Effect of zinc deficiency on zinc and carbohydrate metabolism in genetically diabetic (C57BL / KsJ db+/ db+) and original strain (C57BL / KsJ) mice

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Abstract

Male, 4 -5 week-old, genetically diabetic (C57B/LKsJdb+/db+) and non-diabetic original strain (C57BL/KsJ) mice were fed on a diet containing 1 mg Zn/kg (low-Zn groups) or 54 mg Zn/kg (control groups) for 27 days. Food intakes and body weight gain were recorded regularly. On day 28, after an overnight fast, animals were killed and blood glucose and insulin concentration, glycogen, and femur and pancreatic Zn concentration were determined. The consumption of the low-Zn diet had only minimal effect on the Zn status of the diabetic mice as indicated by growth rate, food intake and femur and pancreatic Zn concentration. In fact, diabetic mice fed on the low-Zn diet had a higher total food intake than those fed on the control diet. The low-Zn diabetic mice had also higher fasting blood glucose and liver glycogen levels than their control counterparts. However the growth rate and femur zinc concentration of the original strain mice were affected by low-Zn diet. The present study demonstrates an adverse effect of reduced dietary Zn intake on glucose utilization in the genetically diabetic mice, which occurred before any significant tissue Zn depletion became apparent and it was also showed that the original strain of mice was more susceptible to the low-Zn diet than the diabetic mice.

Key words: Strain, Zinc, Diabetic mice (C57BL/KsJdb+/db+), non diabetic mice (C57BL/KsJ)

Introduction

The effect of zinc deficiency on insulin and carbohydrate metabolism has been studied in some detail in laboratory animals (1-6) but in many instances the findings are difficult to interpret because of the effect of dietary Zn depletion on food intake (7). Consequently, there is evidence demonstrating that inadequate dietary Zn is associated with changes insulin secretion and action, although microscopic studies indicate that the *B-cell* of Zn deficient animals have decreased granulations and histochemically detectable insulin (8). An effect of low Zn status on hormonal action may, however, become, apparent in conditions where insulin secretion and function are already abnormal. For example, it is has been suggested that the altered Zn metabolism and reduced Zn status reported to occur in type II (maturity onset) diabetic subject (9-11) may aggravate the insulin resistance which is characteristic of this condition. It has been speculated that where there is evidence of poor Zn status a programme of Zn repletion may improve insulin sensitivity and reduce the severity of some of the complications of this disease (12). There appears to be little information, however, on the effect of varying dietary Zn intake on the progression and severity of this form of diabetes.

In the present study a semi-synthetic diet containing either 1or 54 mg Zn/kg was fed to 4-5 week-old male, genetically diabetic mice (C57BL/KsJ db/db). Diabetes (db) is inherited as a unit autosomal recessive for the diabetic gene (db/db) display symptoms similar to non-insulin- dependent diabetes in man: hyperglycaemia, hyperinsulinaemia, polyuria (13). Original strain mice (C57BL/KsJ) cannot be distinguished morphologically or physiologically from normal and were used for comparison in the present study. Growth rate, food intake, Zn status, blood glucose and insulin concentration, were measured in the two dietary groups of each mouse genotype.

Material and methods

Animals and diets:

The experiment was performed in which 4 -5 week old male diabetic mice C57BL/KsJdb/db and original strain mice C57BL/KsJ were each randomly allocate into two groups. Approximately half of each genotype received a diet containing 1 mg Zn/kg (low Zn groups) and the remaining animals received a diet containing 54 mg Zn/kg (control groups). The recommended dietary zinc concentration for both mice and rats is 12-30 mg/kg (14). The composition of diet was similar to that described previously by Southon *et al.* (15) .The low zinc diet was prepared by omitting zinc carbonate from the mineral mix.

In this experiment mice were caged singly in polypropylene cages with stain-less still gridded tops and bottoms and stain-less-steel food hoppers, in a room at 21C° having a 12 hours light/ 12 hours dark

cycle. Trays were placed under each food hopper to collect split food. Food and distilled water were provided *ad-lib.* Food intake was measured daily and body weight recorded twice weekly for 28 days.

