982) observed more excretion of zinc in urine by pregnant women in late pregnancy as compared to non-pregnant controls. In the present study also, although non-significant difference was observed in the mean urinary zinc excretion in case of normal and pregnant women in the third trimester, the latter had slightly higher value as compared to the control women.

4.2.12 Hair zinc: Significant difference ($P<0.05$) was observed in the hair zinc level of the subjects in the selected conditions. The mean hair zinc levels (µg/g) in ascending order were 145.5 $\pm$ 17.38, 165.7 $\pm$ 5.49, 169.9 $\pm$ 10.18, 180.5 $\pm$ 6.01 and 184.9 $\pm$ 10.39, respectively, for controls, pregnant women in third trimester, lactating women and pregnant women in second and first trimester of pregnancy (Table 4.43).

Table 4.43 Urinary and hair zinc level of the experimental female subjects during physiological conditions.

	Urinary zinc (µg/24 hr)					Hair zinc (µg/g)				
		Pregnancy (in trimester)					Pregnancy (in trimester)			
S.No	Control	1st	2nd	3rd	Lactation	Control	1st	2nd	3rd	Lactation
1	428.0	402.0	398.2	485.4	528.0	139.0	176.0	181.0	168.5	175.0.
2	540.5	418.8	416.0	525.0	533.5	165.5	170.5	189.5	160.0	162.5
3	499.0	429.5	456.5	529.5	459.0	119.6	188.6	177.5	172.5	181.0
4	480.0	398.2	502.0	602.0	487.4	165.0	182.5	172.8	170.0	184.0
5	512.0	398.2	463.5	478.4	598.0	123.6	178.8	181.0	168.5	163.5
6	438.0	423.0	463.5	480.0	528.0	133.4	183.0	183.0	162.2	156.5
7	469.0	449.5	528.6	508.6	447.0	165.0	208.0	182.5	162.2	160.0
8	522.7	402.0	532.8	595.5	422.5	158.8	192.5	174.0	158.5	163.5
9	560.0	416.6	486.0	424.4	556.0	139.0	188.6	174.0	161.0	170.0
10	562.0	398.2	451.5	564.0	549.0	146.0	180.4	189.5	173.5	183.0
Mean	501.1	413.6	469.9	519.3	510.8	145.5	184.9	180.5	165.7	169.9
S.D.(±)	47.28	17.14	43.89	56.04	55.08	17.38	10.39	6.01	5.49	10.18
C.V. (%)	9.43	4.14	9.34	10.79	10.78	11.94	5.62	3.33	3.31	5.99
Overall Mean			482.9					169.3		
Overall SD (±)			58.86					17.32		
Overall CV (%)			12.2					10.2		
CD (5%)			29.38					6.86		

Table 4.44 Analysis of variance for urinary zinc level of the female subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Treatment	4	74086.400	18521.600	8.711*
Error	45	95684.020	2126.312	
Total	49	169770.420	3464.70	

* Significant at 5% level

Table 4.45 Analysis of variance for hair zinc level of the female subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Treatment	4	9483.482	2370.871	20.428*
Error	45	5222.723	116.061	
Total	49	14706.205	300.126	

* Significant at 5% level

The results recorded were within the normal range reported by Smith (1967) of 92-255 µg/g for adults.

The results of the present study disclosed a progressive decline in the mean hair zinc level of the pregnant subjects with advancing pregnancy and the decline between second and third trimester was significant. This finding is in total agreement with those of Vir *et al.* (1981). The values for hair zinc recorded during the second and third trimester of pregnancy in the present investigation were also comparable to those given by Vir *et al.* (1981) of 183 ± 26 and 170 ± 26 µg/g during the second and third trimester of pregnancy. Kohrs *et al.*(1977) found highest concentration of hair zinc in the third trimester of pregnancy. The results of the present study were in complete contrast to the above finding as mean hair zinc concentration was found to be lowest in the last trimester of pregnancy in comparison with the first and second trimester.

4.2.13 Milk zinc: The mean milk zinc level (µg/dl) of lactating subjects was 311.5 ± 84.22 (Table 4.46). The coefficient of variation came out to be 27.03 per cent indicating considerable disparity in the milk zinc concentration of the female subjects under study.

Underwood (1971) gave the normal range of milk zinc to the tune of 300-500 µg/dl in lactating women. The mean value of milk zinc obtained in the study is within this specified range but inclines more towards the lower limit. Five out of

Table 4.46 Breast milk zinc concentration of the lactating subjects

S.No	Milk zinc (µg/dl)
1	228.0
2	200.5
3	395.0
4	453.0
5	312.2
6	231.6
7	289.0
8	277.0
9	320.4
10	408.4
Mean	311.5
S.D.(±)	84.22
CV (%)	27.0

Table 4.47 Age (years) of a controls and adolescent (menstruating) female sub

Control	Menstruating
25	18
26	18
23	17
24	19
24	19
27	18
26	19
24	18
22	17
25	17
24.6	18.0
1.50	0.81
6.1	4.5
Overall Mean	21.3
Overall SD (±)	4.66
Overall CV (%)	21.8

ten individual values, however, were lower than 300 ug/dl. Krebs and Hambidge (1986) reported progressive decline in human milk zinc with duration of lactation. This might possibly be the reason for lower than normal values obtained for some of the subjects.

4.3 DURING MENSTRUAL CYCLE PHASES:

4.3.1. Age, Height and weight:

Age: The mean ages of adult controls and menstruating adolescent subjects were 24.6 ± 1.50 and 18.0 ± 0.81 years, respectively. The overall coefficient of variation emerged out to be 21.8 per cent showing heterogenity in the ages of the subjects (Table 4.47).

Height and Weight: Non-significant difference was observed in both heights and weights of the subjects. The mean height (cm) of adult controls and adolescent subjects was 152.6 ± 2.46 and 154.5 ± 3.07, respectively. The corresponding values for weight (kg) were 46.2 ± 1.75 and 45.8 ± 1.31 (Table 4.48).

The results of the study indicated lower weight for height in female subjects as compared to the ICMR (1990) standard of 50 kg weight for 151 cm height for adult women. Similar findings were made by Kochar and Renu (1991) in case of rural adolescent females of Kangra valley. Qumra *et al.*(1990) stated that socio-economic status and dietary intake had a significant effect on the physical growth of adolescent girls. Poor dietary patterns and nutritional

ignorance might be responsible for the lower than normal weights recorded in the present study.

4.3.2 Body Mass Index (BMI): The mean BMI (kg/m^2) of adult controls and menstruating adolescent subjects was 19.84 $\pm$ 0.79 and 19.20 $\pm$ 0.97 respectively (Table 4.48). Non-significant difference was observed in the BMI values of the female subjects.

The findings of the present study revealed that the mean BMI values were less than the Indian standards i.e. 20-25 kg/m^2 for adult women (Nutrition News, 1991). However, in case of adolescent girls, the results are comparable with Gopalan (1989) who reported BMI values for girls in Delhi as 19.45-21.0 kg/m^2. Values of BMI less than 25 kg/m^2 in girls was also recorded by Goni *et al.*(1989) and in rural girls of Kangra Valley by Kochar and Renu (1991). The mean values of BMI recorded in the present study were well below the lowest BMI value of 25 kg/m^2 above which obesity starts (Garrow, 1981).

4.3.3 Basal Metabolic Rate (BMR): Non-significant difference was observed in the BMR values of the female subjects. The mean value (Kcal/24 hr) in case of adult controls and menstruating adolescent subjects was 1117.8 $\pm$ 24.51 and 1112.2 $\pm$ 18.43, respectively (Table 4.48).

The BMR values obtained in the present study were much lower than the normal values reported by Best and Taylor (1969). They explained and related the below normal BMR values to the conditions of starvation and undernutrition.

Table 4.48 Height, weight, Body Mass Index (BMI) and Basal Metabolic Rate (BMR) of adult controls and adolescent (menstruating) subjects.

	Height (cm)		Weight (kg)		BMI (kg/m²)		BMR (Kcal/24 hr)	
S.No	Control	Menstrual cycle	Control	Menstrual cycle	Control	Menstrual cycle	Control	Menstrual cycle
1	152.0	150.0	48	46	20.77	20.44	1143	1115
2	155.0	152.5	49	45	20.39	19.35	1157	1101
3	150.0	157.5	45	46	20.00	18.55	1101	1115
4	150.0	152.5	44	48	19.55	20.64	1087	1143
5	152.5	155.0	47	48	20.21	19.98	1129	1143
6	152.5	155.0	48	45	20.64	18.73	1143	1101
7	157.0	152.5	46	45	18.66	19.35	1115	1101
8	150.0	157.5	46	44	20.44	17.74	1115	1087
9	155.5	160	45	46	18.73	17.96	1101	1115
10	152.0	152.5	44	45	19.04	19.35	1087	1101
Mean	152.6	154.5	46.2	45.8	19.84	19.20	1117.8	1112.2
S.D.(±)	2.46	3.07	1.75	1.31	0.79	0.97	24.51	18.43
CV (%)	1.6	1.9	3.8	2.9	4.0	5.1	2.2	1.7
Overall Mean		153.6		46.0		19.52		1115.0
Overall SD (±)		2.87		1.52		0.92		21.30
Overall CV (%)		1.9		3.3		4.7		1.9
CD (5%)		N.S		N.S		N.S		N.S

NS = Non-significant

Table 4.49 Analysis of variance for height of the female subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Treatment	1	17.113	17.113	2.208
Error	18	139.525	7.751	
Total	19	156.638	8.244	

* Significant at 5% level

Table 4.50 Analysis of variance for weight of the female subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Treatment	1	0.800	0.800	0.333
Error	18	43.200	2.400	
Total	19	44.000	2.315	

* Significant at 5% level

Table 4.51 Analysis of variance for body mass index of the female subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Treatment	1	2.010	2.010	2.534
Error	18	14.277	0.793	
Total	19	16.287	0.857	

* Significant at 5% level

Table 4.52 Analysis of variance for basal metabolic rate of the femal subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Treatment	1	156.800	156.800	0.333
Error	18	8467.200	470.400	
Total	19	8624.000	453.890	

* Significant at 5% level

The same may also be the reason for the lower BMR values recorded in the present investigation for adult controls and menstruating adolescent subjects.

