Observations on glucose tolerance and plasma levels of free fatty acids and insulin in the zinc-deficient rat

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1. The glucose tolerance and plasma free fatty acid and insulin concentrations were compared in zinc-deficient rats and rats given a Zn supplement.

2. The glucose tolerance of Zn-deficient rats was not different from that of rats given a Zn-supplemented diet.

3. The plasma insulin concentration after a dose of glucose tended to be lower in Zn-deficient rats than in those given a Zn supplement.

4. During the development of Zn deficiency there was a transient rise in plasma free fatty acid concentration.

Zinc is closely associated with insulin in the characteristic dark-staining granules of the β-cells of the islets of Langerhans (Logothetopoulos, Kanek, Wrenshall & Best, 1964; Petkov & Galabova, 1969) and it was considered possible that changes in the Zn status of an animal could affect the production, storage or release of insulin, especially since the rat pancreas as a whole suffers a greater decrease in Zn content than most tissues when the animals become Zn-deficient (Williams & Mills, 1970).

In the Zn-deficient animal, changes have been observed in adipose-tissue metabolism (Quarterman, Mills & Humphries, 1966; Quarterman, 1969) and in carbohydrate metabolism (Quarterman *et al.* 1966; Hove, Elvehjem & Hart, 1937). These changes, however, are the sort which could be affected by differences in feeding pattern between an experimental, e.g. Zn-deficient, animal with a reduced food intake and a pair-fed control receiving a diet supplemented with Zn. The deficient animal will have a fairly constant rate of food intake in any one day, but the control animal, receiving a quantity of food much less than its appetite requires, will adopt a meal-eating pattern of feeding. Some effects of feeding pattern and previous day's food intake on glucose tolerance and plasma free fatty acid (FFA) concentration and plasma insulin levels have been described in the preceding paper (Florence & Quarterman, 1972). In the present paper the observations of changes in carbohydrate and lipid metabolism in Zn deficiency are re-examined and extended, taking into account the possible effects of differences in feeding pattern.

EXPERIMENTAL

Most procedures used in this work, including the measurement of glucose tolerance and the imposition of feeding patterns on the experimental rats, are described by Florence & Quarterman (1972).

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Zn-deficient diets and animals

Hooded Lister rats (Rowett strain), maintained under minimal disease conditions, were used in all the experiments. Diets low in Zn were prepared as described by Williams & Mills (1970), and were used when they contained less than 1 μg/g Zn; 40 μg/g Zn as Zn SO_4 was added to the Zn-supplemented diets. Rats given the low-Zn diet lost appetite, ate continuously for 24 h/d and ceased to grow after 3 or 4 d. Thereafter they ate about 5·5 g food a day (Williams & Mills, 1970). Control rats were individually pair-fed with Zn-deficient rats and either meal-fed from a pot or continuously fed by means of a rotary feeder supplying food continuously throughout 24 h (Quarterman, Williams & Humphries, 1970).

Glucose-tolerance tests

The glucose tolerance of ten Zn-deficient rats, 30–50 d of age, was compared with that of ten pair-fed, continuously fed rats of the same age given the Zn-supplemented diet. The tolerance of each rat on the Zn-supplemented diet was measured a day after its Zn-deficient pair-mate so that the two animals were comparable with regard both to feeding-pattern and to the preceding day's food intake.

Analytical methods

Zn determinations were made by atomic absorption spectrophotometry on wet-ashed samples.

Ketone bodies in plasma were estimated by the method of Tanayama & Ui (1963) and cholesterol by a colorimetric method using dimethylbenzene sulphonic acid (Biochemica Test Combination, C. F. Boehringer & Sons Ltd, Mannheim, Germany).

RESULTS

Glucose tolerance

The tolerance curves (Fig. 1) of the two groups were not significantly different.

Plasma FFA

Fig. 2 shows the fasting plasma FFA concentrations of Zn-deficient and pair-fed, meal-eating, Zn-supplemented rats. After about a week the concentration of FFA in the plasma of the Zn-deficient rats after an overnight fast was nearly twice as great as in the controls, but after about 30 d the concentration in the Zn-deficient rats had fallen almost to that in the controls. The concentrations of FFA in the plasma of the deficient rats tended to remain higher than in the controls, but this might be expected, since the deficient rats were continuously fed but the controls were meal-eaters (Florence & Quarterman, 1972).

Plasma ketone bodies and cholesterol

The fasting plasma concentrations of ketone bodies in the Zn-deficient rats were nearly three times as high as in the meal-eating control rats, 6·0 $\pm$ 1·0 mg/100 ml com-

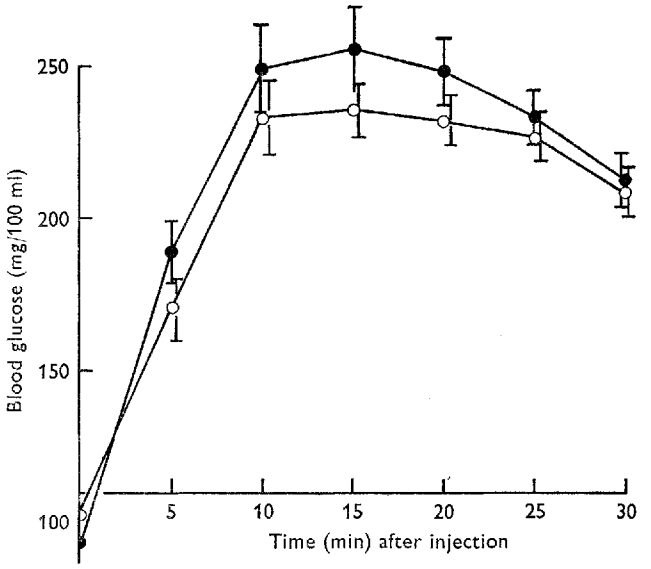

Fig. 1. Changes in tail-blood glucose concentration after intraperitoneal administration of glucose (175 mg/100 g body-weight) to zinc-deficient rats (○—○) and to continuously fed control rats (●—●). The vertical bars represent the standard errors of the mean.

