Discussion
4.1 Obesity

The epidemiological studies on Indian population made during present investigations revealed a positive linear correlation between hair zinc (Zn) concentration and body mass index (B.M.I.) in healthy volunteers indicating that the B.M.I. increases with increase in Zn concentration in hair. These results are in accordance with the report of Taneja et al. (1996) who also observed a rise in height adjusted body weight with increase in Zn concentration in hair of Chandigarh population. Prentice (1993) also recorded an accelerated weight gain, linear growth and synthesis of lean tissue with Zn supplementation during nutritional rehabilitation of Zn-deficient children; but, in healthy children, the excess Zn in diet had little impact on weight gain or height. However, their body composition was altered due to the increase in the portion of body fat during their 25 weeks period of dietary treatment. Since fat is lighter and duration of this study was shorter, the weight increment in this study is expected to be insignificant compared to control children. But, greater bioavailability of Zn in adults over a period of years in their natural diet as reflected by the hair Zn concentration in the present study was significant enough to reflect the changes in body wt. leading to obesity. The higher the Zn concentration greater was the degree of obesity and vice versa in the volunteers of Chandigarh population. This observation found support from the serum Zn concentration which also exhibited a positive correlation with B.M.I. amongst the volunteers having no disease. Role of Zn in the body wt. increment becomes conspicuous right at the time of birth. A study conducted by Neggers et al. (1990, 1991) revealed a threshold for maternal serum zinc concentration below which the prevalence of low birth weight increased significantly and that the
maternal serum zinc concentration measured early in pregnancy to identify those women at higher risk of giving birth to a low-birth-weight infant.

In contrast to this, Chen et al. (1988) recorded a negative correlation between hair Zn and B.M.I. Atkinson et al. (1978) also observed that obese subjects possessed lower but normal plasma level of Zn compared to non-obese individuals. In fact, the plasma Zn status does not truly reflect the long term body Zn status as it tends to maintain Zn homeostasis, either by increasing or decreasing Zn excretion depending upon Zn intake (Lee et al., 1993).

The normal hair Zn concentration, recorded in some of the populations ranges between 2 to 4.6 μ mol/g (Kennedy and Failla, 1987; Chen et al., 1988 and Di-Martino et al., 1993). This value has been reported from the individuals having B.M.I. < 25 kg/m². If all the volunteers with B.M.I. < 25 kg/m² were classified as normal subjects in the study as did the investigators perse, its mean value, 4.1 ± 0.59 μ mol/g in women and 4.73 ± 0.4 μ mol/g in men, falls close to the upper limit of normal range. Chen et al. (1988) on the other hand, had reported its value between 2.17 to 3.14 μ mol/g in the population of Taiwan which occupies the lower limit of the normal range.

Normal range for the human serum Zn had been reported 10.7 to 23 μ mol/l (Baselt, 1980). Chen described 10.4 ± 0.7 μ mol/l for normal (B.M.I. < 25 kg/m²); 13.6 ± 0.5 μ mol/l for overweight (B.M.I., 25 to 30 kg/m²) and 12.5 ± 0.6 μ mol/l for obese (B.M.I. < 30 kg/m²). The serum Zn concentration observed in Chandigarh, population is within the range extending between 10.7 to 23 μ mol/l as reported by Baselt (1980). However, the serum Zn concentration of normal volunteers (B.M.I. < 25 kg/m²) was approximately equal to the one reported by Chen et al. (1988) but
was significantly higher both in obese and overweight suggesting thereby higher Zn reserves in overweight and obese volunteers in Indians as compared to Taiwanese. The difference of Zn concentration between the obese of Taiwanese population reported by Chen et al. (1988) and that of Chandigarh population in this study may possible be either due to racial difference or due to the difference in amount of bioavailable Zn in diet.