4.3.4 Haemoglobin (Hb): The mean Hb level (g/dl) recorded in adult controls and adolescent girls during follicular, ovulatory and luteal phases of menstrual cycle were 11.4 ± 0.23, 11.2 ± 0.34, 11.3 ± 0.35 and 11.2 ± 0.32, respectively. Analysis of variance revealed non-significant difference ($P < 0.05$) in the Hb level of the subjects.

According to WHO (1972), Hb level of adolescents should be above 12 g/dl of blood and the values lower than this are indicative of anaemia or iron deficiency. Following this criterion, all the subjects in the present investigation could be classified as marginally anemic. Szewczynski (1979) stressed that eating practices could influence the Hb concentration. In India, girls eating a mixed Punjabi diet were reported to have an adequate content of Hb in their blood (Kochar, 1981). Though this part of Kangra Valley was once considered as a part of Punjab, yet its Hb values were quite lower than the Punjab values reported by Kochar (1981). Renu (1990) also reported marginal anaemia in rural adolescent females of Kangra Valley. Hence, poor eating practices along with nutritional ignorance and negligence could be the explanation for lower Hb content.

4.3.5 Plasma protein: Statistically non-significant difference was observed in the plasma protein concentration of the subjects. The mean values for plasma protein (g/dl)

were 7.2 ± 0.53, 7.4 ± 0.64, 7.3 ± 0.49 and 7.4 ± 0.45, respectively for adult controls and adolescent girls during follicular, ovulatory and luteal phases of menstrual cycle (Table 4.53).

All the values obtained in the present study were within the normal range of 6-8 g/dl reported by Oser (1976).

4.3.6 Plasma glucose: Mean plasma glucose levels (mg/dl) during adult controls and adolescent girls during three phases of menstrual cycle were 93.6 ± 5.37, 102.04 ± 10.87 (follicular), 102.3 ± 9.84 (ovulatory)and 101.3 ± 11.02 (luteal), respectively (Table 4.53). Non-significant difference was observed in the plasma glucose levels of the female subjects.

The values for plasma glucose recorded in the present study compared well with the normal range of 90-120 mg/dl given by Oser (1976).

4.3.7 Plasma cholesterol: Statistically non-significant difference was observed in the plasma cholesterol level of the subjects. The mean values (mg/dl) recorded for adult controls and menstruating adolescent subjects during follicular, ovulatory and luteal phases of menstrual cycle were 171.3 ± 11.53, 186.0 ± 27.88, 185.5 ± 21.67 and 185.3 ± 24.67, respectively (Table 4.57).

All the values obtained in the present study were well within the normal range of 150-250 mg/dl specified by Oser (1976).

Table 4.53 Haemoglobin (Hb), Plasma protein and Glucose level of adult controls and adolescent (menstruating) subjects.

	Hb (g/dl)				Plasma protein (g/dl)				Plasma glucose (mg/dl)			
		Menstrual cycle phases				Menstrual cycle phases				Menstrual cycle ph		
S.No	Control	Folli-cular	Ovula-tory	Luteal	Control	Folli-cular	Ovula-tory	Luteal	Control	Folli-cular	Ovula-tory	Lu
1	11.2	11.5	11.7	11.7	6.70	8.05	7.90	7.85	96	110	110	1
2	11.6	10.9	10.9	10.8	6.80	6.90	7.00	6.90	86	94	94	
3	11.0	11.2	11.4	11.4	8.20	6.85	7.05	7.15	105	87	89	
4	11.6	11.8	11.8	11.7	7.50	7.30	7.30	7.25	94	100	105	
5	11.4	11.2	11.4	11.2	7.40	6.65	6.70	7.05	88	115	108	1(
6	11.2	10.8	10.8	10.8	6.85	8.05	7.90	7.90	92	105	98.5	
7	11.4	10.8	11.0	11.0	7.20	6.56	6.75	6.80	96	89	92	E
8	11.5	11.0	11.0	11.2	7.70	7.80	7.65	7.60	89	108.4	110	
9	11.4	11.2	11.3	11.2	6.50	8.30	8.05	8.10	96	118	120	11
10	11.8	11.6	11.6	11.4	6.80	7.15	7.15	7.20	94	94	96.4	9
Mean	11.4	11.2	11.3	11.2	7.2	7.4	7.3	7.4	93.6	102.0	102.3	10
S.D.(±)	0.23	0.34	0.35	0.32	0.53	0.64	0.49	0.45	5.37	10.87	9.84	11.
C.V. (%)	2.0	3.1	3.1	2.9	7.4	8.7	6.8	6.1	5.7	10.7	9.6	1(
Overall Mean		11.3				7.3				99.8		
Overall SD (±)		0.31				0.52				9.88		
Overall CV (%)		2.8				7.1				9.9		
CD (5%)		N.S				N.S				N.S		

NS = Non-significant

Table 4.54 Analysis of variance for blood haemoglobin level of the female subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Treatment	3	0.249	0.083	0.834
Error	36	3.582	0.100	
Total	39	3.831	0.098	

* Significant at 5% level

Table 4.55 Analysis of variance for plasma protein levels of the female subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Treatment	3	0.297	0.099	0.346
Error	36	10.301	0.286	
Total	39	10.599	0.271	

* Significant at 5% level

Table 4.56 Analysis of variance for plasma glucose level of the female subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Treatment	3	518.777	172.926	1.892
Error	36	3291.182	91.422	
Total	39	3809.959	97.69	

* Significant at 5% level

4.3.8 Urinary creatinine: Maximum mean urinary creatinine excretion (g/day) was recorded for adult controls (0.523 ± 6.38) while the values obtained for adolescent girls during three phases of menstrual cycle (Table 4.57) were 0.476 ± 9.81 (follicular), 0.458 ± 6.71 (ovulatory) and 0.450 ± 8.18 (luteal). Difference between the subjects was, however, non-significant ($P < 0.05$).

The values obtained in the present study were well below the range of 0.8-1.5 g/day (Best and Taylor, 1969). Renu (1990) in her study on rural adolescent females also observed the similar results of lower than normal creatinine excretion. Mariyama *et al.*(1987) correlated urinary creatinine with weight. The low body weights recorded for adult controls and menstruating adolescent girls might possibly be responsible for lower than normal creatinine excretion in the subjects.

4.3.9 Serum zinc: Mean serum zinc concentrations (µg/dl) in adult controls and adolescent girls during follicular, ovulatory and luteal phases of menstrual cycle were 97.1 ± 18.38, 99.45 ± 18.21, 92.2 ± 15.48 and 88.6 ± 15.26, respectively (Table 4.57). However, the difference was non-significant.

The mean values for serum zinc obtained in the present study were within the range 72-115 µg/dl as reported by Halsted and Smith (1970) for normal female subjects. The results were also comparable with the value 92 ± 3 µg/dl given by Meret and Henkin (1971) for female subjects.

Table 4.5/ Plasma cholesterol, urinary creatinine and serum zinc level of adult controls and adolescent (menstruating) subj

	Plasma cholesterol (mg/dl)				Urinary creatinine (g/day)				Serum zinc (µg/dl)			
		Menstrual cycle phases				Menstrual cycle phases				Menstrual cycle pha		
S.No	Control	Follicular	Ovulatory	Luteal	Control	Follicular	Ovulatory	Luteal	Control	Follicular	Ovulatory	Lut
1	188	154	161	154	0.542	0.514	0.508	0.495	81.0	83.5	78.0	7
2	172	178	182	183	0.612	0.378	0.402	0.415	77.5	115.5	99.0	9
3	180	166	160	160	0.593	0.429	0.452	0.395	122.0	115.0	105.0	9
4	160	177	184	179	0.466	0.320	0.342	0.320	87.5	89.0	79.5	7
5	176	197	192	192	0.495	0.450	0.428	0.472	116.5	125.0	115.5	11
6	155	216	209	197	0.515	0.558	0.492	0.515	92.0	87.0	86.5	8
7	177	204	198	203	0.525	0.392	0.435	0.370	104.0	76.0	72.5	7
8	184	240	226	238	0.602	0.620	0.558	0.498	78.0	98.0	95.0	8
9	161	174	181	185	0.446	0.525	0.425	0.425	87.5	122.0	112.5	11
10	160	154	162	162	0.438	0.583	0.546	0.602	125.0	83.5	78.0	7
Mean	171.3	186.0	185.5	185.3	0.523	0.476	0.458	0.450	97.1	99.4	92.2	8
S.D.(±)	11.53	27.88	21.67	24.67	6.38	9.81	6.71	8.18	18.38	18.21	15.48	15.
C.V. (%)	6.7	14.9	11.7	13.3	12.2	20.6	14.6	18.2	18.9	18.3	16.8	17
Overall Mean		182.0				0.477				94.3		
Overall SD (±)		22.32				0.08				16.78		
Overall CV (%)		12.2				16.9				17.8		
CD (5%)		N.S				N.S				N.S		

NS = Non-significant

Table 4.58 Analysis of variance for plasma cholesterol level of the female subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Treatment	3	1536.275	512.092	1.030
Error	36	17904.700	497.353	
Total	39	19440.975	498.486	

* Significant at 5% level

Table 4.59 Analysis of variance for urinary creatinine level of the female subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Treatment	3	0.032	0.011	1.700
Error	36	0.224	6.227×10^{-3}	
Total	39	0.256	6.560×10^{-3}	

* Significant at 5% level

Table 4.60 Analysis of variance for serum zinc level of the female subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Treatment	3	714.725	238.242	0.834
Error	36	10280.550	285.571	
Total	39	10995.275	281.930	

* Significant at 5% level

Further observation revealed that in the present study there was a gradual decline in the mean serum zinc level of the subjects during the three phases of menstrual cycle, being higher during the follicular phase and lower during the ovulatory and Luteal phases. Similar findings were also reported by Deuster *et al.*(1987).

