EFFECT OF LOW ZINC DIET ON $^{65}$Zn TURNOVER IN NON-INSULIN DEPENDENT DIABETIC MICE

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SUMMARY - Objective: To investigate the effect of low-Zn diet on $^{65}$Zn turnover and content in non-insulin dependent diabetes (NIDDM).

Material and methods: Male 8-9 weeks old, genetically diabetic mice (C57BLKsJdb/db) and non-diabetic heterozygote litter-mates (C57BLKsJdb/+) were fed on a diet containing 1 mg zinc/kg (low-zinc groups) or 54 mg zinc/kg (control groups), and injected subcutaneously with $^{65}$Zn. Body weight gain was recorded regularly, whereas $^{65}$Zn body loss was measured by whole-body counting in the two mouse genotypes over a 28 days period. At the end of the experiment, pancreatic Zn, femur Zn and non-fasting blood glucose levels were determined.

Results: All mice fed on the low-Zn diet showed a marked reduction in whole-body $^{65}$Zn loss compared with animals fed on the control diet. In the low Zn groups, the loss of $^{65}$Zn from the diabetic mice was significantly greater than that of heterozygote mice. This difference was not observed in the control groups. Body-weight gain was not affected by low zinc diet for the two genotypes. However, blood glucose levels were elevated in the low-Zn groups and the possible reasons for these observations are discussed.

Conclusion: The present study demonstrates that there is almost a dramatic decrease in $^{65}$Zn turnover rate immediately following the introduction of the low zinc diet to the animals, but the diabetic mice were less able to reduce zinc loss compared to non diabetic ones. It was also found that the effect of reduced zinc intake on glucose utilization in the genetically diabetic mouse, occurred before any significant tissue zinc depletion became apparent.

Key-words: diabetic mice, heterozygote mice, $^{65}$zinc, turnover.

RESUME - Turnover du $^{65}$Zn chez les souris génétiquement diabétiques, alimentées par un régime carencé en zinc.

Objectifs : Pour étudier l’effet d’une carence en Zn sur le turnover du $^{65}$Zn, le contenu corporel en Zinc et la glycémie chez des souris diabétiques modèle de diabète non insulino-dépendant.

Matériel et méthodes : Des souris mâles génétiquement diabétiques (C57BLKsJdb/db) âgées de 8 à 9 semaines et hétérozygotes non diabétiques de même lignée (C57BLKsJdb/+) ont été nourries avec un régime contenant 1mg/kg (régime carencé) ou 54 mg/kg de zinc (régime témoin). Le turnover du Zinc a été établi après injection sous-cutanées de $^{65}$Zn. Les variations du poids corporel ont été régulièrement enregistrées, la perte de $^{65}$Zn a été mesurée, dans les deux génotypes durant 28 jours. En fin d’expérimentation le contenu en Zn du pancréas et des fémurs ainsi que la glycémie ont été mesurés.

Résultats : Chez les animaux ayant un régime carencé en Zn, l’élimination du $^{65}$Zn était abaissée mais elle reste supérieure chez les homozygotes (diabétiques) comparée aux hétérozygotes, cette différence n’a pas été observée avec le régime témoin. Le poids corporel n’était pas affecté, pour les deux génotypes. Cependant, la glycémie était augmentée chez les souris ayant une alimentation carencée en zinc et l’origine de cette constatation est discutée.

Conclusion : Ce présent travail montre une diminution marquée du turnover du $^{65}$Zn immédiatement après la mise sous régime carencé en Zinc, mais les souris diabétiques sont moins susceptibles de réduire leur perte en zinc que les souris non diabétiques. Il a été aussi constaté que l’effet de la carence en zinc sur l’absorption du glucose chez les souris génétiquement diabétiques, se produit avant que n’apparaissent des signes mesurables de déplétion en Zinc.

Mots-clés : souris diabétiques, souris hétérozygotes, $^{65}$zinc, turnover.
The absorption, deposition, and excretion of zinc has been studied in several species including mice, rats and dogs [1] following the administration of $^{65}$Zn by injection, or in a meal. These studies indicate that radio-labeled Zn-chloride, administrated by injection is deposited preferentially in the pancreas, liver and spleen, with only a minor deposition in muscles and brain. After a much longer period, a large proportion of $^{65}$Zn was transferred to bone [2].

