Hexose absorption from jejunal loops *in situ* in zinc-deficient and Zn-supplemented rats

By SUSAN SOUTHON, JENNIFER M. GEE and I. T. JOHNSON

AFRC Food Research Institute Norwich, Colney Lane, Norwich NR4 7UA

1. Immature, male Wistar rats were given a low-zinc semi-synthetic diet (2 mg Zn/kg) for 22–28 d. Control groups received a similar diet supplemented with 58 mg Zn/kg either *ad lib.*, or in amounts matched to the consumption of the Zn-deficient group. There was a rapid onset of reduced food consumption and growth retardation in the Zn-depleted animals.

2. Serosal surface area of small intestines taken from Zn-deficient rats was significantly reduced compared with that of control animals. Villi, dissected from samples of proximal jejunum, were markedly smaller than those of control rats and were present in greater numbers per unit area of serosa.

3. Luminal loss of galactose from jejunal loops *in situ* was significantly greater in the Zn-deficient rats compared with controls when expressed in terms of unit dry weight of intestine and serosal or villous surface area. Since only a small proportion of the total galactose remained in the mucosal tissue and associated extracellular space, this loss could only be accounted for by an increased efficiency of net trans-epithelial transport. Differences in total galactose absorption per unit length of jejunum were not so marked.

4. This intestinal adaptation to Zn-deficiency allows the maintenance of normal, and possibly increased, rates of hexose transfer into the body of animals exhibiting severe growth retardation, reduced food utilization and abnormal glucose metabolism.

Previous transport studies in vitro showed that there is a marked enhancement of carrier-mediated galactose and 3-*O*-methyl glucose uptake into mucosal tissue of zinc-depleted rats compared with feed-restricted and *ad lib.*-fed controls (Southon *et al.* 1984). Kinetic analysis of the results demonstrated that this was due to an increase in the maximum transport rate rather than carrier affinity, indicating that larger numbers of carrier sites are present per unit dry weight of small intestine. This finding has important implications since Zn-deficiency has been reported to result in a reduction in both the secretion of and sensitivity to insulin (Quarterman *et al.* 1966) and abnormal glucose metabolism (Reeves & O'Dell, 1983). There are several reports of reduced glucose tolerance in Zn-deficient rats given glucose intraperitoneally; however, this effect was not observed when the dose was given orally (Quarterman *et al.* 1966; Hendricks & Mahoney, 1972). It has been suggested that the normal glucose tolerance curves following an oral dose of glucose could be attributed to poor absorption of nutrients from the gut of Zn-deficient animals (Hove *et al.* 1937), or to an increased stimulation of insulin secretion (Hendricks & Mahoney, 1972). Our findings clearly do not support the first of these hypotheses.

An increased capacity to transport substrate across the brush-border membrane does not necessarily imply an increased rate of trans-epithelial intestinal transport. In the present study we investigated the absorption of galactose from intestinal loops *in situ* in anaesthetized Zn-depleted and control rats. This technique allows studies to be performed on the living animal using lengths of intestine with intact blood supply, thus mimicking the in vivo situation more closely. In addition, luminal loss, intestinal tissue accumulation, and transfer of the radiolabelled hexose into the bloodstream can be measured.

MATERIALS AND METHODS
Animals and diets

Thirty-six immature, male Wistar rats (approximately 100 g) were randomly divided into three groups and housed in polypropylene cages with stainless-steel-gridded bottoms and tops. Rats were caged in pairs to eliminate the stress of isolation. The Zn-deficient group received a semi-synthetic diet containing 2 mg Zn/kg diet *ad lib*. The second and third groups received a similar diet supplemented with 58 mg Zn/kg diet. The first of these control groups was given an amount of food equal to that consumed on the previous day by a matched pair of Zn-deficient rats. This group was designated the feed-restricted group. The third group was fed *ad lib*. The composition of the semi-synthetic diet was similar to that previously described (Southon *et al.* 1984) with the addition of 5 g methionine/kg diet. All rats received distilled water *ad lib*. Food intakes were measured daily and body-weights recorded at twice weekly intervals. The body-weight gain of cage mates was compared at each weighing as an index of the relative amounts of food consumed by each individual. On days 22, 23 and 28 of the study, equal numbers of rats from each group were taken for transport studies, followed by morphological examination of the jejunum.