4.3.10 Saliva zinc: Mean saliva zinc level (ng/ml) of adult controls and menstruating adolescent subjects in follicular, ovulatory and Luteal phases of menstrual cycle were 200.0 ± 19.16, 225.05 ± 42.54, 222.33 ± 33.40 and 223.13 ± 38.58, respectively (Table 4.61) and the difference was found to be non-significant.

The results obtained in the present study were in close association with those reported by Greger and Sickles (1979) i.e. 222 ± 161 and 203 ± 72 ng/ml for adolescent females. The results obtained for adult controls women were also comparable to the value 190 ng/ml given by Freeland-Graves *et al.*(1981) for healthy female subjects.

4.3.11 Urinary zinc: Maximum mean urinary zinc excretion (µg/24 hrs.) was observed in adult controls (501.1 ± 47.27), followed by the adolescent subjects in follicular (483.7 ± 45.24), ovulatory (464.9 ± 50.73) and luteal phases (463.5 ± 45.56) of menstrual cycle (Table 4.61). Statistically, the difference was non-significant.

The values for urinary zinc recorded in the present finding were well within the normal range of 400-600 µg/24 hrs. (Roman, 1969).

Table 4.61 Saliva, urine and hair zinc level of adult controls and adolescent (menstruating) subjects.

	Saliva zinc (ng/ml)				Urine zinc (µg/24 hr)				Hair zinc (µg/g)			
		Menstrual cycle phases				Menstrual cycle phases				Menstrual cycle ph		
S.No	Control	Follicular	Ovulatory	Luteal	Control	Follicular	Ovulatory	Luteal	Control	Follicular	Ovulatory	Lu
1	172.0	262.0	248.8	222.0	428.0	430.0	392.4	397.0	139.0	163.0	163.0	1
2	229.0	203.0	192.5	173.0	540.5	453.4	416.5	425.0	165.5	139.0	127.6	1
3	229.0	315.0	292.5	302.4	499.0	518.5	530.0	497.0	119.6	120.0	117.5	1
4	187.5	246.0	226.5	248.4	480.0	428.5	409.8	438.0	165.0	155.0	147.4	1
5	191.5	179.0	203.0	203.0	512.0	453.4	475.0	428.5	123.6	168.2	160.5	1
6	200.0	192.5	183.0	196.0	438.0	528.0	499.2	515.0	133.4	172.0	172.6	1
7	216.0	225.0	246.5	211.0	469.0	563.0	529.0	542.2	165.0	178.5	170.0	1
8	186.0	178.0	202.0	197.0	522.5	464.0	479.5	458.0	158.8	170.8	165.0	1
9	187.5	209.0	199.0	212.5	560.0	486.6	423.0	446.0	139.0	135.0	143.0	1
10	201.5	241.0	229.5	266.0	562.0	512.0	495.5	488.0	146.0	159.0	148.0	1
Mean	200.0	225.0	222.3	223.1	501.1	483.7	464.9	463.5	145.5	156.1	151.5	1
S.D.(±)	19.16	42.54	33.40	38.52	47.27	45.24	50.73	45.56	17.38	18.90	18.29	1
C.V. (%)	9.6	18.9	15.0	17.3	9.4	9.4	10.9	9.8	11.9	12.1	12.1	
Overall Mean		217.6				478.3				150.6		
Overall SD (±)		34.78				47.99				18.31		
Overall CV (%)		15.9				10.0				12.2		
CD (5%)		N.S				N.S				N.S		

NS = Non-significant

Table 4.62 Analysis of variance for saliva zinc level of the female subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Treatment	3	4182.133	1394.044	1.167
Error	36	42996.227	1194.340	
Total	39	47178.360	1209.700	

* Significant at 5% level

Table 4.63 Analysis of variance for urinary zinc level of the female subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Treatment	3	9465.161	3155.054	1.413
Error	36	80388.574	2233.016	
Total	39	89853.735	2303.940	

* Significant at 5% level

Table 4.64 Analysis of variance for hair zinc level of the female subjects.

Source of variation	df	Sum of squares	Mean Squares	F value
Treatment	3	576.949	192.316	0.554
Error	36	12498.059	347.168	
Total	39	13075.008	335.256	

* Significant at 5% level

4.3.12 Hair zinc: Statistically non-significant difference was observed in the hair zinc level of the subjects. The mean values (ug/g) recorded for adult controls and menstruating subjects during three phases of menstrual cycle were 145.5 ± 17.38, 156.1 ± 18.90 (follicular), 151.5 ± 18.29 (ovulatory) and 149.5 ± 19.86 (luteal), respectively (Table 4.61).

The results obtained were well within the normal range of 92-255 ug/g specified for adults (Smith, 1967).

CORRELATION OF SERUM ZINC WITH SOME BIOCHEMICAL AND NUTRITIONAL PARAMETERS (Tables 4.65, 4.66 & 4.67)

Diseased conditions: Positive but non-significant correlation was observed between serum zinc and plasma proteins in hypertensives and normal (control) female subjects. The results are in agreement with those of Lindeman *et al.*(1978) and Kiilerich *et al.*(1980) who also reported weak but significant correlations between serum zinc and protein in normal subjects. Negative correlation was, however, observed in case of diabetics and normal males. Hagglof *et al.*(1980) found no correlation between serum zinc and albumin in diabetic patients which differed with the findings for diabetic subjects in the present study.

Serum zinc was positively correlated with plasma glucose in normal and hypertensive subjects but the correlation was not significant. Negative correlation was recorded for diabetics (r = -0.04 for males and -0.39 for

females). D'oCon *et al.*(1981) noted close relationship between serum zinc and glucose in diabetic patients while Hagglof *et al.*(1983) found no correlation between the two in diabetic patients. The results obtained for diabetics in the present study did not agree with any of these findings.

Negative correlation was found between serum zinc and plasma cholesterol in diabetics and normal females while positive but non-significant correlation was observed in case of hypertensives and normal male subjects. The results obtained for hypertensives and normal male subjects were in partial agreement while those for diabetics and normal females in contrast to the findings of Koo and Williams (1981) and Koo and Ramlet (1983). Both reported a strong positive correlation between the concentrations of serum zinc and HDL-cholesterol.

Serum zinc was positively correlated with saliva zinc in case of female subjects and the correlation was significant in diabetic (r= 0.65) and hypertensive (r= 0.75) subjects. Negative correlation was, however, recorded for male subjects. Greger and Sickles (1979) found no correlation between zinc levels in serum and in saliva which contrasted with the findings of the present study. They, however, stated that factors which affect serum zinc levels and hence limit their usefulness as indicators of zinc nutritional status, may also affect saliva zinc levels. Freeland-Graves *et al.*(1981) found a moderate but significant inverse relationship between serum and saliva

Table 4.65 Correlation of serum zinc with biochemical and nutritional parameters during diseased conditions.

	Control		Diabetic		Hypertensi	
Parameters	Male	Female	Male	Female	Male	Femal
Plasma protein	-0.58	0.33	-0.12	-0.11	0.21	0.32
Plasma glucose	0.24	o.42	-0.04	-0.39	0.28	0.23
Plasma cholesterol	0.35	-0.10	-0.04	-0.32	0.36	0.06
Saliva zinc	-0.21	0.34	-0.17	0.65	-0.23	0.75
Urine zinc	0.49	0.20	-0.05	-0.34	0.02	-0.44
Hair zinc	0.35	-0.53	-0.01	-0.18	-0.69	-0.33

Critical value (2-tail, 0.05) = + or - 0.63

zinc which was in close association with the results obtained for male subjects in the present study.

Positive but non-significant correlation was observed between serum and urine zinc in case of normal subjects and hypertensive males. Negative correlation was found in case of hypertensive female and diabetic subjects. Hagglof *et al.* (1983) reported association of hyperzincuria in diabetic patients with decreased serum zinc levels. This inverse relationship was also observed in diabetics in the present study.

Hair zinc was negatively correlated with serum zinc in case of diabetics, hypertensives and normal females. The correlation was, however, significant in diabetics (r= -0.01 for males and -0.18 for females). Positive but non-significant correlation observed in case of normal male subjects was in concurrence with the report of Freeland-Graves *et al.*(1981) who also observed poor correlations between serum and hair zinc in healthy adults.

Physiological conditions:

During lactation and second and third trimester of pregnancy, serum zinc was positively correlated with plasma proteins but the correlation was not significant. Weak correlations between serum zinc and proteins have also been reported by Kiilerich *et al.*(1980) in healthy subjects. Negative correlation (r=-0.28) was, however, observed during the first trimester of pregnancy.

Positive but non-significant correlation was observed between serum zinc and plasma glucose during lactation and first trimester of pregnancy while negative correlation was found during second and third trimester of pregnancy. Plasma cholesterol was significantly correlated with serum zinc during first (r = 0.93) and third (r = 0.82) trimester of pregnancy. A strong positive relationship between serum zinc and cholesterol was also observed by Koo and Ramlet (1983). During second trimester of pregnancy and lactation, negative correlation was recorded between serum zinc and plasma cholesterol which contrasted with the above report.

Serum zinc was negatively correlated with saliva zinc in lactating and pregnant women in first and second trimester of pregnancy. Freeland-Graves *et al.*(1981) also reported moderate but inverse relationship in healthy females. Non-significant but positive correlation was observed during third trimester of pregnancy (r=0.30) which was in line with the observation of Greger and Sickles (1979) in normal female subjects.

Positive but non-significant correlation was observed during pregnancy and lactation between serum and urine zinc. Vir *et al.*(1981) reported progressive decline in serum zinc while Swanson and King (1982) found increased urinary zinc excretion with duration of pregnancy. This inverse relationship was not observed in the present investigation during pregnancy.

Table 4.66 Correlation of serum zinc with biochemical and nutritional parameters in physiological conditions.