The effect of reduced dietary zinc intake on the growth, food intake, Zn status, fasting blood glucose and insulin concentration, and fasting liver glycogen concentration of diabetic and non-diabetic mice was investigated. Mice were maintained on the appropriate experimental diet *ad-lib* for 26 days. Fasted overnight and on day 27 given access to food for two periods of 1 hour between 11: 00 -12: 00 hours and 17:00 -18:00 hours so that time of feeding on day before death was similar for all groups.

Mice were then killed between 11:00 and 12:30 hours on day 28. On animal from each group being killed at approximately the same time by exanguination from heart whilst under ethyl-ether anaesthesia . Blood was transferred into an ice cold heparinized vial and a portion taken for whole blood glucose analysis which was performed promptly after exsanguination. The remaining blood was centrifuged and the plasma stored a -20 C° before insulin assay. Liver were rapidly excised weighed freeze-clamped at - 196 C°, ground under liquid nitrogen and stored at -20 C° before glycogen analysis. The 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 the right femur and pancreas were weighed, dried at 80 C° for 16 hours and zinc concentration determined.

Analytical methods

Blood glucose was measured in 10 µl samples of whole blood by the glucose oxidase method using an YSI. Model 27 Glucose analyser. Plasma insulin was determined by a radioimmuno-assay. Glycogen was determined as glucose following an enzymatic hydrolysis with amyloglucosidase (16). The dried pancreas and femur were heated in silica crucibles at 480 C° for 48 hours and the ash taken up in hot hydrolic acid (11.7 M) for Zn analysis by Atomic Absorption (Pye Unicam PU 9000,). The accuracy of zinc recovery using this method was checked by utilising standard references materials (bovine liver and wheat flour). Comparison between the effect of diet and the genotype were made using Student t test.

Results

Body weight gain of diabetic mice fed on a low-Zn diet for 28 days was not significantly different from their controls counterparts. Although the total food intake of the low-Zn diabetic animals was 7 % higher ($p < 0.01$) than that of the control diabetic mice. Body weight gain of original stain fed on low-Zn diet was lower ($p < 0.01$) than their controls. However, the amount of food consumed by the low-Zn and control original strain mice was not significantly different. Values for body weight gain and food intake were significantly higher for diabetic animals compared with the original strain (Table 1).

Table 1. Mean body weight gain (g), total food intake (g), femur zinc concentration (µg/g dry wt), dry weight of pancreas (mg), pancreatic Zn content (mg), and pancreatic Zn concentration (µg/g dry wt) of diabetic and original strain mice given low-Zn (1mg Zn/kg) or control (54 mg Zn/kg) semi-synthetic diet for 28 days.

Genotype	Diabetic				Original strain			
Diet	Control (n 20)		Low-Zn (n 19)		Control (n 8)		Low-Zn (n 8)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Initial body-wt	19.6[a]	0.3	19.5[a]	0.1	15.2[b]	1.1	17.9[b]	0.3
Body-wt gain	11.9[a]	0.8	12.7[a]	0.7	7.8[b]	0.8	4.3[c]	0.4
Total food intake	121[a]	2	130[b]	2	93.8[c]	1.6	87.0[c]	3.1
*Food conversion %	9.9[a]	0.6	9.8[a]	0.7	8.2[a]	0.7	4.9[b]	0.5

a, b, c values within a horizontal line with different superscript letters were significantly different ($p < 0.05$),

Femur and pancreatic Zn concentration taken as an index of Zn status, indicated that both the low-Zn diabetic and original strain mice were able to maintain a similar status to that of their control group, except for a significant reduction in femur zinc concentration in the original strain (C57BL/KsJ) mice fed on low-Zn diet compared to their controls (Table 2). Total pancreatic Zn was significantly lower in low-Zn diabetic mice compared with the controls. Pancreatic Zn concentration was significantly lower in all the diabetic animals compared with the original strain. However, because of the increased dry weight of pancreases from diabetic animals, differences in total Zn content were

not so marked. In this experiment mean femur Zn concentration for diabetic mice were lower than those for original strain, but the difference was not always significant (Table 1).

