Zinc Supplementation Attenuates Insulin Secretory Activity in Pancreatic Islets of the ob/ob Mouse

NICOLE BÉGIN-HEICK, MARTHE DALPÉ-SCOTT, JOANNE ROWE, AND H. M. C. HEICK

SUMMARY
The purpose of this study was to establish whether a relationship may exist between the hyperinsulinemia, the exaggerated insulin secretion, and the resistance to insulin characteristic of the obese-hyperglycemic syndrome and the zinc status of the ob/ob mouse. To this end, mice were given control and zinc-supplemented diets, and the effects of zinc supplementation on insulin secretion in vivo and in vitro as well as on glucose tolerance were studied. These data were compared with those obtained with oxytetracycline treatment, which is known to ameliorate the insulin sensitivity and glucose tolerance of these animals. The levels of zinc were measured in several tissues of lean and obese mice and the results show that zinc supplementation attenuated the exaggerated insulin secretion in vivo and in vitro without improving the tolerance to glucose. Zinc levels were significantly higher in the tissues of the obese than of the lean mice, with the exception of bone and pancreas. The results suggest a maldistribution of zinc in the tissues of the obese mouse. DIABETES 1985; 34:179-84.

A relative or absolute zinc deficiency has been suggested to play a role in the pathogenesis of diabetes mellitus in humans. Zinc is known to enhance the binding of insulin to hepatocyte membranes and to have an additive effect to that of insulin on lipogenesis in rat adipocytes. Furthermore, zinc-deficient animals are less sensitive to insulin, have an impaired glucose tolerance, and have degranulated islets of Langerhans. The observation that offspring of zinc-deficient dams do not develop islets of Langerhans is a further indication of the importance of zinc to the normal development of the processes surrounding insulin production and insulin action. Insulin resistance, impaired tolerance to glucose, and degranulated islets of Langerhans are also characteristics of the ob/ob mouse. In addition, the immune system, which is known to require an adequate zinc status has been reported to be defective in this animal.

Oxytetracycline (OTC) interacts strongly with divalent cations, particularly zinc, and has been shown to form stable complexes with zinc and insulin. We have previously found that chronic treatment with OTC produces normoglycemia, normoinsulinemia, and improves glucose tolerance in the ob/ob mouse. In addition, the exaggerated insulin secretion, characteristic of isolated islets from the ob/ob mouse, is attenuated by previous treatment of the animals with OTC. In an attempt to explain the beneficial effect of OTC, we offered the hypothesis that zinc may be interacting with OTC within the islets and in other tissues. We therefore investigated the zinc status and the effect of dietary zinc supplementation on insulin secretion and glucose tolerance in the obese mouse.

MATERIALS AND METHODS
Animals. Male C57BL/6J-ob/ob mice and their lean controls (+/?) were obtained from the Jackson Laboratory, Bar Harbor, Maine. The mice were used in the various experiments at 9-12 wk of age. Unless otherwise specified, they were maintained on Purina Chow and water ad libitum. All animals were kept in a temperature-controlled room at 24 ± 1°C with 12-h light cycles.

Treatments. The mice were housed in individual cages during the treatment periods. They were assigned to the following experimental groups: (1) lean control (In-CTL), (2) lean OTC-treated (In-OTC), (3) obese control (ob-CTL), (4) obese OTC-treated (ob-OTC), (5) food-restricted obese controls (ob-FR), (6) Zn-supplemented obese mice (ob-Zn), and (7) obese OTC-treated and Zn-supplemented (ob-Zn-OTC). Groups of lean and obese mice were treated for 7 days...
Zinc supplementation plus OTC treatments—obese mice differ in size, shape, and degree of granulation. The data are means ± SEM from eight mice in each group. In a given column, values followed by different symbols are significantly different from each other (P < 0.05) between control and treated groups.

with oxytetracycline (Terramycin, Pfizer, New York, New York) via intramuscular injections as described previously. The food restriction schedule was also described previously.

Zinc supplementation. Zinc carbonate was added to ground Purina chow to increase the zinc content from approximately 50 to 1000 ppm. Each of the three batches of diet prepared for these experiments was analyzed in triplicate. The control diet contained 47.5 ± 0.5 and the supplemented diet 964 ± 100 µg Zn/kg diet. This level of zinc was chosen because it was reported that laboratory animals can tolerate 1000-2000 ppm zinc in the diet, approximately 100 times the required dietary level (National Research Council, 1972), without showing any sign of toxicity. The diet was fed ad libitum for 4 wk.