Transport study

On the day before they were killed, feed-restricted control rats consumed their daily ration of food between 16.00 and 17.00 hours. All animals were then fasted overnight and transport studies performed between 10.00 and 12.00 hours on the following day. Animals were anaesthetized by intraperitoneal injection of sodium barbiturate (Sagatal: May & Baker, Dagenham, Essex; 1 ml/kg body-weight), placed on a heated operating table at 37° and the abdomen opened. The small intestine was ligatured 100 mm from the pyloric sphincter and a further ligature was positioned 50 mm distally along the jejunum. The isolated loop was filled with 40 mM-galactose in Krebs bicarbonate ringer buffer containing [^{3}H]galactose (1·0 μCi/ml) and ^{14}C-labelled polyethylene glycol (PEG; molecular weight 4000; 0·5 μCi/ml), and replaced in the abdomen. After 8 min the isolated loop was removed intact, with approximately 10 mm of additional jejunum, which was removed to fixative for morphological examination. The loop was rinsed, trimmed free of mesentery and fat, blotted lightly and weighed on a torsion balance. The loop was then cut open and the lumenal contents collected. The loop was reweighed, placed in a preweighed vial and dried at 85° for 18 h. Blood was collected by cardiac puncture and placed in a heparinized vial.

Dried tissue samples were digested in concentrated nitric acid (0·4 ml; 70° for 15 min) in tightly capped vials, cooled and mixed with Trizma base (3·6 ml; 0·75 M). Portions (0·5 ml) were diluted to 2·0 ml with distilled water and added to 18 ml scintillation mixture (Cocktail T Scintran; BDH, Poole, Dorset). Preliminary experiments in which samples of intestine were either acid-digested and counted, or combusted and counted, showed no loss of ^{3}H or ^{14}C during acid digestion. Lumenal contents were made up to 2·0 ml with distilled water and added to 18 ml scintillant. Whole blood samples (0·2 ml) were digested at room temperature in 1·5 ml Soluene-350 (Packard, Reading, Berks)-propan-2-ol (1:1, v/v). After 48 h the samples were decolorized by the addition of 0·5 ml hydrogen peroxide (300–350 ml/l) and counted in 18 ml 0·5 M-hydrochloric acid–insta-gel (Packard) (1:9, v/v) after dark adaptation.

Prepared tissue and fluid samples were counted in polythene vials using a Philips PW 4700 liquid-scintillation spectrometer and a dual-label counting programme without automatic quench compensation. Counting efficiency was measured by external standard channels ratio.

Mucosal extracellular space and fluid transport were calculated on the basis of the amount

of the non-transported ^{14}C-labelled PEG associated with the tissue, and the change in the lumenal concentration of ^{14}C-labelled PEG respectively. Hexose transfer into the blood-stream was calculated by correcting the change in lumenal [^{3}H]galactose concentration for mucosal accumulation of the radiolabel.

Morphological study

Samples of jejunum, obtained from animals in the transport study, were fixed by immersion in a mixture of absolute ethanol and glacial acetic (75:25, v/v) for 24 h, followed by storage in ethanol–water (75:25, v/v). Subsequently, each sample was slit open and measured under a dissecting microscope, fitted with a graduated eyepiece, to obtain an estimate of the width across the serosal surface and the number of villi per unit area of serosal surface. A total of ten villi were then removed from each sample of jejunum by microdissection with sharpened needles. Estimates of the maximum height, basal width and thickness were made using the graduated eyepiece. From the individual villous surface area and the density of the villous population, it was possible to estimate the villous surface per unit area of serosa (VSA) as follows:

$$\text{VSA} = n\,(wh + 2th'),$$

where n is the number of villi per unit area of serosa, w is the basal width, h is the maximum height, t is the thickness and h' is the length of the edge from base to tip of individual villi. This formula is based on the assumption that villous shape in the proximal jejunum conforms roughly to that of a wedge, with absorbing surfaces on four sides.

Statistics

The significance of differences between means for the Zn-deficient group and the feed-restricted control group was estimated using Student's paired t test, comparing average values for each cage of rats. All other comparisons were made using Student's unpaired t test.

RESULTS

The mean values for body-weight gain and food intake over the first 21 d feeding period are shown in Table 1. Rats given a low-Zn diet consumed on average 44% less food over this period than the *ad lib.*-fed control rats. The growth rate, as judged by body-weight gain, of the feed-restricted controls was significantly greater than that of the Zn-deficient group. Comparisons of individual rates of body-weight gain showed that growth rates of cage mates within each group were similar, indicating that there were no large variations in food intake between rats housed together. The Zn-deficient rats exhibited the characteristic symptoms of the deficiency over the experimental period, including erratic patterns of feeding, hair loss, skin lesions and lethargy.