Since the relationship between zinc and insulin was recognized [3], zinc metabolism in the diabetic state has been studied by many investigators, including Killierich, et al. [4] who found the absorption of $^{65}$Zn tended to be lower in patients with insulin-dependent diabetes compared with healthy people, but did not reach the level of statistical significance. It has been also reported that diabetic subjects have excessive loss of zinc in the urine [5, 6]. In addition, it is documented that inadequate dietary zinc leads to a decrease in zinc concentration in some tissues and in body weight gain [7, 8].

In view of the alteration in zinc metabolism associated with the diabetic state, therefore in this study, it has been decided to investigate whole-body zinc absorption and turnover-rate in both low-zinc and control mice given a subcutaneous dose of $^{65}$Zn. The study was designed in order to compare $^{65}$Zn turnover between different dietary groups, diabetic and non-diabetic animals.

**Materials and Methods**

**Animals and diet**

Male diabetic mice and non-diabetic mice heterozygote littermates 8-9 week-old were randomly allocated into two groups each. Approximately half of each genotype received a diet containing 1mg Zn/kg (low-Zn groups) and the remaining animals received a diet containing 54 mg Zn/kg (control groups). The composition of the diet was similar to that described previously by Southon, et al. [9], but with egg albumin as the protein source. The low Zn was prepared by omitting zinc carbonate from the mineral mix. In this experiment however, mice were ears clipped and housed three to four per cage. Food was provided ad-lib, but because of excessive spillage and contamination of food with urine, it was not possible to estimate food intake. Body-weight gain was recorded twice weekly over an experimental period of 28 days.

The effect of dietary Zn intake on $^{65}$Zn loss from diabetic mice was investigated and compared with that of heterozygote mice. Animals were fed on the control semi-synthetic diet ad-lib for 5 days, after which mice were injected subcutaneously into scruff with 37kBq $^{65}$Zn in 0.1ml saline (9g sodium chloride/l). Immediately after injection, the mice were restrained within a well-ventilated Perspex box (100/100/120mm) and counted in a small animal whole body gamma counter to determine the exact dose of isotope given to each individual. However, counting geometry was within ± 0.5% of the overall mean.

After the injection, half of these animals continued to receive the control diet and the other half were given the low-Zn diet, and the amount of whole-body $^{65}$Zn lost over a 27 days period of the experiment was measured by whole-body counting. On day 28 the mice were killed between 10.00-13.00 hours by intraperitoneal injection of sodium pentobarbitone (0.3 ml, 200 mg/ml), one mouse from each group being killed at approximately the same time.

Before the administration of pentobarbitone a tail-blood sample was taken from each mouse and, after death, the pancreas and femur were removed from a random selection of mice from each group. Then pancreas was washed with isotonic saline (9 g sodium chloride/l distilled water) and blotted to dry. The right femur was taken and connective tissues and muscle were removed. After that pancreases and femurs were weighed and stored separately in plastic vials at -20°C until analyzed for zinc.

**Analytical methods**

Blood glucose was measured in 10 µl samples of whole blood by the glucose oxidase method, using a YSI Model 27 glucose analyzer. The dried pancreas and femur were heated in crucibles at 480°C for 48 h and the ash taken up in hot hydrolic acid (11.7 M) for Zn analysis by Atomic Absorption spectrophotometer (PU 9000; Pye Unicam). The accuracy of zinc recovery using this method was checked by using standard reference materials; bovine liver and wheat flour. Comparison between the effect of diet and genotype groups was made using Student’s unpaired t test.

**Results**

The body-weight gain was similar in the low-Zn and control groups, with the diabetic mice having the greatest weight gain. Pancreatic and femur Zn concentration in both diabetic and heterozygote mice were unaffected by the level of dietary Zn (Table I). However, pancreatic zinc was lower in the diabetic mice than the heterozygote ones.

Whole-blood glucose concentration was found to be significantly higher in each of the low-Zn groups killed after a 27 days feeding period (P < 0.05), compared with mice fed on control diet (Table I). A whole-body $^{65}$Zn study, performed between approximately 8 and 12 weeks of age, showed that $^{65}$Zn loss was markedly lower at all time points for the animals fed on the low-Zn diet (Fig. 1). Rate of $^{65}$Zn loss was similar in both the control groups, but low-Zn diabetic
mice had a significantly greater whole-body $^{65}$Zn loss ($p < 0.05$) over the 28 days period of study compared with heterozygotes fed on the same diet (Fig. 1). Regression analysis of the log$_{10}$ per cent rate of $^{65}$Zn loss, over the last 9 days indicated that the mean daily $^{65}$Zn loss was 2 and 1.3% for low-Zn diabetic and heterozygote mice, respectively.