Parameters	Pregnancy (Trimesters) Ist	2nd	3rd	Lactation
Plasma protein	-0.28	0.07	0.26	0.38
Plasma glucose	0.21	-0.39	-0.44	0.27
Plasma cholesterol	0.93	-0.25	0.82	-0.28
Saliva zinc	-0.34	-0.28	0.30	-0.21
Urine zinc	0.22	0.12	0.18	0.04
Hair zinc	0.04	0.42	0.22	-0.27
Milk zinc				-0.19

Critical value (2-tail, 0.05) = + or - 0.63

Serum zinc was positively correlated with hair zinc during pregnancy but the correlation was not significant. A positive relationship between serum and hair zinc was also suggested by Vir *et al.*(1981) as they observed a significant decline in both serum and hair zinc with duration of pregnancy. Negative correlation was found between serum and hair zinc in lactating subjects.

A significant negative correlation (r=-0.19) was observed between serum zinc and milk zinc during lactation in the present study.

Menstrual cycle phases:

Serum zinc was positively but non-significantly correlated with plasma proteins during luteal phase while negative correlation was observed during the follicular and ovulatory phases. Lindeman *et.al.* (1978) reported low but significant correlations between serum zinc and proteins in control subjects. The result obtained for adolescent subjects in luteal phase of menstrual cycle in the present study were similar to those reported above.

Plasma glucose was positively correlated with serum zinc in all the three phases but the correlation was not significant. Positive but non-significant correlation was observed during the ovulatory and luteal phases between plasma cholesterol and serum zinc while significant negative correlation ($r = -0.04$) was found during the follicular phase. Koo and Williams (1981) reported strong positive correlation between the concentration of zinc in serum and

Table 4.67 Correlation of serum zinc with biochemical and nutritional parameters during menstrual cycle phases

Parameters	Menstrual cycle phases		
	Follicular	Ovulatory	Luteal
Plasma protein	-0.09	-0.02	0.14
Plasma glucose	0.34	0.32	0.27
Plasma cholesterol	-0.04	0.04	0.07
Saliva zinc	-0.15	-0.22	-0.16
Urine zinc	-0.27	-0.03	-0.26
Hair zinc	-0.61	-0.41	-0.49

Critical value (2-tail, 0.05) = + or - 0.63

plasma cholesterol. The results obtained for ovulatory and luteal phases in the present investigation were in partial agreement while those for follicular phase in contrast to the above report.

Serum zinc was negatively correlated with saliva, urine and hair zinc during the three phases of menstrual cycle. Inverse relationship between saliva and serum zinc and poor but positive correlation between serum and hair zinc has been reported by Freeland-Graves *et.al.*(1981) in case of female subjects. The latter report was in contrast while the former in agreement with the results recorded in the present study during menstrual cycle.

ZINC IN LOCALLY GROWN AND CONSUMED FOODS

Locally grown and consumed vegetables as well as cereals and pulses were analysed for their zinc content (Table 4.68).

Vegetables: Among leafy vegetables, on fresh matter basis, highest zinc content was found in mustard leaves (15.94 mg/100g) followed by spinach (10.66mg/100g) and cabbage (7.8mg/100g). Highest zinc content on dry matter basis was again recorded for mustard leaves (120.8mg/100g). The values for cabbage and spinach were 98.0 and 79.0 mg/100g, respectively.

Under roots and tubers, potato had higher zinc content on fresh as well as dry matter basis as compared to onion. The values for potato and onion were 201.6 and 188.8 mg/100g

on dry matter basis and 20.1 and 15.8 mg/100g on fresh matter basis.

Highest zinc content among other vegetables on fresh matter basis was recorded for ladies fingers (17.41mg/100g) followed by tomato (13.44mg/100g) and broad beans (13.22 mg/100g). The values in milli gram per cent for cauliflower, brinjal, cucumber, french beans, giant chillies, peas, lungru, and lasura were 9.00, 5.33, 3.89, 16.1, 7.68, 2.15, 4.54 and 4.12, respectively. On dry matter basis, tomato had highest zinc content (224.4mg/100g). For remaining vegetables, the values (mg/100g) were in the order of 123.3 (cauliflower), 86.0 (brinjal), 139.0 (cucumber), 41.5 (broad beans), 166.0 (french beans), 74.6 (giant chillies), 219.0 (ladies fingers), 9.4 (peas),64.0 (lungru) and 38.2 (lasura).

Cereals and pulses: Among cereals, wheat had higher zinc content than rice and the corresponding values were 0.98 and 0.87 mg/100g. Among pulses, highest zinc content was recorded in whole black gram (1.4mg/100g) and lowest in Bengal gram dhal (0.88mg/100g). The values for Bengal gram (whole), green gram (whole) and lentil dhal were 1.08, 1.1 and 1.25 mg/100g, respectively.

The values obtained for zinc in the present study for vegetables as well as cereals and pulses were much lower than those listed by Gopalan *et al.*(1989). The reason is attributed to the low zinc content of the soil. Soil of low and mid hill regions of the state of Himachal Pradesh has

Table 4.68 Zinc content of locally grown and consumed foods

Vegetable	Moisture content (%)	Zinc content (mg/100g)	
		Dry matter basis	Fresh matter basis
	Leafy vegetables:		
Cabbage *Brassica oleracea* (var. capitata)	92.04	98.0	7.8
Mustard leaves *Brassica campestris* (var. sarson)	86.8	120.8	15.94
Spinach *Spinacia oleracea*	86.5	79.0	10.66
	Roots and tubers:		
Onion *Allium cepa*	91.6	188.8	15.86
Potato *Solanum tuberosum*	90.03	201.6	20.1
	Other vegetables:		
Cauliflower *Brassica oleracea* (var. botrytis)	92.7	123.3	9.0
Brinjal *Solanum melongena*	93.8	86.0	5.33
Cucumber *Cucumis sativus*	97.2	139.0	3.89
Broad beans *Vicia faba*	68.14	41.5	13.22
French beans *Phaseolus vulgaris*	90.3	166.0	16.1
Giant chillies (capsicum) *Capsicum annuum* (var. grossa)	89.7	74.6	7.68
Ladies fingers *Abelmoschus esculentus*	92.05	219.0	17.41
Peas *Pisum sativum*	77.12	9.4	2.15
Tomato *Lycopersicon esculentum*	94.01	224.4	13.44
Lungru *Diplazium esculentum*	92.9	64.0	4.54
Lasura *Cordia dichotoma*	89.2	38.2	4.12

Cereals and Pulses	Zinc content (mg/100g) Dry matter basis
CEREALS AND PULSES	
Wheat *Triticum aestivum*	0.98
Rice *Oryza sativa*	0.87
Bengal gram.(whole) *Cicer arietinum*	1.08
Bengal gram dhal *Cicer arietinum*	0.88
Black gram (whole) *Phaseolus mungo* Roxb.	1.40
Green gram (whole) Phaseolus aureus Roxb.	1.10
Lentil dhal *Lens esculenta*	1.25

been reported to be zinc deficient (Kanwar *et al.*,1983). Randhawa (1988) also pointed out wide ranging deficiencies of micro nutrients in soils of Himachal Pradesh. Underwood (1971) stated that zinc content of plants was influenced by the soil type and the fertilizer treatment. Halsted *et al.*(1974) found wide range of zinc content in the same food and attributed it to the difference in analysis, source and variety.

SUMMARY

SUMMARY

The objective of the present investigation was to study the zinc status including anthropometric and biochemical measurements of local human population in health and diseases. In addition, zinc content was also determined in locally grown and consumed foods. The results of this study are summarised under three headings:

a) During diseased conditions:

Significant differences were observed between sexes for height and weight and the corresponding overall mean values for male subjects were 167.35 cm and 60.2 kg while those for female subjects 153.0 cm and 46.8 kg, respectively. These values were lower than the reported Indian standards. No influence of diseased conditions was observed on the BMI of the subjects and the mean values were between 20.87 to 22.35 kg/m^2 in males and 19.83 to 20.35 kg/m^2 in females.

The mean values of BMR (kcal/24hr) ranged from 1460.8 to 1553.9 in males and 1117.8 to 1183.1 in females. The values of mean diastolic and systolic blood pressures (Bp), were higher during different diseased conditions (Mean

diastolic Bp, 89.3 to 114.0 mm Hg: systolic Bp, 118.4 to 141.5 mm Hg).

Significant difference was observed between sexes for haemoglobin (Hb) content. The mean values of Hb(g/dl) in both sexes were lower (males, 12.2 to 12.8; females 10.4 to 11.4) than the WHO standards. The mean plasma protein concentrations were within normal range (7.16 to 8.23 g/dl) in all the subjects.

Mean plasma glucose and cholesterol was significantly higher in diabetics and the values were of the order of 275.6 and 222.3 mg/dl in males and 334.6 and 224.3 mg/dl in females, respectively. In hypertensive patients, the respective values recorded were 120.5 and 210.1 mg/dl for males and 126.1 and 183.4 mg/dl for females. The urinary output of creatinine in the subjects was considerably lower than the reported standard values.

Mean serum zinc concentration ranged from 88.7 to 93.2 ug/dl in hypertensive subjects while it was considerably higher in diabetics (139.1 µg/dl in males and 168.5 µg/dl in females). Average saliva zinc was comparable to the reported values and ranged from 205.4 to 225.8 ng/ml in males and 189.7 to 252.8 ng/ml in females.

Urinary zinc excretion was normal in hypertensive subjects (540.8 to 541.1 µg/24hr in males and females, respectively) while hyperzincuria was observed in diabeties (944.3 and 1034.8 µg/24hr in males and females,

respectively). Mean hair zinc was within normally reported range (116.4 to 145.5 µg/g).

Serum zinc was significantly correlated with saliva zinc in diabetic and hypertensive female subjects but it was negatively correlated in diabetic male subjects.

b) During different physiological stages:

Zinc status and anthropometrical and biochemical measurements were recorded in pregnant and lactating subjects.

It was observed that mean body weight and body mass index recorded during third trimester of pregnancy (56.6 kg, 24.62 kg/m^2) and lactation (54.8 kg, 23.13 kg/m^2) were significantly higher than the respective control values (46.2 kg, 19.84 kg/m^2). Lower weight for height as compared to reported Indian standards was observed in all the subjects. Mean values of BMR ranged from 1238.2 to 1262.7 kcal/24hr.