Morphological study

There was a significant reduction in the circumference of small intestines from Zn-deficient animals compared with both groups of controls, which resulted in a 15–20% decrease in serosal surface area per unit length of jejunum (Table 2). Villi dissected from samples of proximal jejunum of Zn-depleted rats were significantly shorter, narrower and consequently of smaller surface area than those from control animals. However, they were present in larger numbers per unit area of serosa, with the result that the villous surface per unit area of serosa was not significantly different from that of the controls (Table 2). Villous density in the jejunum of feed-restricted rats was also significantly greater than in the *ad lib.*-fed controls but the increase was not as marked as in the Zn-deficient animals.

Table 1. *Body-weight gain and food intake over the first 21 d of the study for zinc-deficient, feed-restricted and* ad lib.*-fed control rats**

(Values are means with their standard errors for twelve rats)

Dietary treatment...	Zn-deficient		Feed-restricted		*Ad-lib.*	
	Mean	SE	Mean	SE	Mean	SE
Body-weight gain (g)	43·4ᵃ	3·0	102·5ᵇ	5·0	153·2ᶜ	3·9
Average food intake (g)†	462·4ᵃ	11·8	479·0ᵃ	5·8	818·0ᵇ	16·9

a, b, c Mean values with different superscript letters were significantly different ($P < 0.05$).
* For details of dietary treatments, see p. 194.
† Average intake of six pairs of rats.

Table 2. *Morphology of jejunum and microdissected villi from zinc-deficient, feed-restricted and* ad lib.*-fed control rats**

(Values are means with their standard errors for twelve rats)

Dietary treatment...	Zn-deficient		Feed-restricted		*Ad-lib.*	
	Mean	SE	Mean	SE	Mean	SE
Jejunal loop						
Circumference (mm)	7·7ᵃ	0·2	9·0ᵇ	0·2	9·5ᵇ	0·4
Serosal surface area† (cm²/5 cm)	3·85ᵃ	0·1	4·5ᵇ	0·1	4·75ᵇ	0·2
Villi						
Height (μm)	500ᵃ	10	570ᵇ	20	560ᵇ	10
Width (μm)	440ᵃ	20	520ᵇ	30	580ᵇ	30
Thickness (μm)	63ᵃ	4	75ᵇ	4	76ᵇ	3
Density (no./cm²)	2010ᵃ	60	1820ᵇ	40	1550ᶜ	60
Surface area per villus (mm²)	0·29ᵃ	0·02	0·39ᵇ	0·03	0·42ᵇ	0·02
Villous surface area per unit area serosa (mm²/mm²)	5·8ᵃ	0·3	7·1ᵃ	0·4	6·5ᵃ	0·3

a, b, c Mean values with different superscript letters were significantly different ($P < 0.05$).
* For details of dietary treatments, see p. 194.
† Surface area per loop.

Transport study

Data for one pair of rats per group was not available for the transport study due to death of animals whilst under anaesthetic, or loss of lumenal contents. Observations were therefore restricted to five pairs of rats per group.

Animals maintained on a low-Zn diet showed a significant increase in the rate of lumenal loss of galactose per unit dry weight, serosal surface and villous surface of jejunum compared with control rats. This was accompanied by an increase in the concentration of the radiolabel in the blood (Table 3). The concentration of galactose in the intestinal tissue and associated extracellular space of the Zn-depleted rats was, however, no higher than in tissue from control animals. Differences in the total amount of galactose transported from the jejunal loop during the incubation period were not so marked. Nevertheless, the rate of galactose absorption per unit length of jejunum in the Zn-depleted rats was significantly

Table 3. *Lumenal loss, mucosal accumulation and net transport of galactose over 8 min from jejunal loops in zinc-deficient, feed-restricted and* ad lib.*-fed control rats**

(Values are means with their standard errors for ten rats)

Dietary treatment...	Zn-deficient		Feed-restricted		*Ad-lib.*	
	Mean	SE	Mean	SE	Mean	SE
Lumenal loss (μmol galactose/cm^2 villous surface area)	0·55[a]	0·05	0·37[b]	0·02	0·37[b]	0·04
Mucosal accumulation (μmol galactose/cm^2 villous surface area)	0·01[a, b]	0·003	0·02[a]	0·003	0·01[b]	0·002
Net transport† μmol galactose/cm^2 villous surface area	0·52[a]	0·04	0·34[b]	0·02	0·34[b]	0·03
μmol galactose/g dry weight jejunum	163[a]	7	123[b]	7	108[b]	6
μmol galactose/mm length jejunum	0·224[a]	0·01	0·213[a, b]	0·01	0·185[b]	0·01
Blood concentration (μmol galactose/ml whole blood)	0·15[a]	0·02	0·1[b]	0·01	0·09[b]	0·01

[a, b] Mean values with different superscript letters were significantly different ($P < 0.05$).