Zinc supplementation plus OTC treatments. Oxytetracycline was administered as described above for the last 7 days of the 4-wk zinc supplementation period.

Preparation of islets. Islets were isolated from fed animals by a method based on the collagenase method of Lacy and Kostianovsky as described previously.

Sizing of islets. Islets were sized on a dissecting microscope with the help of an eyepiece micrometer. The longest diameter of each islet was used for computing the data reported in Figure 1 and Table 3.

Measurements of insulin secretion from isolated islets. These were done exactly as described previously.

Measurement of insulin. Plasma and tissue incubation media were assayed for insulin with a double-antibody radioimmunoassay after appropriate dilution of the sample with the assay buffer.

Expression of results. The islet population in the lean and obese mice differ in size, shape, and degree of granulation. In the lean mice, the islet population is fairly homogeneous; in the obese, within one pancreas, islets of different sizes and/or different degrees of granulation are found. Since insulin secretion is highly correlated with insulin content of the islets, it was decided to express the results as insulin secreted per unit time as a fraction of the total insulin content of the islets, a method that has been used by others.

Glucose tolerance tests. Twelve hours before the test, at 2100 h, food was removed from the cages. At 0900 h the following day, blood samples were taken from the tail vein into heparinized capillary tubes (0-time sample). Glucose (1 g/kg) was injected intraperitoneally and blood samples were withdrawn at 30 and 60 min after the injection of glucose. The experiments were performed without anesthesia.

Glucose determinations. Plasma glucose was measured by the glucose-oxidase method, using a Beckman Glucose Analyzer (Beckman Instruments, Fullerton, California). After separation of the plasma by centrifugation, a 5-µl portion of plasma was diluted with 10 µl of distilled water before analysis.

Zinc analysis. Tissues were removed and washed in normal saline made with deionized water. They were blotted dry and frozen individually. The tissues were lyophilized and prepared for zinc analysis by wet washing. Zinc content was determined by atomic absorption spectrophotometry in an International Laboratories Atomic Absorption Spectrophotometer, Model 457 (Fisher Scientific, Mississauga, Ontario). Zinc content of food was monitored with a similar method by analyzing portions of powdered Purina Chow with or without zinc carbonate added. The zinc content of drinking water was determined directly. Plasma was diluted 1:10 with 1 M HCl before analysis. Care was taken to ensure that no hemolysis was present in the samples of plasma tested.

Reagents. Bovine serum albumin (fraction V, RIA grade) was obtained from Sigma Chemical Co. (St. Louis, Missouri); collagenase and hyaluronidase were from Worthington Bio-

TABLE 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial Body wt (g)</th>
<th>Final Body wt (g)</th>
<th>Fat (%)</th>
<th>H2O (%)</th>
<th>Food/day (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>In-CTL</td>
<td>23.1 ± 0.9</td>
<td>48.3 ± 0.8</td>
<td>11.3 ± 1.9</td>
<td>62.1 ± 0.9</td>
<td>30 ± 0.1</td>
</tr>
<tr>
<td>In-Zn</td>
<td>22.9 ± 0.7</td>
<td>47.4 ± 0.8</td>
<td>9.1 ± 2.0</td>
<td>64.7 ± 0.8</td>
<td>37.0 ± 1.0</td>
</tr>
<tr>
<td>ob-CTL</td>
<td>45.1 ± 0.3</td>
<td>48.9 ± 0.7</td>
<td>52.1 ± 0.8</td>
<td>33.6 ± 0.8</td>
<td>9.4 ± 0.2</td>
</tr>
<tr>
<td>ob-Zn</td>
<td>44.5 ± 0.7</td>
<td>46.7 ± 0.4</td>
<td>51.2 ± 0.6</td>
<td>33.7 ± 0.6</td>
<td>7.2 ± 0.3</td>
</tr>
</tbody>
</table>

The mice were fed their respective diets for a period of 4 wk. The composition of the diet was as given in the text. All values were significantly different between lean and obese mice.

