Summary
The present research work entitled, 'Studies on the Involvement of Zinc in Obesity, Diabetes and Hypercholesterolemia in Humans,' was carried out in two steps:

(I) Epidemiological studies on the human population.
(II) Experimental studies on the mice.

5.1 Epidemiological Studies

The volunteers for this work were recruited randomly from the General Hospital, Sector 16, Chandigarh and Post Graduate Institute of Medical Education and Research, Chandigarh.

A brief history of the volunteers was recorded and their hair, urine and serum samples were collected for further analysis. On the basis of their disease and family history, they were divided into following groups:

(i) **Control group**: Apparently healthy people having no family history of NIDDM with a normal post prandial plasma glucose ( < 140 mg/dl) and cholesterol (< 240 mg/dl) and a normal arterial blood pressure (systolic pressure < 135 mm Hg and diastolic pressure < 95 mm Hg) were considered as control. They were further divided into 'lean', 'normal', 'overweight' and 'obese'.

(ii) **Group I**: Hyperglycemic (Plasma glucose (pp) >140mg/ dl) and glucosuric NIDDM patients.

(iii) **Group II**: Hyperglycemic, glucosuric and hypertensive (systolic pressure >135 mm Hg and diastolic pressure >95 mm Hg) NIDDM patients.
(iv) **Group III**: Hyperglycemic, glucosuric and hypercholesterolemic (serum cholesterol > 240 mg/dl) NIDDM patients.

(v) **Group IV**: Hypercholesterolemic with no other ailment.

(vi) **Group V**: Hypercholesterolemic with hypertension.

(vii) **Group VI**: Hypertensive with no other ailment.

5.1.1 **Zinc**

Zn concentration in the above mentioned groups was estimated in hair, urine, and serum and compared with those of their respective control groups.

5.1.1.a **Healthy Control**

The mean Zn concentration in healthy women and men was recorded as 5.6 ± 0.73 and 4.62 ± 0.513 μmol/g in hair; 16.63 ± 0.3 and 17.39 ± 0.67 μmol/l in serum and 2.3 ± 0.19 and 3.39 ± 0.22 μmol/l in urine respectively. There was a wide variation in B.M.I and Zn concentration in the volunteers. The data was therefore rearranged depending upon their B.M.I. The Zn concentration was found to be least in lean individuals having B.M.I. ≤ 20 kg/m² and it increased as the B.M.I. increased. Maximum concentration of Zn in hair and serum was recorded in obese volunteers (B.M.I. >27 kg/m²). Both hair and serum Zn observed a linear positive correlation with B. M. I. while urine Zn exhibited a negative correlation with it. The data revealed that the B.M.I increased with increase in Zn concentration in hair as well as in serum. The zinc in urine decreased with rise in B.M.I, indicting its increased retention in the body. These results suggest a positive role of Zn in the aetiology of obesity.
5.1.1 b Group I

The women and men having hyperglycemic and glucosuric NIDDM recorded 3.99 ± 0.54 and 3.87 ± 0.47 μ mol/g Zn in hair; 12.75 ± 0.996 and 11.65 ± 0.54 μ mol/l Zn in serum and 21.3 ± 1.71 and 20.6 ± 1.21 μ mol/l of Zn in urine respectively. The data revealed that hair and serum Zn reduced in patients which suffered with NIDDM as compared to B.M.I. matched control counterparts. The statistical analysis of data displayed a negative correlation between hair Zn and B.M.I. and a positive correlation between urine Zn and B.M.I. a situation opposite to that of the control volunteers. Since the amount of Zn in hair and serum was lesser and more in urine, it could, therefore, be safely concluded that tissues instead of retaining Zn, lose it through urine in NIDDM patients. The tendency seems to cause Zn deficiency like conditions in the body which might have a physiological significance in the aetiology of NIDDM.

5.11.c Group II

The volunteers included in this group were NIDDM complicated with hypertension. The hair Zn concentration in this group of volunteers was slightly higher and serum Zn concentration was significantly higher compared with their control counterparts. On the other hand, the urine concentration was lower compared to those of the NIDDM patients who didn't have complication of hypertension. However, Zn concentration in urine was significantly higher in them than that of Zn lost by control but lower than group- I volunteers. This implies that the Zn lost by tissues is not excreted at the same rate in NIDDM hypertensive patients as it occurs in simple NIDDM patients. Part of it is lost through urine showing hyperzincuria and a part of it is retained in the blood plasma showing
hyperzincemia. The Zn fraction associated with blood plasma seems to play a vital role in the of hypertension.