The segment of Indian population belonging to the upper middle or high income group during this study consumes wheat flour poor in bran, legumes, milk products, low fibred vegetables and complete lack of soybean based products that constituted the bulk of the diet of these volunteers. These food components possessed factors such as phytates and fibres, less in amount, which bind Zn in the lumen of intestine (Agte et al., 1994) in contrast to fibre rich leafy vegetable and phytate based soybean diet containing high Zn binding components of Taiwanese (Tian et al., 1995) and Western world (Biro et al., 1996 and Ballew and Sugarman, 1995) possibly contributed to this difference. Agte et al. (1994) also reported that certain dietary components used along with diet by Indians such as hemicellulose, cereal protein, niacin and milk proteins act as significant enhancers in zinc absorption while legume proteins had inhibitory effect on copper absorption. Since the consumption of cereal proteins and milk product in diet is rather high in North Indian population, their presence in diet perhaps may be the cause of high Zn concentration obtained in hair and blood serum of volunteers during present investigation.

Although the present data showed that Zn aggravates fat deposition in the body but these data did not find conclusive support from other studies on human
population. In order to differentiate between the two contradictory observations, experimental studies conducted on mice gave a better picture. Feeding of mice on isocaloric diet containing Zn as 20, 40, 60 and 80 mg/kg diet, a consistent rise of adipocytes in subcutaneous and abdominal tissue with increase in Zn in diet was discernible in Sudan black - B preparations meant to localise total fat. The biochemical studies of these mice not only supported the high fat content in these tissues but also in liver, despite the fact that there had been an equal amount of sucrose, casein and fat contents besides vitamins and other minerals in diet. The histochemical studies supported by biochemical estimations amply clarify that the amount of fat deposition increases with increase in Zn content in diet and thus support our observation obtained in human population of Northern India. Welsh et al. (1985); Kennedy et al. (1986) and Prohaska et al. (1988) conducted similar studies on animal models and observed a reduced level of Zn in obese than in lean littermates. A difference in the metal status especially of Zn level, where Zn has been shown to decrease in obese, has been attributed to the dilution of trace metals due to the presence of high concentration of neutral lipids in chronically obese mice (Kennedy et al., 1986). Kennedy et al. (1986) showed that 22 weeks old genetically ob/ob mice possessed 75% of excess Zn in liver compared to those of lean mice when the metal status was measured after the extraction of neutral lipids in them, despite the fact its level was lower in obese than that of lean mice when estimated without extraction of triglycerides. Similar results were obtained by Begin-Heick et al. (1985) who observed higher concentration of Zn in fat free liver and triceps muscles from ob/ob mice than lean control. The relationship of Zn absorption and deposition of fat in ob/ob and high fat induced ICR mice has been
documented by Chen et al. (1995). They have reported that the mice (ICR) fed a high fat diet containing Zn in higher amount resulted in a significantly higher body wt. identical to obese mice than their control counterparts fed on high fat but low amount of Zn in diet. They suggested that the dietary Zn-supplementation increases fat deposition via metabolic regulation of utilization of dietary fat and favouring this condition for obesity. Lopez et al. (1991) associated obesity with an increase in Zn values of serum. The increased transfer of orally administered Zn from the lumen of gastrointestinal tract to the blood plasma of obese mice has been attributed to the altered characteristics of Zn metabolism since they have greater potential of absorbing Zn from a simple solution and 60% more of glucose absorption in them than those of lean controls (Kennedy and Failla, 1987; Begin-Heick et al., 1985 and Lin et al., 1992).

The increased absorption of glucose caused by excess Zn in diet alone cannot account for the aggravation of fat deposition in mice studied during present investigations. There was an increase in plasma glucose, plasma triglycerides, plasma cholesterol and increase in the total lipids, glycogen, triglycerides and phospholipids in various tissues which reflected not only the excessive glucose absorption but also other nutrients. The nutrients absorption is dependent upon their rate of hydrolysis in the gastrointestinal tract depending upon the amount of enzymes liberated from the associated digestive glands. The ultrastructure of pancreas of the mice fed on a diet containing 40 mg Zn/Kg diet showed greater amount of zymogen granules in the acinar cells of the of mice fed on 20 mg Zn/kg diet indicating thereby that the excess Zn stimulates the acinar cells of pancreas to synthesize more of zymogen granules leading to their excessive release in the
pancreatic juice. Since the rate of hydrolysis of a substrate depends upon the enzyme concentration in the medium, excessive liberation of zymogen is likely to result into availability of larger amount of nutrients for their absorption by the intestine. Their excessive absorption leads to the increased amount of fat in the adipocytes resulting their growth and also in other tissues which in fact has been reflected in the adipocytes of mice during present investigation.