*Significant difference (P < 0.05) between control and treated groups.
TABLE 3

Insulin content of isolated islets

<table>
<thead>
<tr>
<th>Group</th>
<th>Insulin (ng/5 islets)</th>
</tr>
</thead>
<tbody>
<tr>
<td>In-CTL</td>
<td>160 ± 10.7*</td>
</tr>
<tr>
<td>In-OTC</td>
<td>158 ± 8.2*</td>
</tr>
<tr>
<td>In-Zn</td>
<td>268 ± 26†</td>
</tr>
<tr>
<td>ob-CTL</td>
<td>79 ± 8.5*</td>
</tr>
<tr>
<td>ob-OTC</td>
<td>430 ± 34</td>
</tr>
<tr>
<td>ob-Zn</td>
<td>219 ± 13†</td>
</tr>
<tr>
<td>ob-Zn-OTC</td>
<td>660 ± 55§</td>
</tr>
<tr>
<td>ob-FR</td>
<td>218 ± 15†</td>
</tr>
</tbody>
</table>

Insulin was measured as described in the text. The data are means ± SEM of a minimum of 25 determinations. Within the lean and the obese groups, values followed by different symbols are significantly different from each other (P < 0.05). In addition, the following differences between lean and obese groups receiving the same treatment were significant (P < 0.05): In-CTL > ob-CTL, In-OTC < ob-OTC, and In-Zn > ob-Zn.

RESULTS

Body weight, body composition, and food intake. The zinc-supplemented diet did not produce significant effects on body weight or body composition of either lean or obese mice as compared with their respective, nonsupplemented controls (Table 1). The only significant effect of zinc supplementation was a decrease in food intake in the obese group.

Plasma glucose and insulin response after a glucose load. Data comparing the effects of zinc supplementation, OTC treatment, and a combination of both treatments to values obtained from controls and food-restricted controls are shown in Table 2. All treatments produced a significant decrease of fasting plasma glucose and insulin levels (0-time values). After the administration of the glucose load, blood glucose values rose to levels that were not significantly different from controls in all groups except for the ob-OTC group (30-min value), and the ob-OTC and ob-Zn-OTC groups (60-min value). Similar fasting plasma insulin values were obtained from the ob-CTL and ob-FR groups. The OTC and/or zinc treatments all led to significantly lower plasma insulin levels compared with control values. The lowest insulin levels were found in the ob-OTC group. There were no effects of the zinc-supplemented diet on glucose tolerance and insulin secretion in the lean mouse.

Insulin content and size of islets. The insulin content of islets is given in Table 3. Data on islets from lean mice (control and OTC-treated) and from food-restricted obese mice are included for comparative purposes. In the obese mouse, zinc supplementation, food restriction, and OTC treatment (alone or in combination with zinc supplementation) resulted in a significant increase in insulin content when compared with controls.

FIGURE 1. Islet distribution in lean and obese mice. The value given on the ordinate is the percentage of the total number of islets of the size indicated on the abscissa. The data were obtained by measuring the largest diameter of a minimum of 100 islets for each group. (A) Comparison of the effect of OTC treatment on islets of lean and obese mice. In-CTL = O; In-OTC = □; ob-CTL = ▲; ob-OTC = △; ob-FR = △. (B) Comparison of the effect of OTC treatment with or without zinc supplementation on islets of obese mice. ob-CTL = ▲; ob-OTC = △; ob-Zn = □; ob-Zn-OTC = △.
with control values. The highest islet insulin content was obtained with a combination of the two treatments (Zn-OTC). In the lean mouse, zinc supplementation alone, but not OTC treatment, increased the insulin content of the islets.

Figure 1 (A and B) represents profiles of the size distribution of islets from different groups of mice. In accordance with previous reports on rat and mouse islets, the islets were found to be asymmetrically distributed. A nonparametric statistical test (the Kolmogorov-Smirnov test) was used to evaluate whether islets from one population were significantly larger or smaller than islets from another population. The results of the statistical analysis are given in Table 4. The majority of islets was small (76–152 μm) in lean mice (In-CTL and In-OTC). Islets of intermediary size (152–229 μm) were the most numerous in the ob-CTL group. Treatment of the obese mouse with OTC (ob-OTC group) resulted in a distribution profile of the islets resembling that seen with islets of lean mice. Only 25% of the islets of the ob-OTC group had a diameter exceeding 152 μm as compared with 50% in islets of the ob-CTL. Zinc supplementation of the obese mouse (ob-Zn group) did not lead to a significant difference in the size distribution of the islets. The distribution profile found for the ob-Zn-OTC group was not significantly different from that of the ob-OTC group. Food restriction resulted in a slight increase in the number of smaller islets in the obese mouse. Taken as a whole, however, the islet population in the FR group was not significantly different from that of the obese controls.