Marginally low content of Hb was recorded (10.4 to 11.4 g/dl) as compared to the WHO standards. Mean plasma protein and glucose concentration of the subjects was within normal range (7.16 to 7.67 g/dl and 93.6 to 105.4 mg/dl, respectively).

Lower than normal plasma cholesterol concentrations were observed during the first trimester of pregnancy (mean 142.2 mg/dl) while normal values were recorded for other subjects (171.3 to 183.4 mg/dl). The creatinine excretion in all the subjects was much lower than the standard values.

The mean serum zinc concentrations ranged from 58.2 to 102.6 µg/dl and a progressive decline in serum zinc level with duration of pregnancy was observed. Mean saliva zinc values were comparable to normally reported range (200.0 to 272.1 ng/ml).

Urinary and hair zinc values were within normal range (overall mean 482.9 µg/24hr and 169.3 µg/g, respectively). Decline in hair zinc was observed with increasing duration of pregnancy. Mean milk zinc concentration (311.5 µg/dl) was also within normal range.

Positive and significant correlation was observed between serum zinc and plasma cholesterol during first and third trimester of pregnancy. Significant negative correlation was found between serum and milk zinc.

c) During menstrual cycle phases:

Anthropometric parameters did not differ significantly among various subjects. The overall mean values for height, weight and body mass index were 153.57 cm, 46.0 kg and 19.52 kg/m^2, respectively. The values recorded were lower than the reported Indian standards. Mean BMR was 1117.8 and 1112.2 kcal/24hr, respectively, for adult control and adolescent menstruating subjects.

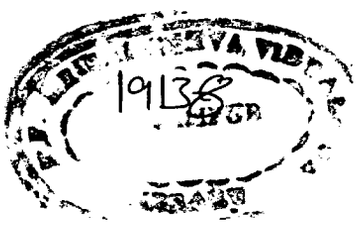
Marginal anaemia was observed in the subjects and haemoglobin (Hb) level ranged from 11.2 to 11.4 g/dl. Mean plasma protein, glucose and cholesterol were within normal range (overall mean 7.3 g/dl, 99.8 mg/dl and 182.0 mg/dl, respectively).

Mean urinary output of creatinine was considerably lower than the reported standards (0.450 to 0.523 g/day). The serum zinc concentration ranged from 88.6 to 99.4 µg/dl and progressive decline in serum zinc concentration was observed during the three phases of menstrual cycle.

Saliva, urine and hair zinc concentrations were within normal ranges as per reports (overall mean 217.6 ng/ml, 478.3 µg/24hr and 150.6 µg/g, respectively).

Serum zinc was negatively correlated with saliva, urine and hair zinc and positively correlated with plasma glucose in all the three phases of menstrual cycle.

Maximum zinc content was recorded in tomato (224.4 mg/100g) and potato (20.1 mg/100g) on dry and fresh matter basis, respectively among vegetabls. Wheat (0.98 mg/100g) and whole black grm (1.4 mg/100g) had highest zinc content among cereals and pulses.



REFERENCES

REFERENCES

Abdulla,M. (1983). Zinc deficiency in human subjects. Alan R. Liss, INC, N.Y. p 171.

Anthony, W.L. and Hsu, J.M.(1973). Zinc deficiency and free amino acid in rat plasma and skin. Fed. Proc. 32: 895.

Apgar,J.(1968). Amer.J.Physiol. 215:160.

Argemi,J.; Serrano,J.; Gutierrez,M.C.; Ruiz,M.S. and Gil,A. (1988). Serum zinc binding capacity in pregnant women. Annal. Nutr. Metabol. 32: 121-126.

Atkinson, S.A.;Whelan,D.;Whyte, R.K. and Lonnerdal,B.(1989). Abnormal zinc content in human milk: Risk for development of nutritional zinc deficiency in infants. Am. J. Dis. Child. 143:608-611.

Baer,M.T. and King,J.C.(1978). Experimental zinc depletion in young men. Fed. Proc. 37:216.

Barr,D.P.; Russ,E.M. and Eder, H.A. (1951). Protein-Lipid relationship in human plasma. II Atherosclerosis and related conditions. Am. J. Med. 11: 480-93.

Bergmann, K.E. and Bergmann, R.L. (1985). Gestational zinc deficiency. Am.J. Clin.Nutr.42: 343-344.

Bertrand, P.V.; Rudd,B.T.; Weller, P.H. and Day,A.J. (1987). Free cortisol and creatinine in urine of healthy children. Clin. Chem.33:2047-2051.

Best, C.H. and Taylor, N.B. (1969). The Physiological Basis of Medical Practice. Waverly Press Inc. U.S.A. p 1793.

Bilinski,D.L.;Ehrenkranz,R.A.; Cooley-Jacobs,J. and McGuire, J. (1987). Symptomatic zinc deficiency in a breast-fed, premature infant. Arch.Dermatol.123:1221-1224.

Bjorksten,F.; Aromaa,A.; Knekt.P. and Malinen,L. (1978). Serum zinc concentrations in Finns. Acta Med. Scand. 204:67-74.

Blamberg,D.L.; Blackwood,U.B.; Supplee,W.C. and Combs,G.F. (1960). Effect of zinc deficiency in hens on hatchability and embryonic development. Proc. Soc. Exp. Biol. Med. 104:217-220.

Boyett,J.D. and Sullivan, J.F.(1970). Distribution of protein-bound zinc in normal and cirrhotic serum. Metabolism 19: 148-157.

Brosbin,H.W.; Worthington Roberts,B.S. and Knopp,R.H. (1983). First trimester serum zinc concentrations in human pregnancy. Am.J.Clin.Nutr. 38:943-953.

Buchanan,P.J. and Hsu,J.M. (1968). Zinc deficiency and DNA synthesis in the rat liver. Fed. Proc. 27:483.

Campbell-brown, M.; Ward, R.J. and Haines, A.P. (1985). Zinc and copper in Asian pregnancies- is there evidence for a nutritional deficiency? Br. J. Obstet. Gynaecol. 92: 875-885.

Canfield, W.K.; Hambidge,K.M. and Johnson,L.K. (1984). Zinc nutriture in type I diabetes mellitus: relationship to growth measures and metabolic control. J. Pediatr. Gastroenterol. Nutr. 3:577-584.

Casey, C.E.; Neville,M.C. and Hambidge,K.M. (1989). Studies in human lactation: secretion of zinc, copper, and manganese in human milk. Am. J. Clin. Nutr. 49: 773-785.

Castelli,W.P.; Doyle,J.T. and Gordon,T. (1977). HDL-Cholesterol and other lipids in coronary heart disease: the cooperative lipoprotein phenotyping study. Circulation 55:767-772.

Chen,M.D.; Lin,P.Y.; Lin,W.H. and Cheng,V. (1988). Zinc in hair and serum of obese individuals in Taiwan. Am. J. Clin. Nutr. 48:1307-1309.

Chesters, J.K. and Will, M.(1981). Zinc transport proteins in plasma. Br. J. Nutr. 46:111-118.

Cronk, C.E. and Roche, A.F.(1982). Am. J. Clin. Nutr.35:351-354.

Czerniejewski,C.P.; Shank,C.W.; Bechtel,W.G. and Bradley, W.B. (1964). The minerals of wheat, flour, and bread. Cereal Chem. 41:65-72.

D'oCon,C.; Alonso De Armino, V. and Frasquet, I. (1987). Serum zinc and copper values in a diabetic population. Revista Espanola de Fisiologia 43: 335-338.

Dawes, C. (1970). Effect of diet on salivary secretion and composition. J. Dental Res. 49: 1263.

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

Deuster,P.A.; Dolev,E.;Bernier,L.L. and Trostmann,U.H. (1987). Magnesium and zinc status during the menstrual cycle. Am J. Obst. Gyne. 157: 964-968.

Dreosti, I.E.; Grey, C. and Wilkins, P.J. (1972). Deoxyribonucleic acid synthesis, protein synthesis and teratogenesis in zinc- deficient rats. South African Med. J. 46: 1585.

Eberhardt, M.J. and Halas,E.S. (1987). Developmental delays in offspring of rats undernourished or zinc deprived during lactation. Physiol. Behavior 41: 309-314.

Eltohamy, M.M. (1987). Zinc deficiency during pregnancy in rabbits. J.Egyp. Vet. Med. Asso. 47: 489-496.

Engelbart,K. and Kief,H. (1970) Uber das funktionelle Verhalten von Zink und Insulin in den B-Zellen des Rattenpankreas. Virchows Arch [Zellpathol] 4:294-302.

Everett, G.A. and Apgar, J.(1979). Effect of zinc status on salivary zinc concentrations in the rat. J.Nutr. 109: 406-411.

Fairweather- Tait, S.J.; Wright, A.J.A.; Cooke, J. and Franklin, J. (1985). Studies of zinc metabolism in pregnant and lactating rats. Br. J. Nutr. 54: 401-413.

Foley,B.; Johnson, S.A.; Hackley, B.;Smith, J.C. Jr. and Halstead,J.A. (1968). Zinc content of human platelets. Proc. Soc. Exp. Biol.Med. 128: 265-269.

Folin and Wu (1965). Blood analysis. In: Hawk's Physiological Chemistry (14th ed.). Edited by Oser, B.L. Tata McGraw-Hill Publishing Co.Ltd. New Delhi. P 1052.

Forbes,G.B. (1988). Body composition:influence of nutrition, disease, growth, and aging. In: Modern Nutrition in Health and Disease. Edn 7. (Eds. Shils, M.E. and Young, V.R.). Lea and Febiger, Philedelphia.

Freeland- Graves, J.H.; Hendrickson, P.J.; Ebangit, M.L. and Snowden, J.Y. (1981). Salivary zinc as an index of zinc status in women fed a low-zinc diet. Am.J.Clin.Nutr. 34: 312-321.

Fujioka, M. and Lieberman, I. (1964). A Zn^{65} requirement for synthesis of deoxyribonucleic acid by rat liver. J.Biol. Chem. 239: 1164-1167.

Garrow, J.S.(1981). Treat Obesity Seriously. Churchill Livingstone, Edinburgh.

Glover, M.T. and Atherton, D.J. (1988). Transient zinc deficiency in two full-term breast-fed siblings associated with low maternal breast milk concentration. Pediatr. Dermatol. 5: 10-13.