The reported lower concentration of Zn in obese, in contrast to the present study may be attributed to its sequestering from the blood plasma by growing adipocytes that have shown to retain five fold more Zn than their normal counterparts (Kennedy and Failla, 1987). As a consequence of this, the plasma Zn and Zn in other tissues is likely to fall which in fact had been reported in severely obese SMR corpulent rat (Failla and Michaelis, 1984), in obese C578/6J ob/ob mouse (Begin-Heick et al., 1985 and Kennedy et al., 1986) and obese Zucker rat (Donaldson et al., 1987).

Luque-Diaz et al. (1982) reported that obese patients show hyperzincuria and/or normo or hypozincemia. These studies on obesity provide evidences in favour and against the role of zinc in its aetiology. This situation could occur due to insufficient supply of Zn in diet, unable to maintain plasma Zn, in response to the demand of Zn by the growing fat cells. This is particularly true in genetically obese mice where the fat accumulation in the body is far greater than in the non-obese mice (Kennedy and Failla, 1987). During present investigation, the genetically non-obese mice were employed, hence the increase in Zn concentration in tissues with its increase in diet occurred due to smaller amount of fat deposition but more than their control counterparts.
Discussion

Further, in the volunteers of Chandigarh population there was a negative linear correlation between urine Zn and B.M.I. which was more conspicuous in case of men than in women. This inverse relationship between the two indicated the increased retention of Zn in the body with increase in B.M.I. caused by fat deposition identical to the observations made on obese mice (Kennedy and Failla, 1987). The mean Zn concentration in urine in the present set of normal volunteers was recorded as $3.12 \pm 0.41 \mu \text{mol/l}$ in the normal healthy women and $3.25 \pm 0.22 \mu \text{mol/l}$ in the normal healthy men.

The normal Zn excretion in humans has been reported as 500-800 $\mu \text{mol/day}$ (Abu Hamdan et al., 1981). If the total excretion of urine is taken as 1 ml/min., it amounts to 1.5 l/day. The range of Zn in urine accordingly would be 5.1 to 8.16 $\mu$ mol/l for the reported value of Zn in urine in healthy population in Western countries. In contrast to this, the value of urine Zn in Chandigarh population was 1.5 times lower than the normal population reported elsewhere. The fall of Zn concentration in urine in Northern Indian population coincided well with higher Zn concentration in hair reflecting a genetic difference between the population of different ethnic groups. Its fall in overweight and obese volunteers further point out this difference between North Indian population and that of Taiwan where its concentration in obese was reported higher, close to the one reported by Abu Hamdan et al. (1981).

The variations recorded in hair Zn, serum Zn and urine Zn within the four B.M.I. group may, therefore, appear to depend upon the nutritional state of body reflected in terms of B.M.I. The B.M.I. being an index of total fat content with the correlation co-efficient generally above 0.5 (Bray, 1992), its positive correlation with
hair Zn not only associates the role of body fat in Zn retention but also signifies its physiological importance. The higher Zn concentration recorded in obese volunteers of North Indian population, observed during present investigation than those of their lean controls clearly associated the role of Zn in the development of obesity. Large increases in body fat deposition in mice fed a high Zn in high fat diet, identical to ob/ob mice (Chen, et al., 1996) and increase in subcutaneous fat in healthy children fed a diet containing double the amount of Zn than, did their control (Prentice, 1993) lend further support to this concept.

The reported lower concentrations of Zn in chronic obese (Chen et al., 1988 and Pleban and Matsushige, 1980) in contrast to this concept may possibly be the result of the sequestering of Zn from blood plasma by the growing adipocytes which tend to retain higher amount of Zn than the healthy volunteers. Consequently the growing adipocytes tends to retain higher amount of Zn than the healthy volunteers. Accordingly, the blood plasma Zn is likely to fall reflecting a reduction in it as the B.M.I. increases. The return of serum Zn from lower to normal in obese on Zn therapy (Di-Martino et al., 1993) and reduction in urinary Zn excretion with increase in B.M.I. observed during present epidemiological studies on Chandigarh population provide further support to this.