On day 27 of the study, time of feeding was controlled to allow a more accurate comparison of blood glucose and insulin concentration between groups on day 28 when the mice were killed following an overnight fast. Food intake measurements showed that there was no difference between genotypes or dietary groups in the amount of food consumed at either of the two meals on the day before sampling. Weights of food consumed at last meal (mean ± SEM) were (g) control groups: diabetic (0.9 ± 0.05), original strain (1± 0.1), low-Zn groups: diabetic (0.8 ± 0.07), original strain (0.8 ± 0.02).

Analysis of fasting blood glucose and plasma insulin concentration and liver glycogen content, indicated that low-Zn diabetic mice had a similar plasma insulin concentration, a higher fasting blood glucose level ($p < 0.05$) and an increased liver glycogen content ($p < 0.05$) compared with the control diabetic animals (Table 2). The higher liver glycogen values in the low-Zn diabetic group were associated with a significant increase in liver weight ($p < 0.05$) together with a trend towards a higher glycogen concentration. Liver weight and liver glycogen content were similar in both low-Zn and control groups of the original strain mice (Table 2).

Table 2. Mean fasting blood glucose (m mol), plasma insulin (μ units/ml), liver fresh wt (g), total liver glycogen (mg), and liver glycogen concentration (mg/g fresh wt) of diabetic and original strain mice given a low-Zn (1mg Zn/Kg) or control (54 mg Zn/kg) semi-synthetic diet for 28 days.

Genotype	Diabetic				Original strain			
Diet	Control (n 20)		Low-Zn (n 19)		Control (n 8)		Low-Zn (n 8)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Femur Zn Concentration	120^{a}	3	121^{a}	6	149.6^{b}	2.9	116.7^{a}	3.8
Pancreas dry wt	59^{a}	3	57^{a}	3	34^{b}	1	33^{b}	24
Pancreatic Zn content	3.2^{a}	0.1	2.7^{b}	0.1	3.6^{ac}	0.2	3.4^{ac}	0.11
Pancreatic Zn concentration	56.5^{a}	4.1	49.0^{a}	3.8	108.1^{b}	6.1	106.0^{b}	5.9

a, b, c values within a horizontal line with different superscript letters were significantly different ($p < 0.05$),

Genotype	Diabetic				Original strain			
Diet	Control (n 20)		Low-Zn (n 19)		Control (n 8)		Low-Zn (n 8)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Blood glucose	12.4^{a}	0.4	15.3^{b}	0.9	6.97^{c}	0.5	6.9^{c}	0.3
Plasma insulin	52.1^{a}	5.6	46.0^{a}	5.4	15.2^{b}	3.3	9.4^{b}	1.4
Liver fresh wt	1.86^{a}	0.08	2.1^{b}	0.5	0.84^{c}	0.04	0.81^{c}	0.0
Total Liver glycogen	22.1^{a}	3.5	32.7^{b}	3.9	5.64^{c}	1.3	3.77^{c}	1.4
Liver glycogen concentration	11.4^{a}	1.5	15.7^{a}	1.9	6.57^{b}	1.5	4.54^{b}	1.7

a, b, c values within a horizontal line with different superscript letters were significantly different ($p < 0.05$).