5.1.1 d Group III

The group- III that included volunteers having hyperglycemic, glucosuric and hypercholesterolemic conditions didn't show any significant difference in hair Zn concentration as compared to their control counterparts but they possessed significantly higher Zn concentration in their blood serum and urine, a condition which was identical to that of group- II volunteers. In this case also, the hyperzincemia and hyperzincuria were the main features suggesting that a part of Zn is retained by blood plasma and remaining is excreted out through urine revealing a close association of group-III volunteers to those of group-II.

5.1.1.e Group IV

The hypercholesterolemic volunteers with no other complications of hypertension or NIDDM were the members of this group. Their hair recorded higher Zn concentration than those in group, I, II and III patients. However, Zn in serum and urine was not significantly different from those of the control counterparts. Most volunteers in this group were in the category of obese suggesting thereby that excess Zn reserves in the body results in hypercholesterolemia. On comparison with volunteers having hypercholesterolemia associated with NIDDM (Group III), it was observed that neither there was hyperzincemia nor hyperzincuria in the volunteers suffering only with hypercholesterolemia. This comparison reflects that initially there is an
accumulation of Zn in the tissue reserves which leads to obesity followed by hypercholesterolemia.

5.1.1.f Group V

Hypercholesterolemic and hypertensive patients were included in this group and belonged to the category of overweight and obese. Their hair Zn was almost identical to their control counterparts but serum Zn was significantly higher in them than the control volunteers. The urine Zn concentration in them was significantly lower than those of control volunteers. This data suggest that hypertension is the result and effect of accumulation of Zn in the blood plasma.

5.1.1.g Group VI

This group included the volunteers who were hypertensive and did not suffer with any other ailments. Their hair Zn concentration was slightly lower than those of their controls but the serum Zn amount was more than double, suggesting thereby that the volunteers lose Zn from their tissues which is retained in the blood plasma in contrast to the NIDDM patients complicated with hypertension who exhibited hyperzincuria along with hyperzincemia.

5.1.1.h Conclusion

The study of Zn status in different groups i.e. controls as well as diseased patients suggest that obesity, hypercholesterolemia and NIDDM are associated with excess bioavailability of Zn. the absorbed Zn is transformed to the tissues whose levels continue to increase with increase in B.M.I leading to obesity. The hypercholesterolemia is the resultant effect of obesity. In diseased conditions, such as NIDDM or hypertension, the absorbed Zn does not find its way into the tissues.
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probably due to the failure of transmembrane cation (Zn$^{2+}$) pump. Instead, there seems to occur a leaching of Zn from the tissues which adds to already existing Zn in blood plasma. In case of simple NIDDM, blood plasma maintains Zn homeostasis through its excretion via kidney. In the patients of NIDDM complicated with arterial hypertension Zn homeostasis becomes partially ineffective as a part of it is retained by the plasma as evidenced by associated hyperglycemic and hyperzincuric condition. Rise of Zn concentration in blood due to ineffective homeostatic mechanism perhaps adds to the conditions favouring hypertension in the patients.

5.1.2 Copper

The data of this study on Zn status revealed an association of excess Zn absorption in the aetiology of obesity and associated diseases. The absorption of Zn effects the mechanism of Cu uptake in the intestine due to their antagonistic interaction. It was, therefore, considered essential to study the status of Cu in association with Zn. Accordingly, the Cu concentration in the hair and urine were studied in the same patients whose Zn was estimated. Following data were recorded in control and diseased patients.

5.1.2.a Healthy Control

The women and men in this group recorded mean Cu concentration as 0.32 ± 0.28 and 0.79 ± 0.60 μ mol/g in hair; 13.75 ± 0.62 and 13.75 ± 0.58 μ mol/l in serum and 0.56 ± 0.04 and 0.64 ± 0.07 μ mol/l in urine respectively. Like Zn, hair Cu concentration was lowest in lean and was significantly more in overweight and obese volunteers. Both serum and urine Cu exhibited a linear positive correlation
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with B.M.I. in contrast to a negative correlation of urine Zn in normal population. The data of Cu concentration suggest that Cu concentration in the body also increases along with increase in body mass.

5.1.2.b Group-I

The group-I volunteers that included hyperglycemic and the glucosuric patients possessed a lower hair and serum Cu concentration. In their urine, however the Cu concentration was not different from that of their control group. The lower hair and serum Cu concentration is this group of patients suggest a reduction in the absorption of Cu which has been attributed to the excess absorption of Zn indicating a Cu-Zn antagonistic interaction.

5.1.2.c Group II

The NIDDM patients suffering from hypertension constituted group-II. Their hair and serum possessed lower amount of Cu and Cu in urine was however significantly higher compared to those in control group and group-I volunteers. The data suggest that this group of patients lose Cu profusely in urine. Since the Zn in blood plasma of these volunteers was higher, the excessive Cu excretion may possibly be due to the replacement of Cu²⁺ in plasma proteins by Zn²⁺. The imbalance of these two elements in plasma playing a vital role in the complications of hypertension has been discussed.