The analysis of Cu concentration in hair, serum and urine samples of male and female volunteers provided interesting information relating to obesity and Zn-Cu interaction in them. Hair Cu in women with B.M.I. < 25 kg/m² was 0.305 ± 0.05 µ mol/g and in men 0.72 ± 0.098 µ mol/g. These values of Cu in hair were slightly higher than those of healthy volunteers reported previously (Durak et al., 1990 and Piccinnini et al., 1996) who recorded the range of hair Cu between 0.186 to 0.24 µ

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mol/g in hair. The mean serum Cu was slightly lower in present set of volunteers with B.M.I. < 25 kg/m² (11.5 ± 0.781 μ mol/l) against 14.3 to 17.36 μ mol/l reported previously (Litzman et al., 1995 and Piccinnini et al., 1996). However, serum and hair Cu concentration increased linearly with B.M.I., the former being more strong.

Similarly the mean urine Cu concentration in the normal healthy women with B.M.I. < 25 kg/m² recorded as 0.39 ± 0.02 μ mol/l and 0.475 ± 0.05 μ mol/l in men which on comparison with the previous reported value of 0.36 ± 0.05 μ mol/l (Chen et al., 1995) did not differ significantly.

Thus the data of this study suggest that the Zn and Cu concentrations in tissues increase with increase in the degree of obesity (B.M.I.). This may possibly be due to the increase in the output of pancreatic enzymes resulting in the increased hydrolysis and absorption of nutrients and their subsequent deposition in adipocytes as revealed by the experimental data on mice.

4.2 NON-INSULIN DEPENDENT DIABETES MELLITUS

The data of NIDDM patients with hyperglycemia and glucosuria (group I) revealed a low Zn concentration in serum (hypozincemia) and in hair and a five fold rise in its concentration in urine (hyperzincuria) in both the sexes than those of their controls. The Cu content, on the other hand was significantly less in serum (hypocupremia) and hair but it remained unchanged in urine (normocupruria). This data reflected a significant loss of Zn in tissues through urine and reduction of Cu without loss in NIDDM patients compared to those of healthy volunteers, inspite of the fact that the B.M.I. in the two groups was identical.

These results are consistent with the reports of the previous workers who also observed hyperzincuria which appears to be a consistent feature in all the
diabetics, both NIDDM and IDDM patients (Chen. et al., 1995; Walter et al., 1991; Sjogren et al., 1986; Raz and Havivi, 1989; Kinlaw et al., 1983; Levine et al., 1983; McNair et al., 1981; Cunningham et al., 1994; Heise et al., 1988 and Brun et al., 1988) consequently resulting in hypozincemia (Sjogren et al., 1986; Kumar and Rao, 1974; Schlienger et al., 1988; Thompson and Godin, 1995; Kinlaw et al., 1983; Golik et al., 1993 and Car et al., 1992; Walter et al., 1991 and Chen. et al., 1995). However normozincemia was reported by Kiiierich (1985); Pidduck et al. (1971); Tarui (1963); Davies et al. (1968); Rosner and Gorfien (1968); Pidduck et al. (1970); Alexander (1979) and Cunningham et al. (1994), and hyperzincemia by Conston et al. (1964); Mateo et al. (1978) and Chooi et al. (1976) in both NIDDM and IDDM patient.

The hyperzincemia and normozincemia may possibly represent the early stages prior to the onset of hypozincemia. Zinc perturbation was also reported in erythrocytes (Kumar and Rao, 1974; Raz and Havivi, 1989 and Rosner and Gorfien, 1968) and leucocytes (Sjogren et al., 1986 and Rosner and Gorfien, 1968). Type I (IDDM) and type II (NIDDM) diabetic subjects have lower concentration of Zn in lymphocytes, granulocytes and platelets compared with those of control subjects (Pai and Prasad, 1988) and lower Zn fraction in erythrocytes (Chen. et al., 1995) which represents a situation identical to that of hair. The depletion of Zn in tissues and high zinc excretion appears to cause vulnerability from moderate to severe zinc deficiency in NIDDM patients as the age advances.

The NIDDM patients included in this study possessed a reduced Cu concentration in their hair and serum than the healthy volunteers. In contrast to this observation, most of the previous worker have recorded either normocupremia
(Pidduck et al., 1970) or hypercupremia (Chen et al., 1995; Schlienger et al., 1988; Mateo et al., 1978; Culebras et al., 1978 and Walter et al., 1991) in diabetic patients. The Russian diabetics however were found to have low serum Cu but those with gangrene had its high levels (Kuleshova, 1973).