In vitro glucose-induced insulin secretion. The results of these experiments are shown in Figure 2. Zinc supplementation alone significantly diminished the abnormally high insulin secretory response to glucose of the ob/ob mouse islets (at 10 mM, P < 0.001; at 15 mM, P < 0.025; and at 20 mM, P < 0.05). The secretion of insulin was maximally stimulated at 15 mM glucose in the islets of the ob-Zn mouse as was the case in the obese controls. When zinc-supplemented obese mice were also treated with OTC (ob-Zn-OTC group), the effect of the two treatments together was greater than that of either alone in attenuating the exaggerated secretion of insulin. The results obtained with the islets from the ob-OTC-Zn group were the closest to those obtained with the islets of lean mice, although the insulin secretory activity was still significantly greater at all the glucose concentrations studied.

Zinc content of plasma and tissues. In a first experiment, zinc levels were measured in the plasma, liver, and femur of zinc-supplemented lean and obese mice and their non-supplemented controls (Table 5). Plasma zinc values did not differ significantly between lean and obese mice. Whereas these values were increased significantly by zinc supplementation in the lean mouse, no significant change was found in the obese mouse. On a fat-free weight basis, the zinc content of the liver was significantly higher in the obese than in the lean mouse. Zinc supplementation did not alter the levels significantly in either group. In contrast, the zinc content of the femur was significantly lower in the obese than in the lean mouse. Although the weight of the bone was significantly increased in both groups as a result of zinc supplementation, the proportion of zinc in the tissue was not altered. In a second experiment, zinc levels were measured in the triceps muscle, brown adipose tissue (BAT), and pancreas of lean and obese mice (Table 6). In the obese mouse, the muscle was significantly smaller but contained twice as much zinc per unit of dry weight as did the lean mouse. BAT, which is much larger in the obese because of the excessive fat deposition in the tissue, contained considerably more zinc on a fat-free, dry-weight basis. On the other hand, the pancreas of the obese mouse contained significantly less zinc than did that of the lean.

DISCUSSION

The results described in this paper demonstrate that dietary zinc supplementation of the ob/ob mouse results in a significant attenuation of the abnormally high insulin secretory response of the islets to glucose in vitro, and a significant decrease of fasting plasma glucose levels. Fasting plasma insulin levels and the plasma insulin levels produced during a glucose tolerance test were also lower in the animals that received the zinc-supplemented diets than in the controls. When added in vitro, zinc inhibits glucose-induced insulin
these responses. Tissue-related defects that account for the discrepancy in concentration in muscle was almost twice that found in the lean. Elevation of plasma glucose with a simultaneous elevation on glucose tolerance. The data on insulin secretion and glucose tolerance taken together indicate that there may be significantly lower in the obese mouse, the other soft tissues. Zinc content of tissues in lean and obese mice.

**TABLE 5**

The zinc content of plasma and tissues in control and zinc-supplemented lean and obese mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma</th>
<th>Liver</th>
<th>Femur</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zinc*</td>
<td>wt (g)</td>
<td>Zinct</td>
</tr>
<tr>
<td>ln-CTL</td>
<td>1.35 ± 0.09†</td>
<td>1.27 ± 0.7†</td>
<td>118.2 ± 2.8†</td>
</tr>
<tr>
<td>ln-Zn</td>
<td>1.60 ± 0.17§</td>
<td>1.32 ± 0.4§</td>
<td>130.5 ± 9.5§</td>
</tr>
<tr>
<td>ob-CTL</td>
<td>1.28 ± 0.08‡</td>
<td>3.26 ± 0.3§</td>
<td>156.6 ± 8.1§</td>
</tr>
<tr>
<td>ob-Zn</td>
<td>1.47 ± 0.09‡</td>
<td>2.80 ± 0.3§</td>
<td>174.1 ± 9.0§</td>
</tr>
</tbody>
</table>

The data presented are means ± SEM for six animals in each group. Values in the same column followed by different symbols are significantly different from each other.

*Zinc values are given as |xg/g fat-free dry wt for the liver and |xg/g dry wt for the femur.

The zinc content of plasma and tissues in control and zinc-supplemented lean and obese mice. This may represent a regulatory function for zinc, as the inhibitory effects of zinc can be reversed by increasing the calcium concentration of the medium. The physiologic significance of these phenomena has been questioned, because the zinc concentration (60 |xM) required to achieve significant inhibition is far greater than the levels found in plasma (10–20 |xM). As rightly pointed out by Ghalghazi et al., however, the intraskeletal zinc concentration is not known and may be greater than the plasma concentration. Additionally, zinc uptake by islets in vitro is a very slow process, but it results in zinc concentrations that are 30 times greater than those in the external medium. High extracellular concentrations of zinc may, therefore, be needed to produce significant changes in the intracellular concentration over short incubations.