Gofman,J.W.; deLalla,O.F.; Kovich,E.L.; Lowe,O.; Martin,W.; Piluso,D.L.; Tandy,R.K. and Upham,F. (1964). Chemical elements of the blood of man. Arch. Environ. Health 8: 105-109.

Golub,M.S.;Gershwin,M.E.;Hurley,L.S;Baly,D.L. and Hendrickx, A.G. (1984). Studies of marginal zinc deprivation in rhesus monkeys.II pregnancy outcome. Am. J. Clin. Nutr. 39: 879-887.

Goni,I.; Garcia- Diz,L.; Cuesta,C.; Sanchez-Muniz, F.J. and Castro,A. (1989). Relationships between anthropometrical measurements, lipemia and apolipoproteinemia in a sample of Madrid Secondary School adolescents. Nutr. Rep. Int. 39: 1167-1175.

Gopalan, C. (1989). Growth of affluent Indian girls during adolescence. Scientific report 10. Nutrition Foundation of India, New Delhi. p 74.

Gopalan, C.; Rama Shastri, B.V. and Balasubramanian, S.C. (1989). Nutritive Value of Indian Foods. NIN Hyderabad.

Greger,J.I. and Sickles, V.S. (1979). Saliva zinc levels: potential indicators of zinc status. Am. J. Clin. Nutr. 32: 1859-1866.

Griffith, P.R. and Alexander, J.C. (1972). Effect of zinc deficiency on amino acid metabolism of the rat. Nutr. Rep.Int. 6:9.

Gupta, S.P. (1988). Statistical Methods. Publishers, Sultan Chand & Sons; 23, Daryaganj, New Delhi-110002.

Hagglof,B.; Hallmans,G.; Ludvigsson,J.and Falkmer,S. (1983). Prospective and retrospective studies of zinc concentrations in serum,blood clots, hair and urine in young patients with insulin-dependent diabetes mellitus. Acta Endocrinol. 102:88-95.

Halsted, J.A. and Prasad, A.S. (1960). Syndrome of iron deficiency anemia, hepatosplenomegaly, hypogonadism, dwarfism and geophagia. Trans.Amer.Clin.Climat.Ass. 72: 130-149.

Halsted,J.A. and Smith,J.C.,Jr.(1970).Plasma zinc in health and disease. Lancet 1:322-324.

Halsted,J.A.;Smith,J.C. and Irwin,M.I. (1974). A conspectus of research on zinc requirements of man. J. Nutr. 104: 345-378.

Hambidge,K.M. and Droegemueller, W. (1974). Changes in plasma and hair concentrations of zinc, copper, chromium and manganese during pregnancy. Obstet. Gynecol. 44: 666-672.

Hambidge,K.M.; Hambidge,C.; Jacobs,M. and Baum,J.D. (1972). Pediatrc. Res. 6:868.

Hambidge,K.M.; Hambidge,C.; Jacobs,M. and Baum,J.D. (1972). Low levels of zinc in hair,anorexia,poor growth, and hypogeusia in children. Pediat.Res.6: 868-874.

Hambidge,K.M.;Krebs, N.F.; Jacobs,M.A.; Favier,A.; Guyette, L. and Ikle,D.N. (1983). Zinc nutritional status during pregnancy: a longitudinal study. Am. J. Clin. Nutr. 37: 429-442.

Hambidge,K.M.;Neldner,K.H.;Walravens,P.A.;Weston,W.L.;Silver-man,A.;Sabol,J.L. and Brown,R.M.(1978). In 'Zinc and copper in clinical medicine' Hambidge,K.M. and Nichols, B.L. (Eds).Spectrum Publications,New York, pp.81-98.

Harding,M.M.; Hodgkin,D.C.; Kennedy,A.F.; O'Connor,A. and Weitzmann, P.D.J. (1966). The crystal structure of insulin.II. An investigation of rhombohedral zinc insulin crystals and a report of other crystalline forms.J.Molec. Biol. 16: 212-216.

Hartoma,T.R.;Sotaniemi,E.A. and Maattanen,J.(1979). Effect of zinc on some biochemical indices of metabolism. Nutr. Metab. 23: 294-300.

Hendricks,D.G. and Mahoney,A.W. (1972). Glucose tolerance in zinc deficient rats. J.Nutr. 102: 1078-1084.

Henkin, R.I.;Schechter,P.J.;Hoye, R.and Mattern, C.F.T. (1971). Idiopathic hypogeusia with dysgeusia, hyposmia, and dysosmia. A new syndrome. J.Amer.Med.Asso. 217:434-440.

Henkin,R.I.;Mueller,C.W. and Wolf,R.O.(1975). Estimation of zinc concentration of parotid saliva by flameless atomic absorption spectrophotometry in normal subjects and in patients with idiopathic hypogeusia. J Lab. Clin. Med. 86: 175-180.

Henry,R.J. (1968). Clinical Chemistry-Principles and Technics. Harper & Row, 5th Reprint.

Higashi,A.; Tajiri,A.; Matsukura,M. and Matsuda,I.(1988). A prospective survey of serial maternal serum zinc levels and pregnancy outcome. J.Pediatr. Gastroenterol. Nutr. 7: 430-433.

Ho,S.K. and Hidiroglou,M.(1977). Effects of dietary chelated and sequestered zinc and zinc sulphate on growing lambs fed a purified diet. Can.J.An.Sci. 57: 93-99.

Hooper,P.L.;Visconti,L.;Garry,P.J. and Johnson,G.E.(1980). Zinc lowers high-density lipoprotein-cholesterol levels. JAMA 244: 1960-1961.

Howard,J.M.H.(1990). Serum, leucocyte sweat and hair zinc levels-a correlational study.J.Nutr.Med.1: 119-126.

Hsu,J.M. and Anthony,W.L.(1975). Effect of zinc deficiency on urinary excretion of nitrogenous compounds and liver amino acid catabolizing enzymes in rats. J.Nutr.105:26.

Hsu,M.H. and Woosley,R.L.(1972). Metabolism of L-methionine-^{35}s in zinc deficient rats. J.Nutr. 102:1181.

Huber,A.M. and Gershoff,S.N. (1973). Effect of zinc deficiency in rats on insulin release from the pancreas. J. Nutr. 103: 1739-1744.

Hunter,D. and Bomford,R.R.(1968). Hutchisonis Clinical Methods.(15thed.) Bailliere Tindall and Cassell,London.

Hurley, L.S. (1976). In 'Trace elements in human health and disease'.Vol.1. Prasad,A.S. (Ed.) Academic Press, New York, pp.301-314.

I.C.M.R.(1990). Nutrient Requirement and Recommended Dietary Allowances for Indians. Indian Council of Medical Research, New Delhi.

Jackson, S.M. (1981). Anatomy and Physiology for Nurses. The English Language Book Society & Bailliere Tindall London P 147.

Jameson, S. (1976). Effects of zinc deficiency in human reproduction. Acta Med. Scand. 593 : 1-89.

Jeliffe, D.B. (1966). The assessment of the nutritional status of the community. World Health Organisation Monograph series No.53.

Johnson, P.J.; Freeland, J. and Ebangit, M.L. (1978). Saliva: a diagnostic tool for evaluating zinc status in man. Fed. Proc. 37: 217.

Johnson,C.M. and Ulrich,A. (1959). Analytical methods for use in plant analysis. calif.Agric.Expt.Sta.Bull. 766: 54.

Kannel, W.B. and Gordon, T. (1979). Physiological and medical concomitants of obesity : the framingham study. In : Obesity in America. Edited by G.A. Bray. New York, National Institutes of Health Publication.

Kanwar, B.B.; Tripathi, B.R. and Kanwar, B.S. (1983). Occurence of zinc, copper, iron and manganese in soils and wheat plants of important valleys of Himachal Pradesh. Him. J. Agric. Res. 9: 75-81.

Kapoor, R.K.; Misra, P.K.; Dixit, S.; Wakhlu, I.; Sharma, B. and Seth, T.D. (1988). Zinc and intrauterine growth. Ind. Pediatr. 25: 972-976.

Karlinskii, V.M. (1973). Contents of zinc in blood serum, erythrocytes and leucocytes of healthy people. Problemy Gematologii i Perelivaniya Krovi. 18: 28-32.

Kay, R.G. and Tasman-Jones, C. (1975). Lancet 2: 605.

Khalil, N.; Samny, S.G. and Farag, R.S. (1981). Estimation of serum and tissue zinc level in normal Egyptian subjects. J. Egyp. Med. Asso. 64: 439-446.

Khattab, M.; Danasoury, M.; Sharaf, M. and Sherbeny, A.E. (1976). Zinc in serum and urine of diabetics. J. Egypt. Med. Assoc. 59: 597-601.

Kiilerich, S.; Christensen, M.S.; Naestoft, J. and Christiansen, C. (1980). Determination of zinc in serum and urine by atomic absorption spectrophotometry: Relationship between serum levels of zinc and proteins in 104 normal subjects. Clin. Chim. Acta. 105: 231-239.

Kinlaw, W.B.; Levine, A.S.; Morley, J.E.; Silvis, S.E. and Mc Clain, C.J. (1983). Abnormal zinc metabolism in type-II diabetes mellitus. Am. J. Med. 75: 273-277.

Klevay, L.M. (1973). Hypercholesterolemia in rats produced by an increase in the ratio of zinc to copper ingested. Am. J. Clin. Nutr. 26 : 1060-1068.

Klevay, L.M. (1975). Coronary heart disease: the zinc/copper hypothesis. Am. J. Clin. Nutr. 28 : 764-774.

Kochar, G.K. (1981). Effect of Dietary fibre on the utilization of various nutrients. M.Sc. Thesis. P.A.U. Ludhiana, India p 179.

Kochar, G.K. and Renu, S. (1991). Nutritional status of adolescent girls of Kangra Valley. Proceedings of 78th Indian Science Congress.

Kohrs, M.B.; Choh, K.H. and Nordstrom, J.W. (1986). Dietary and clinical factors related to concentrations of zinc and copper in hair from healthy pregnant women. Nutr. Res. 6: 889-903.