Since the individual investigated consumed almost same type of food, the possibility of low copper content in diet of NIDDM patients was unlikely to exist. The increased absorption of Zn may be the because of the known Cu - Zn antagonistic interaction (Fisher et al., 1981; Ogiso et al., 1979 and Hall et al., 1979).

NIDDM patients exhibited normocupruria in the current studies which is in accordance with the findings of Chen et al. (1995). The low Cu levels in hair and serum and normal excretion of Cu in urine suggest that these patients are deficient in Cu possibly due to either reduced bioavailability of Cu in diet or excessive presence of Zn in it. There are increasing evidences which suggest a Cu-deficiency conditions in tissues, more particularly so in muscles, as revealed by the muscle biopsies of diabetic patients (Sjogren et al., 1986) and in the erythrocytes (Chen et al., 1995).

The NIDDM patients complicated with either hypertension (group II) or hypercholesterolemia (group III) revealed different results. The hair Zn values of hypertensive NIDDM and hypercholesterolemic NIDDM patients were insignificantly lower from their control counterparts but it was higher than that of the NIDDM patients with no other complication. The serum zinc on the other hand in hypertensive NIDDM patients was quite high while the serum zinc of NIDDM without complication was less than their control counterparts.
Discussion

All the NIDDM patients with or without any other complication exhibited hyperzincuria and excreted Zn in the order of: Healthy controls < NIDDM with hypercholesterolemia < NIDDM with hypertension < NIDDM without any other complication and the levels of serum zinc were in the order of: NIDDM without any complication < healthy controls < NIDDM with hypertension and NIDDM with hypercholesterolemia.

These results depict that NIDDM with hypercholesterolemia or hypertension were prevalent in overweight or obese diabetics and they exhibited hyperzincuria and hyperzincemia which differentiated them from plasma and its excessive excretion may perhaps be responsible for the manifestation of these additional disorders associated with NIDDM.

4.3 Hypercholesterolemia and Hypertension

The copper level in hypercholesterolemic and hypertensive NIDDM patients was significantly lower in hair as well as in blood plasma but they exhibited hypercupruria. There was a general trend of Cu loss in hypertensive and hypercholesterolemic NIDDM patients. The low level of Cu in tissues and its higher excretion appeared to have made it a two way loss; firstly reduction of Cu absorption at the intestinal level caused by Zn - Cu antagonism due to high Zn absorption, as displayed by the presence of hyperzincemia and hypocupremia in these two groups and secondly, a high rate of Cu excretion. The excessive Cu loss might have resulted in the manifestation of the disease associated with hypertension and hypercholesterolemia, since the NIDDM without any complication exhibited normocupruria.
Discussion

An analysis of the metal status of hypercholesterolemic patients without diabetes (Group IV) and hypercholesterolemic without diabetes but complicated by hypertension (Group V), usually associated with a higher range of B.M.I., were mainly in the category of obese revealed an insignificantly different Zn concentration in hair, serum and urine from those of their control counterparts. Urine zinc exhibited a negative correlation and hair Zn a positive correlation with B.M.I. in hypercholesterolemic patients suggesting an increased retention of Zn in tissues with increase in body mass index. The serum Zn was significantly higher in hypertensive hypercholesterolemic patients than in their control counterparts as well as from those of hypercholesterolemic patients without any complication which suggests a positive role of serum Zn in the aetiology of hypertension.

In contrast to Zn, the Cu concentration in hair and serum was significantly less in hypercholesterolemic and hypertensive complicated hypercholesterolemic patients compared with those of control counterparts. The excretion of Cu through urine was however greater in them (hypercupruria). This reflects a lower amount of retention of Cu causing a copper deficiency in them.

The hypertensive patients who had no other complication possessed zinc concentration in hair identical to those of their control counterparts. But the zinc fraction in serum was recorded 2 to 2.5 times more, highest of all the patients investigated, while the Cu levels in hair, serum and urine was comparable to those complicated with hypercholesterolemia.