5.1.2.d Group-III

Group-III included the volunteers who were hyperglycemic, glucosuric and hypercholesterolemic patients. Their Cu status was not different from those of group-II volunteers as they were exhibiting hypocupremia and hypercupruria. The
hypercholesterolemic condition observed in these patients has been associated with Cu-deficiency and seems to appear prior to the onset of hypertension.

5.1.2.4 Group IV, V and VI

Similar situations exhibiting hypocupremia and hypercupruria have been observed in hypercholesterolemic (group IV); hypercholesterolemic suffering with hypertension (group V) and simple hypertensive (group VI) patients.

5.1.3 Zinc and Copper Status in the Descendants of NIDDM Patients

Since imbalance of Zn and Cu was observed in NIDDM and hypercholesterolemic patients, a separate study was conducted in the descendants of NIDDM parents so as to ascertain the Cu and Zn status prior to the onset of this disease. The data of this study revealed that hair Zn concentration was highest in NIDDM descendants and minimum in NIDDM patients. Both NIDDM descendants and patients exhibited hyperzincuria. Hair Cu on the other hand, was maximum in controls and minimum in NIDDM patients and in between these two extremes, its concentration was recorded in NIDDM descendants. Concentration of Cu in urine was highest in NIDDM patients, lowest in controls and moderate in NIDDM descendants.

The data of this study suggest that NIDDM descendants possess high Zn reserves probably associated with excess Zn absorption that prevents Cu absorption.

5.1.4 Conclusions of Epidemiological Studies

The results of epidemiological studies suggest that the body mass index increases with increase in Zn concentration in the body. The individuals more likely
to develop NIDDM (NIDDM- descendants) absorb and accumulate relatively high Zn concentration in their tissue. Owing to the excessive absorption of Zn, the absorption and retention of Cu in them decreases relatively due to Cu-Zn interaction. The continuous influx of excessive Zn in the body over a period of time, results in extremely high concentration of Zn in tissues and may result the cells saturated with Zn\(^{2+}\). Its further increase may prove toxic. The observed hyperzincuria in diabetics seems to be on adaptive measure of the cells against Zn toxicity. The NIDDM may possibly be the result of Zn toxicity.

As long as Zn continue to pass through urine and blood is able to maintain Zn-homeostasis, NIDDM remains limited to hyperglycemic and glucosuric condition (glucose intolerance condition).

Continuous influx of Zn and inhibition of Cu-absorption results in Cu-deficiency which aggravate NIDDM complicated with hypercholesterolemia. As the severity of NIDDM increases body becomes unable to maintain Zn - homeostasis and a part of Zn gets associated with plasma proteins leading to hypertension in them.

### 5.2 Experimental Studies

The conclusions drawn from the epidemiological studies on human were confirmed experimentally using mice (Lacca strain) in the laboratory. The experiment was carried out in two phases; in Phase-I the mice were divided into four groups namely, ZS-I, ZS-II, ZS-III and ZS-IV. They were fed on semisynthetic diet containing all ingredients in equal amounts except for Zn. The ZS-I group was
fed on diet containing 20 mg; ZS-II, containing 40 mg; ZS-III, containing 60 mg and ZS-IV containing 80 mg of Zn/ kg diet. These animals were fed for a period of 8 weeks. Thereafter, half of the animals were sacrificed for their various biochemical, histochemically and trace metal analysis from each group and half of these were shifted to the phase-II of the experiment.

During phase-II, the animals were fed on a diet which contained all the ingredients including Zn similar to the phase-I but Cu was added equal to an amount of 48 mg in ZS-I (CS-I); 96 mg in ZS-II (CS-II); 144 mg in ZS-III (CS-III) and 192 mg in ZS-IV (CS-IV) group of mice/ kg diet. They were also fed on the diet for a period of 8 weeks.

There was no significant difference in dry food intake/ 100 g body weight among the four groups but ZS-II, III and IV mice recorded a higher body weight gain compared with ZS-I group at the end of phase-I experiment.

During phase-II, the dry food intake/ 100 g body weight was not significantly different. The CS-I group recorded maximum weight gain in phase-II but yet it was significantly lesser than the weight gained by mice of ZS-I, II, III, and IV groups in phase-I.

5.2.1. Detection of the onset of NIDDM

The urine of mice in group ZS-I, II, III and IV was collected at regular interval. With the help of Benedict's test, the presence of glucose in it was tested and it was found that the mice of group-III and IV showed the presence of traces of glucose in their urine at the end of the 6 weeks of the experiment of the phase-I. The amount
increased in their urine as the duration of the experiment increased from 6 to 8 weeks.