Kohrs, M.B.; Nordstrom, J.; Lorah, E.; Sauvage, J.; Shank, R.E. and Brennan, R. (1977). Concentrations of copper, zinc and magnesium in hair and serum of black pregnant women. In: Trace substances in environmental health. 11 Columbia, MO. USA; University of Missouri pp 55-61.

Koo, S.I. and Lee, C.C. (1988). Compositional changes in plasma high-density lipoprotein particles in marginally zinc-deficient male rats. Am. J. clin. Nutr. 47: 120-127.

Koo, S.I. and Ramlet, J.S. (1983). Dietary cholesterol decreases the serum level of zinc : further evidence for the positive relationship between serum zinc and high-density lipoproteins. Am. J. Clin. Nutr. 37: 918-923.

Koo, S.I. and Williams, D.A. (1981). Relationship between the nutritional status of zinc and cholesterol concentration of serum lipoproteins in adult male rats. Am. J. Clin. Nutr. 34: 2376-2381.

Krebs, N.F. and Hambidge, K.M. (1985). Zinc supplementation during lactation: Effects on maternal zinc status and milk zinc concentrations. Cited from proceedings of the fifth international symposium on trace elements in man and animals- Tema 5. Edited by C.F. Mills, I. Bremner and J.K. chesters. Commonwealth agricultural bureau, p 419.

Krebs, N.F. and Hambidge, K.M. (1986) Changes in milk concentrations with duration of lactation in relation to zinc requirements of breast-fed infants. In : Human lactation. 2. Maternal and environmental factors (Edited by Hamosh, M. and Goldman, A.S.) N.Y. USA; Plenum Press. 359-362.

Kumar, S. and Jaya Rao, K.S. (1974). Blood and urinary zinc levels in diabetes mellitus. Nutr. Metabol. 17 : 231-235.

Lazebnik, N.; Kuhnert, B.R.; Kuhnert, P.M. and Thompson, K.L. (1988). Zinc status, pregnancy complications, and labor abnormalities. Am. J. obstet. Gynecol. 158 : 161-166.

Leek, J.C.; Vogler, J.B.; Gershwin, M.E.; Golub, M.S.; Hurley, L.S. and Hendrickx, A.G. (1984). Studies of marginal zinc deprivation in rhesus monkeys.V Fetal and infant skeletal effects. Am. J. Clin. Nutr. 40 : 1203-1212.

Lindeman, R.D.; Baxter, D.J.; Yunice, A.A. and Kraikitpanitch, S. (1978). Am. J. Med. Sci. 275 : 17-31.

Lindeman, R.D.; Clark, M.L. and Colmore, J.P. (1971). J. Gerontol. 26: 358-363.

Lukaski, H.C.; Klevay, L.M. and Bolonchuk, W.W. (1982). Influence of dietary lipids on iron, zinc and copper retention in trained athletes. Fed. Proc. 41 : 275a.

Mahajan, S.K.; Prasad, A.S.; Rabbani, P.; Briggs, W.A. and McDonald, F.D. (1979). J. Lab. Clin. Med. 94 : 693.

Mariyama, M.; Saito, H. and Kabuto, M. (1987). Relationship between twenty four hour urinary creatinine excretion and weight, or weight and height of Japanese children. 33: 185-193.

Martin Mateo, M.C.; Bustamante Bustamante, J. and Quiros, F.B. De. (1974). HIgh zinc in urine and metalloenzymes in diabetes mellitus. Revista Clinica Espanola 132 : 313-318.

Martin Mateo, M.C.; Bustamante Bustamante, J.; Quiros, J.F.B. De and Ortiz Manchado, O. (1975). A study of the metabolism of zinc, its metalloenzymes in diabetes mellitus. Biomed. (Express) 23: 134-136.

McBean, L.D.; Mahloudji, M.; Reinhold, J.G. and Halsted, J.A. (1971). Correlation of zinc concentrations in human plasma and hair. Am. J. Clin. Nutr. 24 : 506-509.

McMichael, A.J.; Dreosti, I.E.; Gibson, G.T.; Hartshorne, J.M.; Buckley, R.A. and Colley, D.P. (1982). A prospective study of serial maternal serum zinc levels and pregnancy outcome. Early Hum. Dev. 7 : 59-69.

McNair, P.; Kiilerich, S.; Christiansen, C.; Christensen, M.S.; Madsbad, S. and Transbol, I. (1981). Hyperzincuria in insulin treated diabetes mellitus-its relation to glucose homeostasis and insulin administration. Clin. Chim. Acta 112: 343-348.

Meadows, N.J.; Ruse, W.; Smith, M.F.; Day, J.; Keeling, P.W.N.; Scopes, J.W.; Thompson, R.P.H. and Bloxam, D.L. (1981). Zinc and small babies. Lancet 2 : 1135-1137.

Meret, S. and Henkin, R.I. (1971). Simultaneous direct estimation by atomic absorption spectrophotometry of copper and zinc in serum, urine, and cerebrospinal fluid. Clin. Chem. 17: 369-373.

Michell, C.O. and Lipschitz, D.A. (1982). Am. J. Clin. Nutr. 35 : 398-406.

Miller, W.J. and Miller, J.K. (1963). Zinc content of certain feeds, associated materials, and water. J. Dairy Sci. 46 : 581-583.

Mills, C.F.; Quarterman, J.; Chesters, J.K.; Williams, R.B. and Dalgarno, A.C. (1969). Metabolic role of zinc. Amer. J. Clin. Nutr. 22 : 1240-1249.

Mitchell, R.L. (1964). Trace elements in soils. In : Chemistry of the soil, Ed. 2 (Bear, F.E., ed.), pp 253-285, Reinhold Publishing Corp., New York.

Mittal, R.; Bhargawa, U.; Anand, S. and Vir, S.C. (1982). Zinc status of Indian pregnant women of low income group and its effect on outcome of pregnancy. Ind. J. Nutr. Diet. 19: 117-124.

Monceda, N.P. De; Bonilla, L.C. De; Villasmil, J.J.; Esteves, J. and Bonilla, E. (1989). Zinc in serum of normal subjects. Investigacion Clinica 30 : 101-109.

Mukherjee, M.D.; Sandstead, H.H.; Ratnaparkhi, M.V.; Johnson, L.K.; Milne, D.B. and Stelling, H.P. (1984). Maternal zinc, iron, folic acid, and protein nutriture and outcome of human pregnancy. Am. J. Clin. Nutr. 40 : 496-507.

Munro, C.S.; Lazaro, C. and Lawrence, C.M. (1989). Symptomatic zinc deficiency in breast-fed premature infants. Br. J. Dermatol. 121 : 773-778.

Mutch, P.B. and Hurley, L.S. (1974). Effect of zinc deficiency during lactation on postnatal growth and development of rats. J. Nutr. 104 : 828-842.

NNMB (1980). Report for the year 1974-1979, NIN Hyderabad.

Nobels, F.; Rillaerts, E.; D'Hollander, M.; Gaal, L. Van. and Leeuw, I. De.(1986). Plasma zinc levels in diabetes mellitus : relation to plasma albumin and amino acids. Biomedicine and Pharmacotherapy. 40 : 57-60.

Nutrition News (1991). 12(2). National Institute of Nutrition. Hyderabad.

O'Dell, B.L. and Savage, J.E.(1957). Potassium, zinc and distillers dried solubles as supplements to a purified diet. Poultry Sci. 36: 459-460.

Oberleas, D. and Prasad, A.S. (1969). Growth as affected by zinc and protein nutrition. Amer. J. Clin. Nutr. 22 : 1304-1314.

Oser, B.L. (1976). Hawk's Physiological Chemistry. Tata McGraw Hill Publishing Company Ltd., New Delhi.

Osis, D.; Kramer, L.; Wiatrowski, E. and Spencer, H. (1972). Dietary zinc intake in man. Amer. J. Clin. Nutr. 25: 582-588.

Pai, L.H. and Prasad, A.S. (1988). Cellular zinc in patients with diabetes mellitus. Nutr. Res. 8 : 889-897.

Parisi, A.F. and Vallee, B.L. (1970). Isolation of a zinc alpha-2 macroglobulin from human serum. Biochemistry 9: 2421-2426.

Passmore, R. and Eastwood, M.A. (1986). Human Nutrition and Dietetics (8th ed.) EIBS Churchill Livingstone.

Patel, P.B.; Chung, R.A. and Lu, J.Y. (1975). Effect of zinc deficiency on serum and liver cholesterol in the female rat. Nutr. Rep. Int. 12 : 205-210.

Pidduck, H.G.; Wren, P.J.J. and Price Evans, D.A. (1970). Hyperzincuria of diabetes mellitus and possible genetical implications of this observation. Diabetes 19: 240-247.

Pories, W.J.; Henzel, J.H.; Rob, C.G. and strain, W.H. (1967). Lancet 2: 121.

Prasad, A.S. (1989). Human zinc deficiency. Proc. Nutr. Soc. India 35 : 1-12.

Prasad, A.S. and oberleas, D.(1970). Binding of zinc to amino acids and serum proteins in vitro. J. Lab. Clin. Med. 76 : 416-425.

Prasad, L.S.N.; Ganguly, S.K. and Vasuki, K. (1974). Role of zinc in foetal nutrition. Ind.Pediatr. 11: 799-802.

Prema, K. (1980). Predictive value of serum copper and zinc in normal and abnormal pregnancy. Indian J. Med. Res. 71: 554-560.

Quarterman, J.; Humphries, W.R.; Morrison, J. and Jackson, F.A. (1973). Effect of zinc deficiency on intestinal and salivary mucins. Biochem. Soc. Trans. 1 : 101.

Quarterman, J.; Mills, C.F. and Humphries, W.R. (1966). The reduced secretion of, and sensitivity to insulin in zinc deficient rats. Biochem. Biophys. Res. Commun. 25 : 354-358.

Qumra, S.R.; Mehta. S. and Deodhar, S.D. (1990). Physical growth in school girls. 2. Relationship to socio-economic status and dietary intake. Ind. Pediatr. 27: 1051-1065.