A unique observation of presence of hyperzincemia in the hypertensive individuals with or without any complication in Chandigarh population recorded is in conformity with those of Lopez et al. (1991); Ripa and Ripa (1994) and Davydenko
et al. (1995,b) who also have suggested that high level of Zn was related with the high level of arterial hypertension. The zinc levels of hypercholesterolemic patients haven’t exhibited much perturbations except for displaying the marked Cu-deficiency in them reported earlier also by Klevay (1982); Lau and Klevay (1981) and Harvey and Allen (1981). Collectively, the above observations provide a reasonable case for a disposition towards an imbalanced Zn-Cu status in disease inflicted individuals.

4.4 Experimental Results

The experimental studies conducted on mice displayed a NIDDM like situation in the mice of phase-I. The increase of Zn in diet, resulted in the proportional rise of Zn in plasma, skin, liver and hair until group-III (ZS-III) followed by its fall in these tissues of group-IV (ZS-IV) mice a situation identical to one observed in NIDDM descendants and NIDDM patients, respectively. The glucose appeared in urine in mice of group-IV (ZS-IV) of phase-I on the beginning of 7th week and continued thereafter. These results provided the evidence that excess bioavailability of Zn triggers the onset of NIDDM which is consistent with those of NIDDM patients wherein excessive Zn absorption and retention in NIDDM descendants continue to occur until the onset of NIDDM. The increase of Zn in diet, resulted in a proportional fall in the amount of Cu in the tissues of mice including blood plasma leading to Cu-deficiency condition attributed to Zn-Cu antagonism. This was further supported by Zn-Cu ratio in blood plasma which increased in animals of ZS-I to ZS-IV of phase-I. Animals of ZS-IV that displayed glucose in urine, possessed lower Zn-concentration in tissues compared to those of ZS-III, despite higher Zn concentration in diet may be attributed to excessive Zn loss in
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urine just like that observed in diabetic humans. The hyperzincuria observed in experimental mice and diabetic patients on the onset of disease and thereafter seems to be an adaptive protective measure against Zn-toxicity owing to its high degree of deposition in tissues attributed to its excessive bioavailability earlier.

The supplementation of Cu to the mice of phase-I restored the Cu-Zn ratio to normal levels in CS-III and CS-IV group of mice after eight weeks of feeding during phase-II of experiment. Interestingly, the plasma glucose status in them was restored to normal level. However, in CS-II mice in phase-II developed hyperglycemia like situation. This might be due to inability of dietary Cu to prevent excessive absorption to Zn due to former's insufficient amount to counteract the delayed hyperglycemia.

Further, the excessive Zn resulted in the onset of NIDDM was evidenced by the glycogen level in liver which increased significantly in ZS-I to ZS-III of phase-I mice followed by its reduction in ZS-IV group that had developed NIDDM like condition. The loss of glycogen in liver has been frequently reported in NIDDM patients (Beck-Nielson, 1992; Bogordus et al., 1984 and Damsbo et al., 1988). Again Cu-supplementation to the Zn-loaded mice of phase-I, prevented further loss of glycogen in group IV mice (CS-IV) in phase-II, estimated after eight-ten weeks of the onset of NIDDM. Instead, a slight increase of its level was recorded in them. However, the CS-II mice of phase-II on Cu-supplementation recorded almost two fold increase of glycogen in them and similar amount of glycogen was found in control mice for phase-II experiment. This increase of glycogen level in liver might not only be related to age factor but also suggest that the Cu-supplementation less than double the amount than its RDA value, did not produce significant effect in
blocking the Zn influx. Amount of Cu greater than two fold than R.D.A. reduced Zn-influx leading to restoration of glycogen level in liver as was indicated in group-III mice in phase-II.

That excess Zn promotes fat deposition in adipocytes resulting in obesity and obesity associated disorders were evident when the data of lipids were analysed. The triglyceride levels increased steadily in skin, liver and abdominal muscles in group ZS-II to III of phase-I. Its level in abdominal muscles and liver decreased in ZS-IV group of mice, a situation identical to NIDDM patients with policosanol, wherein the triglycerides have been reported to fall, though insignificantly (Torres et al., 1995). However, the subcutaneous fat was not affected in group-IV of phase-I mice.