5.2.2 Analysis of Blood

The blood plasma of the mice in phase-I showed a significant rise in its fasting glucose concentration in groups ZS-III and IV mice, indicating the onset of diabetic condition in them. The cholesterol and triglycerides concentrations also exhibited an increase, maximum being in ZS-III group. Thereafter they became static. Thus, the hyperglycemia, hypercholesterolemia and hypertriglyceridemia were evident in mice fed a diet containing Zn in high amount suggesting aggravating effect of Zn on these components of blood.

5.2.3 Other Tissues

The onset of hyperglycemic, hypercholesterolemic and hypertriglyceridemic conditions could occur when transfer of nutrients of the tissues is depressed. Accordingly, the liver, skin and abdominal muscles were studied biochemically.

The data revealed that the glycogen in skin and abdominal muscles did not observe much variations in the four groups. But, liver recorded a rise in its glycogen levels to group ZS-III, followed by a fall in its concentration in group ZS-IV.

Total lipids fraction, on the other hand, recorded a steady rise from ZS-I to ZS-IV group but in liver and abdominal muscles, its concentration fell significantly in ZS-IV group than that in ZS-III group. Similar situation was observed in these tissues for cholesterol, phospholipids and triglycerides, suggesting thereby that the
tissues were saturated for glycogen and fats in the mice fed on diet containing Zn 40 mg/ kg diet. In ZS-III and IV group of mice, further influx of nutrients into the tissues was depressed resulting in their rise in circulating fluid instead of their retention by the tissues. All these conditions i.e. hyperglycemia, hypercholesterolemia and hypertriglyceridemia are prevalent in individuals suffering with NIDDM and hypercholesterolemia.

Since the NIDDM have been frequently reported to be associated with increase in body mass index (B.M.I.) which is associated with total fat contents of the body, accordingly, the effect of increasing concentration of Zn in diet was studied on the growth of adipocytes located in skin and abdominal muscles of mice histochemically. The study revealed that adipocytes stainable with Sudan black -B increased in number and size with increase in amount of dietary Zn. The deposition of fat in the skin of ZS-IV group was so intense that fatty material was seen moving from the subcutaneous layer towards the underlying muscle layer. Similar situation was observed in abdominal muscles as well.

In order to establish the role of Zn in increased fat deposition in adipocytes and effect of excess Zn on cell organelles, acinar cells of pancreas were studied under electron microscope. This organ was chosen for the study because of its sensitivity to Zn$^{2+}$ and its role in digestion. The electron photomicrographs of acinar cells of pancreas revealed that there was a gradual disintegration of Golgi apparatus, mitochondria and dilatations of endoplasmic reticulum (E.R.) proceeding from ZS-III to ZS-IV group. The zymogen granules which were abundant in ZS-I and II groups of mice were significantly less is ZS-III and almost lacking in ZS-IV
group of mice indicating the increased inflow of pancreatic enzymes from the cells to the duodenal portion of intestine. Since, the enzymatic reaction depends upon the amount of enzyme in the medium, their excess release from the pancreatic acinar cells to the intestine seemed to contribute in the increased hydrolysis of nutrients and increased absorption of the products so released. This coincided well with the increased deposition of fatty material in adipocytes as well as rise in glucose, triglycerides and cholesterol in the blood plasma. The degeneration of Golgi apparatus and mitochondria and appearance of vacuoles in the acinar cells of group ZS-IV support the view of the toxicity of Zn as a cause of their degeneration.

The analysis of Zn and Cu concentration indicated that the rise of Zn in tissues resulted in an equivalent fall of Cu concentration like that observed in NIDDM descendants and patients and there was a direct correlation of severity of NIDDM with that of Zn concentration and fall of Cu-concentration reserves in the experiment investigated. This ionic imbalance lead to the fall of superoxide-dismutase activity in P.M.S. fraction of liver in ZS-IV group. The association of fall of S.O.D. enzyme activity has been recently been reported in NIDDM patients. This data clearly confirm our epidemiological observations that excess Zn absorption and retention in humans leads to Cu-deficiency and subsequent onset of NIDDM.

Since, the experimental data revealed that the excess Zn bioavailability results in obesity and Cu-deficiency which collectively induce NIDDM and hypercholesterolemia, it was therefore considered desirable to find if Cu-supplementation to Zn-loaded mice can ameliorate NIDDM-related symptoms or NIDDM related symptoms are independent of Cu-deficiency. Accordingly, the
phase-II of the experiment considered this aspect. The feeding of NIDDM induced mice on Cu-supplemented diet containing Cu-144 mg / kg (group CS-III) and 192 mg/ kg resulted in the reversal of the changes close to the level of mice in control group of phase-I which confirmed the concept that excess Zn bioavailability results in obesity. The subsequent continuous influx of Zn in the tissues cause Cu-deficiency leading to NIDDM and hypercholesterolemia. The correction of Cu-level ameliorate the NIDDM related diseases.