Raghupathy, L. (1987). Studies on zinc and copper concentrations in hair of human populations from India. In: Trace substances in environmental health. Columbia, MO, USA; University of Missouri pp 88-96.

Randhawa, N.S. (1988). In 5th Convocational address at H.P.K.V., Palampur.

Rea, I.M. (1989). Sex and age changes in serum zinc levels. Nutr. Res. 9: 121-125.

Reeves, P.G. and O'Dell, B.L. (1981). Short term zinc deficiency in the rat and self selection of dietary protein level. J. Nutr. 111: 375.

Reinhold, J.G. (1953). Total protein, albumin and globulin. In: Standard Methods of Clinical Chemistry (Ed. Reiner, M.), Vol.1, Academic Press, New York, P 88.

Reinhold, J.G.; Kfoury, G.A. and Arslanian, M. (1968). Relation of zinc and calcium concentrations in hair to zinc nutrition in rats. J. Nutr. 96 : 519.

Renu, S.K. (1990). Nutritional status of Human Female Population with special Reference to Iodine in Kangra Valley (H.P.). M.Sc. Thesis. HPKV Palampur, India.

Robinson, C.H. and Lawler, M.R. (1982). Normal and Therapeutic Nutrition. (16th ed.), Macmillan Publishing Co. Inc. New York.

Rolland-Cachera, M.F.; Sempe, M. and Guillond-Bataill, M. (1982). Am. J. Clin. Nutr. 36 : 178-184.

Roman, W. (1969). Zinc in porphyria. Am. J. Clin. Nutr. 22: 1290-1303.

Rose, G.A.; Path, M.R.C. and Willden, E.G. (1972). Br. J. Urol. 44: 281-286.

Samman, S. and Roberts, D.C.K. (1988). Zinc and cholesterol metabolism. Nutr. Res. 8: 559-570.

Sandstead, H.; Klevay, L. and Mahalko, J. (1980). Marginal zinc nutriture : effects of lipid metabolism and plasma zinc. Am. J. Clin. Nutr. 33 : 944a.

Sandstead, H.H.; Strobel, D.A.; Logan, G.M. Jr.; Marks, E.O. and Jacob, R.A. (1978). Zinc deficiency in pregnant rhesus monkeys : effect on behaviour of infants. Am. J. Clin. Nutr. 31 : 844-849.

Saudek, C.D. and Young, N.L. (1981). Diabetes 30: 76-81.

Schechter, P.J.; Friedewald, W.T.; Bronzert, D.A.; Raff, M.S. and Henkin, R.I. (1972). Idiopathic hypogeusia : a description of the syndrome and a single-blind study with zinc sulphate. Int. Rev. Neurobiol. 1 : 125-140.

Schroeder, H.A. (1957). Mechanisms of Hypertension. Thomas, Springfield, Illinois.

Schroeder, H.A. (1971). Losses of vitamins and trace minerals resulting from processing and preservation of foods. Amer. J. Clin. Nutr. 24 : 562-573.

Schroeder, H.A. and Nason, A.P. (1969). Trace metals in human hair. J. Invest. Dermatol. 53: 71.

Schroeder, H.A., Nason, A.P.; Tipton, I.H. and Balassa, J.J. (1967). Essential trace metals in man: Zinc. Relation to environmental cadmium. J. Chronic Dis. 20 : 179-210.

Shils, M.E. and Young, V.R. (1988). Modern Nutrition in Health and Diseases (7th ed.) Lea & Febiger Philadelphia.

Shrimpton, R.; Alencar, F.H.; Vasconcelos, T.C. and Rocha, Y.S. (1984). Nutr. Res. (Press). Cited from proceedings of the fifth international symposium on trace elements in man and animals-Tema 5. Edited by C.F. Mills, I. Bremner & J.K. chesters. Commonwealth agricultural bureau, P 90.

Simmer, K. and Thompson, R.P.H. (1985). Maternal zinc and intrauterine growth retardation. Clin. Sci. 68: 395-399.

Sjogren, A.; Edvinsson, L.; Abdulla, M. and Srinivas, U. (1985). Plasma levels of magnesium, copper, zinc and calcium in patients with diabetes mellitus. Cited from proceedings of the fifth international symposium on trace elements in man and animals-Tema 5. Edited by C.F. Mills, I. Bremner & J.K. Chesters. Commonwealth agricultural bureau, p 774.

Sjogren, A.; Edvinsson, L.; Floren, C.H. and Abdulla, M. (1986). Zinc and copper in striated muscle and body fluids from subjects with diabetes mellitus type I. Nutr. Res. 6: 147-154.

Smith, H. (1967). Forensic Sci. Soc. J. 7: 97.

Soltan, M.H. and Jenkins, D.M. (1982). Maternal and fetal plasma zinc concentration and fetal abnormality. Br. J. Obstet. Gynaecol. 89: 56-58.

Somers, M. and Underwood, E.J. (1969). Studies of zinc nutrition in sheep. II. The influence of zinc deficiency in ram lambs upon the digestibility of the dry matter and the utilization of the nitrogen and sulphur of the diet. Austral. J. Agric. Res. 20: 899-903.

Spencer, H. and Samachson, J. (1970). Studies of zinc metabolism in man. In : Trace Element Metabolism in Animals (Mills, C.F., ed.), PP 312-320, E. and S. Livingstone, Edinburgh.

Steele, T.H. (1973). Dissociation of zinc excretion from other cations in man. J. Lab. Clin. Med. 81 : 205-213.

Stephen, J.K. and Hsu, J.M. (1972). Zinc deficiency and DNA metabolism in rat skin. Fed. Proc. 31 : 667.

Strain, W.H.; Steadman, L.T.; Lankau, C.A.; Berliner, W.P. and Pories, W.J. (1966). Analysis of zinc levels in hair for the diagnosis of zinc deficiency in man. J. Lab. Clin. Med. 68: 244.

Sullivan, J.F.; Parker, M.M. and Bonett, J.D. (1969). Incidence of low serum zinc in non-cirrhotic patients. Proc. Soc. Exp. Biol. Med. 130: 591-594.

Swaminathan, M. (1988). Essentials of Food and Nutrition. Vol II (2nd ed.). The Banglore Printing and Publishing Co. Ltd.

Swaminathan, M. (1990). Essentials of Food and Nutrition. Vol.1 (2nd ed.). The Banglore Printing and Publishing Co. Ltd.

Swanson, C.A. and King, J.C. (1982). Zinc utilization in pregnant and nonpregnant women fed controlled diets providing the zinc RDA. J. Nutr. 112: 697-707.

Swanson, C.A. and King, J.C. (1987). Zinc and pregnancy outcome. Am. J. Clin. Nutr. 46 : 763-771.

Szewczynski, J. (1979). Determinants of the concentration of haemoglobin in girls from 15-19 years old with particular regard to socio-economic factors. 1. Characteristics of socio-economic conditions and qualitative assessment of the diet. 30: 535-543.

Tao, S.H. and Hurley, L.S. (1971). Changes in plasma proteins in zinc deficient rats. Proc. Soc. Exp. Biol. Med. 136: 165.

Todd, W.R.; Elvehjem, C.A. and Hart, E.B. (1934). Zinc in the nutrition of the rat. Amer.J. Physiol. 107: 146-156.

Tucker, H.F. and Salmon, W.D. (1955). Parakeratosis or zinc deficiency disease in the pig. Proc. Soc. Exp. Biol. Med. 88: 613-616.

Umoren, J and Kies, C. (1982). Menstrual blook losses of iron, zinc, copper and magnesium in adult female subjects. Nutr. Rep. Int. 26 : 717-726.

Underwood, E.J. (1971). Zinc. In : Trace elements in human and animal nutrition. 3rd ed. New York, NY : Academic Press pp 208-252.

Vallee, B.L. (1959). Biochemistry, physiology and pathology of zinc. Physiol. Rev. 39: 443-490.

Vir, S.C.; Love, A.H.G. and Thompson, W. (1981). Zinc concentration in hair and serum of pregnant women in Belfast. Am. J. Clin. Nutr. 34 : 2800-2807.

Vuori, E. and Kuitunen, P. (1979). The concentrations of copper and zinc in human milk : A longitudinal study. Acta Paediat. Scand. 68 : 33-37.

Warkany, J. and Petering, H.G. (1972). Congenital malformations of the central nervous system in rats produced by maternal zinc deficiency. Teratology 5: 319-334.

Wegener, W.S. and Romano, A.H. (1963). Zinc stimulation of RNA and protein synthesis in Rhizopus nigricans. Science 142: 1669-1670.

WHO study Group on 'Iron Deficiency Anaemia' (1959). WHO Tech. Rep. Ser. 182, WHO, Geneva.

Wight, P.A.L. and Dewar, W.A. (1978). Aspects of the histology of zinc deficiency in domestic birds. In: Trace Element Metabolism in Man and Animals -3 (Kirchgessner, M. ed.), pp 215-217.

Williams, R.B.; Mills, C.F.; Quarterman, J. and Dalgarno, A.C. (1965). The effect of zinc deficiency on the in vivo incorporation of ^{32}P into rat-liver nucleotides. Biochem. J. 95 : 29-30.

Winterberg, B.; Bocchicchio, M.; Hossdorf, T.; Lahl, H.; Lison, A.E. and Zumkley, H. (1989). Zinc in the treatment of diabetic patients. Trace elements in Medicine 6: 173-179.

Woods, D.L.; Sive, A.A.; Pocock, F. and Dempster, W.S. (1988). Zinc in the mother and infant at delivery. South African J. Sci. 84 : 212-213.

Wootton, I.D.P. (1974). Micro Analysis in Medical Biochemistry (5th ed.).Churchill Livingstone. Edinburgh and London.

Yasodhara, P.; Ramaraju, L.A. and Raman, L. (1991). Trace minerals in pregnancy. 1. Copper and zinc. Nutr. Res. 11: 15-21.

Zhou, J.P.; Hong, Z.Y. and Zhou, J.D. (1984). Zinc concentrations in human milk and in the blood of infants. Shanghai Med. J. 7: 323-325.