It has been postulated that triglyceride concentration rises in NIDDM conditions. Lopez et al. (1991) established a correlation between zincemia and glycemia, and between glycemia and triglyceridemia in the human population. Zn\(^{2+}\) and insulin have been shown to have a strong relationship and in vitro Zn\(^{2+}\) mimics insulin in stimulating glucose transport (May and Contoreggi, 1982 and Ezaki, 1989) and its role in D-(3-3H) glucose incorporation into lipids (Shechter, 1987) and glucose oxidation by pentose phosphate pathway (May and Contoreggi, 1982). Zn, therefore, clearly plays a positive role towards lipogenesis and increases triglyceride concentration in blood and tissues both in humans and rodents. The results of the present studies on mice, are very close to this interpretation as revealed by an increased dietary Zn in phase-I simultaneously increased the levels of triglycerides and total lipids in tissues from ZS-I to ZS-III stage of phase-I.
Discussion

The fall of total lipids and triglycerides in some tissues appear to occur because of the onset of NIDDM at ZS-IV stage in phase-I in which entry of glucose in the tissues decreased significantly as revealed by a fall of glycogen concentration in these tissues, and increased mobilization of lipids for energy purposes causing their depletion in the tissues. Since the NIDDM in mice was only two weeks old when they were sacrificed the reduction of total lipids and triglycerides in tissues particularly the skin were not evident, perhaps their fatty reserves might be metabolized at a later stage.

Plasma cholesterol also increased sharply in ZS-II mice and became static thereafter despite increasing Zn concentration in the diet of ZS-IV mice suggesting its role in triggering hypercholesterolemia as was reported by Ripa and Ripa (1994) in human and rodents. Therefore the hypercholesterolemic condition has been reported to occur both in high serum Zn or low Cu-levels in tissues. The supplementation of Zn in high amount in diet results in the equivalent fall of Cu in tissues in this study and the recovery of cholesterol and Cu concentration on supplementation of Cu in diet of mice in phase-II reflect that the hypercholesterolemia is primary to Cu-deficiency and Cu-supplementation restores its normal level despite high concentration of Zn in diet. This is in accordance with the reports of Van Campen (1966) and Reeves et al. (1993) who suggested that high amount of Zn inhibit Cu uptake resulting in its deficiency. The experimentally induced NIDDM in rats through streptozotocin injections further support the fact that Zn concentration increases in liver and kidneys as was reported by Failla and Kiser (1981); Johnson and Evans (1984) and Spittle and Failla (1983). Similar results are also obtain in spontaneous diabetes (Failla and Gardell, 1985). Moreover Oster et
al. (1994) observed elevated concentration of both Cu and Zn in diabetes associated with hyperphagia in diabetic Sprague-dawley rat. Since the mice in phase-I of the experiment were not hyperphagic, hence it is difficult to comment on this at the moment.

Active oxygen species (AOS) are involved in the pathogenesis of a variety of diseases including NIDDM, hypertension and other related diseases (Babiar, 1978; McCord, 1985; Sies, 1985 and Emerit and Packer, 1990) and have been implicated in the aetiology of diabetic complications (Wolff, 1993 and Gillery et al., 1989). The enzyme superoxide dismutase acts as a natural scavenger against these active free radicals of oxygen by its oxygen detoxifying behaviour in human (Yamanaka et al., 1979). Since the SOD activity during present investigation was estimated in cytosol fraction it may possibly be representing Cu-Zn SOD fraction as reported by Rest and Spitznagel (1977). The activity of SOD (Cytosol fraction) declined gradually in the liver of mice in various groups of phase-I, though this fall remained insignificant till group ZS-III stage but in ZS-IV it fell significantly from those of other groups which revealed the association of reduction of SOD with the altered metal status in the tissue. The restoration of Cu-Zn ratio to its control level by Cu supplementation in phase-II not only eliminated but also enhanced the enzyme activity in the experimental animals which revealed its role in the aetiology of NIDDM. This lends support to the work of Robbins et al. (1980) who had reported a protective action of SOD against the onset of induced diabetes mellitus by streptozotocin.