Discussion

In this experiment the body weight gain of diabetic mice was not significantly affected by dietary Zn concentration (1 or 54 mg Zn / kg). Although the diabetic mice fed on the low-Zn diet had a higher food intake than those fed on the control diet. This is surprising in view of the many studies demonstrating reduced appetite in animals fed on low-Zn diets (17-18) and raises the possibility that the degree and direction of response to dietary Zn depletion may be influenced by the metabolic state of the animals. Body weight gain of the original strain (C57BL/KsJ) mice fed on low-Zn diet was lower than that of the control group, this is an agreement with the results which obtained with other animal species fed on inadequate dietary zinc (17-19). There was, however, no significant differences in the amount of food consumed by these two groups of mice, consequently, the food conversion ratio over 27 days was significantly higher in the control group compared to the low-Zn group. This supports the work of Chesters & Quarterman (20) who found in force feeding experiments, that the low growth rate of zinc deficient rats was not entirely due to reduced food consumption, but also to other metabolic process for which zinc is essential. Rate of growth of the diabetic mice, whether fed on a low-Zn or control diet, was markedly higher than for any of the non diabetic groups of animals. This is likely to be related to the high food intake of these mice compared to the non-diabetic animals, or to the possibility that insulin. dependent enzyme activities (glucokinase, citrate lyase, glucose 6 phosphate dehydrogenase) are probably much higher in the adipose tissues of the diabetic (C57BL /KsJdb/db) mice because of their hyperinsulinaemia. They would therefore be capable of converting glucose to carbon dioxide much more efficiency than the non-diabetic mice, thus producing the energy required for free fatty acids and fat synthesis leading to obesity (13, 21, 22). Pancreatic Zn concentration were similar in dietary groups of the same genotype, despite the fact this tissue is generally regarded to be one of the most sensitive to variation in dietary Zn intake (23, 24). There was, however an indication of reduced total pancreatic Zn in diabetic mice fed on the low-Zn diet. The lower pancreatic zinc concentration observed in the diabetic mice fed at both levels of Zn compared with original strain, is probably related to their hyperinsulinaemia, the early onset of *B-cell* degranulation and other pathological changes in this tissue associated with the progression of the condition (13), and is consistent with human studies showing that the pancreatic Zn concentration of diabetics is depressed compared with normals (25, 26). The reduction in femur zinc concentration in the original strain low-Zn group, compared with their controls, is interesting in view of the finding that the growth rate of these animals was also significantly reduced. These mice appear, therefore, to be more susceptible to low dietary zinc intake than the diabetic animals studied. There are two possibilities, which explain the loss of femur zinc in these C57BL/KsJ mice. Less of the absorbed zinc may have been transferred to the relatively non mobilisable pool of zinc in bones, thus preserving zinc in the metabolically active pool, or bone zinc stores may have been mobilised to maintain tissue zinc levels. Despite the apparently minimal effects of consuming the low-Zn diet on growth and Zn status of mice used in the present study, significant differences in glucose metabolism were observed. In this experiment, when the time of feeding was strictly controlled and the amount of food eaten by each animal before an overnight fast was known to be similar, the mean blood glucose concentration in the low-Zn diabetic mice was found to be 23 % higher than that for control diabetic group. This suggests that the lower Zn intake had exacerbated the reduced ability of the diabetic mice to utilize glucose. Results from the present study, and those of a previous studies showing increased blood glucose concentration after oral dosing in rats fed on a marginal zinc diet in late pregnancy (4), suggest a relation between carbohydrate utilization and dietary Zn supply, particularly in condition associated with hyperinsulinaemia and tissue in insulin resistance. Since circulating insulin level in this study was unaffected by the reduced dietary Zn intake, the possibility of increased insulin resistance or reduced physiological potency of the hormone should be considered. Aside from the well known large accumulation of subcutaneous fat in the genetically diabetic mice, the most striking anatomical deviation is the size of the liver, which is in part due to metabolic defects resulting in increased fat and glycogen deposition (13). In the present study, the livers of diabetic mice were two to three times heavier than their original strain and it was noted that livers from low-Zn diabetic mice were significantly heavier than those from their control counterparts. In addition, the fasting glycogen content of the low-Zn diabetic mice was approximately 48 % higher than in the control diabetic animals, although food intake on the day before death was similar for these two groups. This again indicates that the carbohydrate metabolism of these animals is sensitive to reduced Zn intake. It is interesting to not that Reevers & O'Dell (3) found also evidence of increased glycogen synthesis in Zn-deficient rats. The fresh weights of liver from both groups of diabetic mice were higher than for any of the non diabetic groups of animals. This is likely to be related to the higher glycogen content of the livers of these mice and possibly to other compositional changes not considered in this study. The increased glycogen deposition in these mice may be related to the higher food intake of the diabetic mice , the high blood glucose concentration, and possibly to an

increased activity of hepatic enzymes such as pyruvate carboxylase and phosphoenol pyruvate carboxylase, which increase the rate of gluconeogenesis in the liver.

In conclusion, findings presented in the present paper demonstrate that reduced Zn intake had an adverse effect on glucose utilization in the genetically diabetic mouse, although changes in Zn status appeared to be minimal. It appears therefore, that abnormalities in carbohydrate metabolism may occur before tissue Zn depletion become apparent. However the original strain mice were more susceptible to low dietary zinc than diabetic mice in that the original strain mice given a low-Zn diet had lower body weight gain and femur zinc concentration than their controls.

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