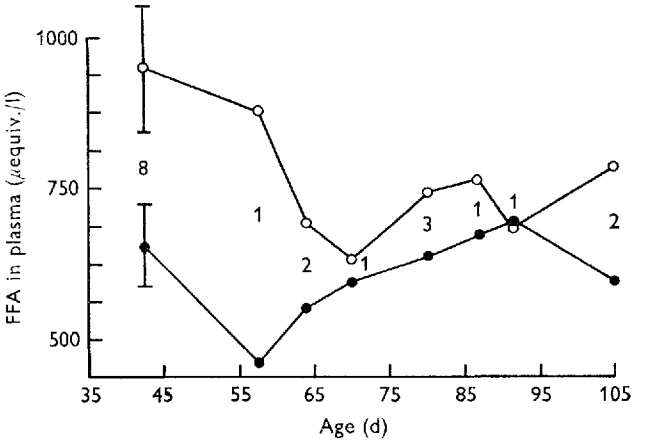

Fig. 2. Changes with time in plasma free-fatty-acid (FFA) concentration of continuously fed zinc-deficient rats (●—●) and meal-eating rats given a Zn-supplemented diet (○—○). The numbers indicate the number of pairs of rats sampled at each time. The difference at 42 d was highly significant ($P < 0.01$) – for this sampling time, the vertical bars represent the standard errors of the mean.

pared with 2.2 ± 0.4 mg/100 ml. There was no difference in the plasma cholesterol concentrations, 77 ± 3 and 73 ± 4 mg/100 ml, between the deficient and control groups. The rats in which plasma ketone bodies were determined had received the experimental diets for 29 d; those used for cholesterol determinations received the diet for 16–101 d.

Plasma insulin concentrations

The concentrations of insulin in plasma taken 15 min after a dose of glucose in six Zn-deficient rats, six continuously fed rats and three meal-eating rats, all 40–50 d of age, were not significantly different, being 54 ± 12, 63 ± 8 and 70 ± 8 μunits/ml respectively.

DISCUSSION

It has previously been reported that there was a poorer glucose tolerance than normal in Zn-deficient rats than controls (Hove *et al.* 1937; Quarterman *et al.* 1966; Hendricks, Mahoney & McClusky, 1971). The observed effect has, however, been attributed to a difference in feeding pattern between Zn-deficient rats eating slowly and continuously and their meal-fed pair-mates which eat their food over a relatively short period (Macapinlac, Pearson & Darby, 1966). We now believe from the evidence presented in this and the previous paper (Florence & Quarterman, 1972) that the amount of food eaten during the day preceding the glucose tolerance test is the decisive factor. The imposition of different feeding patterns on an animal leads necessarily to the consumption of different amounts of food before an overnight starvation. Thus, animals feeding continuously, either because they are given food *ad lib.* (Florence & Quarterman, 1972) or because they are fed by a continuous feeder (Quarterman *et al.* 1970), receive their food from the time of the morning feed until food is withdrawn before starvation, usually for about 8 h, and thereby consume about a third of their day's food. Zn-deficient rats are usually fed *ad lib.* and are in this category of continuous feeders. Meal-eating rats, such as the pair-fed controls in our experiments and those of Macapinlac *et al.* (1966), soon adapt themselves to eating the whole of their day's food in much less than 8 h.

A previous report (Quarterman *et al.* 1966) described a small decrease in plasma insulin of Zn-deficient rats compared with Zn-supplemented rats following an intraperitoneal dose of glucose. The rats were then about 70 d of age and the concentrations were much lower than those observed in the present work where the rats were about 45–50 d of age. A decrease in plasma insulin with age in this age-range has been described by Florence & Quarterman (1972). In both experiments the plasma insulin concentration was lower in the Zn-deficient rats, though in the present work the difference was not statistically significant. This decrease in the Zn-deficient rats is insufficient to affect the glucose tolerance, which changes with age in the same way as in Zn-treated animals (Florence & Quarterman, 1972).

Soon after young rats are given a Zn-deficient diet there is a large increase in the fasting plasma FFA concentration which cannot be accounted for by any aspect of dietary history except Zn-deficiency, since the plasma FFA concentrations of continuously fed rats of the same age on the Zn-supplemented diet are only 760 ± 30 μequiv./l (Florence & Quarterman, 1972). After 2 or 3 weeks the value has fallen to nearly that of the meal-eating controls. The tendency for the concentration in the deficient rats to be higher than in these controls after the 3rd week could be due to the different feeding systems of the two groups. The same considerations probably apply to the plasma

ketone body concentrations. Very small additions of Zn have been shown to affect glucose uptake by rat adipose tissue in vitro (Quarterman, 1969) and the glucose uptake of adipose tissue from Zn-deficient rats is much less than that from rats given a Zn supplement. The changes in FFA and ketone body concentrations are evidence that some disturbance in adipose tissue metabolism is occurring in vivo. This disturbance may be such as to result in degradation of the triglyceride reserves, causing in turn high FFA concentrations in the blood.

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