The results of epidemiological studies suggest that the patients of NIDDM; NIDDM complicated with hypercholesterolaemia; NIDDM complicated with hypertension; hypercholesterolemic without any complication; hypercholesterolemic complicated
with hypertension and those with arterial hypertension only, absorb excessive Zn but are incapable of retaining it in their tissues. Consequently, most of it is excreted through urine except in case of hypertension or NIDDM complicated with hypertension or hypercholesterolemia. The Cu, on the other hand, is absorbed in lesser amount and most of it is retained by the tissues and smaller amount of it is excreted out. This ionic imbalance between Zn and Cu appears to be Cu-Zn antagonism which may occur due to genetic predisposition. The role of genetic component in above mentioned diseases in human population has not been documented so far.

The data of the descendants of NIDDM parents who had not been diagnosed for the disease at the time of investigations showed a higher Zn concentration in their hair and lower concentration in urine and slightly higher hair and urine Cu concentration than those of NIDDM patients. This data suggest that NIDDM susceptible subjects (NIDDM-descendants) appear to be genetically predisposed to excess Zn absorption. The continuous influx of zinc due to its excessive absorption causes Cu-deficiency over a period of time as has been observed in the hair and urine of NIDDM patients. Cu-deficiency is known to cause glucose intolerance, hypercholesterolemia and hypertension (Klevay, 1987 and Klevay et al. 1986 and Klevay, 1990).

Thus the data of this study suggest that NIDDM occurs due to metabolic imbalance of Cu and Zn (Cousins, 1985). These two metallic ions are regulated in the body by metallothionein. The metallothionein are low molecular wt. proteins with multiple cystein residues (Irato et al., 1996). Their synthesis is induced by some of the metals to which they bind and by various agents such as glucocorticoids (Hager
and Palmiter, 1981) and stress (Hamer, 1986). Zinc binds with cysteine residues on its molecules which serve as a mean of sequestering Zn. This property of the protein provides opportunity to sequester Zn when in excess, serving Zn reservoir and delivers on event of its deficiency to the tissue and thus play a major role in the maintenance of Zn homeostasis (Kelly et al., 1996) in the body. Oberleas (1996) reported that the pancreas is an important organ involved in Zn homeostasis. Dalton et al. (1996) found that the metallothionein concentration was the greatest in pancreas in transgenic mice that had the overexpression of metallothionein I (MTI). The pancreatic metallothionein concentration has been considered exceptionally sensitive to Zn status in the body as Zn administration has been observed to increase its level in pancreas (Wang et al., 1996). The electron micrographs of acinar cells of pancreas revealed the disintegration of Golgi apparatus, degeneration of mitochondria, disorganisation of E.R. and vacuolization of cytoplasm which pointed towards the fact that toxicity of Zn resulted in the arrest of synthetic machinery in the cells. In the absence of MT in pancreas and perhaps in other tissues, Zn could not be retained as revealed by its level in the different tissues investigated. Whether the release of stress hormone prior to the onset of NIDDM as observed in number of studies (Brindley et al., 1981; Amatruda et al., 1983 and Plested et al., 1987) is a response to the degenerative changes in pancreas or is a response to the extraordinary growth of adipocytes accumulating zones, was not clear in this study. High absorption of Zn induces the metallothionein synthesis in pancreas and thionein in the liver (Wang et al., 1996). The thionein binds Cu in intestinal mucosa and prevents the Cu from serosal transfer (Fischer, 1981) leading to Cu deficiency in them. Cu on the other hand, is
less effective than Zn in stimulating metallothionein mRNA transcription (Culotta and Hamer, 1989 and Durnam and Palmiter, 1981). Irato et al. (1996) confirmed that both Zn and Cu induce metallothionein synthesis in liver, kidney and intestine but Zn is 100% more effective than Cu. This implies that pharmacological doses of Zn given orally may induce metallothionein synthesis in the epithelial cells of gut which may promote Zn absorption but the induction of thionein synthesis in response to metallothionein synthesis blocks the Cu absorption leading to Cu-Zn imbalance in the body.

The result of the study, therefore suggest that NIDDM, hypercholesterolemia and hypertension occur due to the genetic predisposition of high absorption of Zn attributed to the overexpression of genes responsible for the synthesis of metallothionein at a young age. The manifestation of clinical symptoms occur due to Zn toxicity developed due to continuous inflow of excessive Zn which in turn creates Cu deficiency in the body. Our experimental data show that Cu supplementation triple the amount than R.D.A. (144 mg/Kg diet) is likely to reduce or ameliorate the severity of disease.