Zinc-deficient animals have been reported to be glucose intolerant and zinc has been shown to enhance the effect of insulin in certain tissues. On the other hand, acute administration of zinc to normal animals produces a transient elevation of plasma glucose with a simultaneous elevation of plasma glucagon and fall of plasma insulin. In our experiments, zinc supplementation did not alter peripheral glucose tolerance in the obese or in the lean mouse (Table 2). In addition, the combination of OTC treatment and zinc supplementation was significantly less effective than OTC alone on glucose tolerance. The data on insulin secretion and glucose tolerance taken together indicate that there may be tissue-related defects that account for the discrepancy in these responses.

Bone and muscle zinc levels are believed to be the best indicators of zinc status. It was, therefore, interesting to find a significantly lower zinc concentration in the femur of the obese than of the lean mouse. On the other hand, zinc concentration in muscle was almost twice that found in the lean mouse. Except for the pancreas, where the zinc levels were significantly lower in the obese mouse, the other soft tissues were similar to muscle. These results show unequivocally that there is a maldistribution of zinc in the tissues of the obese mouse. It is interesting that significantly lower levels of femur zinc have been reported for the db/db mouse. The same authors found little difference in liver zinc between the control and diabetic mice on a dry-weight basis. As the liver of the db/db mouse contains more fat than that of the lean, elevated liver zinc levels would be expected on a fat-free, dry-weight basis. In contrast to the ob/ob mouse, zinc supplementation of the diet restored femur zinc levels to normal in the db/db mouse. No differences in tissue zinc levels were found between streptozocin-diabetic mice and their controls, whereas higher liver and kidney zinc levels have been reported for streptozocin-diabetic rats.

Tissue zinc is partially bound to metal-binding proteins, the metallothioneins (MT). Although the functions of MT in metabolism are only beginning to be studied, it is believed that they may afford a mechanism to sequester zinc into specific tissues. The synthesis of these proteins can be induced to high levels by factors such as stress, infections, heavy metals, and hormones. Zinc itself is an inducer of MT. Corticosteroids induce the formation of MT, so that in hypercorticosteroidism, more of the zinc in any given target tissue may exist as a zinc-MT complex.

The corticosteroid levels are abnormally high in the obese mouse. We offer the hypothesis that these high levels maintain MT at elevated levels in target tissues. Consequently, those tissues contain high levels of zinc complexed to MT. The exception is the bone, where zinc levels were lower in the obese mouse. It is possible that bone zinc is lower in the obese mouse because of the catabolic effects of corticosteroids on that tissue. The exocrine pancreas also had lower levels of zinc in the obese. This may be an indication that zinc is sequestered in corticosteroid target tissues at the expense of other tissues. MT levels of lean and obese mouse tissues are currently under investigation.

**TABLE 6**

Zinc content of tissues in lean and obese mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Muscle</th>
<th>BAT</th>
<th>Pancreas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>wt (mg)</td>
<td>Zinct</td>
<td>wt (mg)</td>
</tr>
<tr>
<td></td>
<td>Zinc*</td>
<td>wt (mg)</td>
<td>Zinc*</td>
</tr>
<tr>
<td>Lean</td>
<td>181.1 ± 11</td>
<td>159.4 ± 17</td>
<td>85 ± 5.6</td>
</tr>
<tr>
<td>Obese</td>
<td>126.5 ± 10</td>
<td>337.3 ± 34</td>
<td>283 ± 24</td>
</tr>
</tbody>
</table>

The data presented are means ± SEM for five mice in each group. The muscle used was the triceps. All values are significantly different between lean and obese mice.

*Zinc values are given as |xg/g dry wt for the muscle and pancreas and as |xg/g fat-free dry wt for the brown adipose tissue (BAT).
indicate a positive relationship between zinc and MT in liver and pancreas.\(^{45}\)

OTC possesses two properties that may be of importance in understanding the metabolism of zinc in the ob/ob mouse. It interacts with membranes and has a great avidity for divalent cations, particularly zinc.\(^{12,13}\) It is, therefore, possible that the beneficial effects of OTC in the obese mouse are due to its metal-chelating properties, allowing it to compete with MT.

Although they raise many additional questions, the data presented here show that the availability of zinc to the beta cell may be an important factor in allowing insulin granules to be stored and, thus, in regulating insulin secretion. Second, the data show that absolute tissue zinc levels are modified by hormonal and other factors that need to be taken into consideration when assessing zinc status.

**ACKNOWLEDGMENTS**

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**REFERENCES**