### DISCUSSION

In this experiment the body-weight gain of diabetic and heterozygote mice was not significantly affected by dietary Zn concentration. This is not in good agreement with the results obtained with other animal species fed on inadequate dietary zinc [10, 11]. It is suggested that this genotype may have a more efficient homeostatic control mechanism for zinc, enabling however, the animal to conserve its metabolically active zinc pool. In contrary the body-weight gain of both groups of diabetic mice was greater than for any of the non-diabetic groups, which was in line with that reported earlier by Colman & Hummel [12] using the same strain of diabetic mice. As expected, glucose concentration of both control and low-zinc diabetic mice was found to be markedly higher than for any of the non-diabetic animals. It was also found that the glucose level in whole blood of the diabetic and the non-diabetic mice fed on low-Zn diet was higher than their controls. These results strongly suggest that dietary zinc may be involved in the maintenance of normal glucose homeostasis [13, 14].

On the other hand, zinc analysis of pancreas and femurs, has showed that the animals were not susceptible to reduced dietary zinc intake over the experimental period, despite the fact that these tissues is generally regarded to be one of the most sensitive to variation in dietary Zn intake [15]. It appeared from what mentioned above that these mice have an efficient mechanism for retaining body zinc which results from a homeostatic response to the increased needs caused by the low dietary zinc intake. Such mechanism results in the maintenance of tissue zinc levels in the low-Zn groups, despite the dietary concentration of zinc being fifty times lower than the zinc supplemented groups. It is well known that animals and humans subjected to dietary mineral depletion, are often able to conserve the mineral within certain tissues even in the face of a severe deficiency [16]. The lower pancreatic Zn concentration observed in the diabetic mice fed at both levels of Zn compared with heterozygotes, is probably

### TABLE I.

Mean Body-weight gain (g), pancreatic zinc and femur zinc concentration and blood glucose concentration of diabetic and heterozygote mice given a low-Zn (1mg Zn/kg) or control (54 mg Zn/kg) semi-synthetic diet for 28 days.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Diabetic (n = 15)</th>
<th>Heterozygote (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>Initial body-wt (g)</td>
<td>28.9*</td>
<td>0.5</td>
</tr>
<tr>
<td>Body-wt gain (g)</td>
<td>5.9*</td>
<td>0.5</td>
</tr>
<tr>
<td>Pancreatic Zn*</td>
<td>19.2a</td>
<td>1.2</td>
</tr>
<tr>
<td>Femur Zn**</td>
<td>152ab</td>
<td>9</td>
</tr>
<tr>
<td>Blood glucose***</td>
<td>18.3a</td>
<td>1</td>
</tr>
</tbody>
</table>

* (µg/g fresh wt.)
** (µg/g dry wt.)
*** (mmol/l whole blood)
a, b, c, d values within a horizontal line with different superscript letters were significantly different ($p < 0.05$)
related to their hyperinsulinaemia, the early onset of B-cell degranulation and other pathological changes in this tissue associated with the progression of condition, and is consistent with human studies showing that the pancreatic Zn concentration of diabetics is depressed compared with normal. This is similar to an earlier studies which have demonstrated a 50% drop in pancreatic zinc in diabetic subjects as compared to healthy people [17, 18]. Additionally, a significant reduction in femur Zn concentration in genetically diabetic mice, compared with heterozygotes, was observed only in low-Zn intake group. However, another work proved a reduction in femur zinc in the same strain [5]. The results of 65Zn study clearly indicated the ability of both mouse genotypes to reduce 65Zn loss when dietary zinc intake was reduced. This may have been achieved by decreased endogenous zinc secretion into the gastrointestinal tract [19], or increased efficiency of Zn absorption from the diet [20].

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REFERENCES

8 Oteiza PI, Katheron LO, Cesar GF, Carl LK. Zinc deficiency causes oxidative damage to proteins, lipids and DNA in rat testes. Nutr, 1995, 125, 823-829.
11 McNall AD, Etherton, T D, Fosmire G J. The impaired growth of genetically diabetic mouse (C57BL/KsJdb+/db+).