* For details of dietary treatment, see p. 194.

† Lumenal loss corrected for accumulation of [^{3}H]galactose in mucosal tissue and extracellular space.

Table 4. *Net transport of water over 8 min from jejunal loops in zinc-deficient, feed-restricted and* ad lib.*-fed control rats**

(Values are means with their standard errors for ten rats)

Dietary treatment...	Zn-deficient		Feed-restricted		*Ad-lib.*	
	Mean	SE	Mean	SE	Mean	SE
μl/g dry weight	1485[a]	170	965[b]	140	788[b]	90
μl/cm^2 villous surface	4·85[a]	0·6	2·90[b]	0·4	2·48[b]	0·3
μl/mm length of jejunum	2·28[a]	0·23	1·84[b]	0·20	1·49[b]	0·17

[a, b] Mean values with different superscript letters were significantly different ($P < 0.05$).

* For details of dietary treatments, see p. 194.

greater than the value for the *ad lib.*-fed controls (Table 3). Net fluid transport was consistently higher in Zn-depleted animals compared with both control groups, irrespective of the basis of expressing the values (Table 4).

DISCUSSION

In a previous study we investigated the phloridzin-sensitive uptake of galactose and 3-*O*-methyl glucose in vitro by everted jejunal rings from Zn-deficient and Zn-supplemented rats. The study demonstrated that Zn-deficiency was accompanied by a marked enhancement of carrier-mediated hexose uptake in the jejunal mucosa, which appeared to result from the presence of larger numbers of carrier sites per unit dry weight of intestine (Southon *et*

al. 1984). This increased capacity to transport substrate across the brush-border membrane however does not necessarily imply an increased rate of net trans-epithelial transport. In the present study, galactose loss from jejunal loops *in situ* in anaesthetized rats was investigated, in order to provide direct evidence of an alteration in the rate of hexose absorption in animals consuming a low-Zn diet. This investigation clearly showed that the rate of lumenal loss of galactose per unit dry weight and per unit surface area of jejunum was significantly increased in dietary Zn-depletion. Since galactose is poorly metabolized by the mucosal tissue, and the concentration of the radiolabelled galactose in the mucosa and associated extracellular space of Zn-depleted rats was no higher than in controls, this increased loss from the lumen could only be accounted for by an accelerated transfer of the substrate into the bloodstream.

Morphological studies confirm that Zn-deficiency in the rat is accompanied by a reduction in villous dimensions and an increase in the number of villi present per unit serosal surface area of jejunum. In contrast to our previous findings, villous density was also significantly greater in the feed-restricted controls, but this increase was not as marked as in the Zn-deficient animals. We suggest that this increase is an adaptive response allowing the maintenance of intestinal absorptive surface during prolonged periods of restricted nutrient intake. It has been argued that an increase in villous surface, such as observed in alloxan-induced diabetic rats, is associated with hyperphagia and consequent increased lumenal nutrition (Lorenz-Meyer *et al.* 1977), but in this instance villous density remained unchanged. Alterations in mucosal morphology and cellular proliferation in the Zn-deficient rat are discussed in detail elsewhere (Southon *et al.* 1984, 1985).

The alterations in intestinal structure resulting from inadequate dietary Zn, coupled with the severe growth retardation characteristic of the deficiency, make changes in intestinal function difficult to interpret with respect to rates of nutrient uptake and utilization. No single index or method is satisfactory for characterizing intestinal absorption, particularly when groups of animals differ greatly in levels of food intake, body-weight and intestinal morphology (Levin, 1967). In the present study, although Zn-deficiency is associated with a considerable increase in the rate of galactose absorption when expressed in terms of dry weight or surface area of intestine, differences between Zn-deficient and Zn-supplemented rats are not so great when transport is expressed in terms of unit length. This occurs because dietary Zn-depletion in the rat often results in a reduction in dimensions of the small intestine, which may account for the apparent disparity between our findings and those of other authors who report that animals maintained on a low-Zn diet exhibit similar or lower rates of lumenal loss of glucose in vivo compared with Zn-supplemented controls (Reeves & O'Dell, 1983; Ghishan, 1984). Such absorption studies performed at a single substrate concentration, and without reference to intestinal structural changes, do not lend themselves to investigations of absorption mechanisms at the cellular level. Our investigations, which include both kinetic and morphological studies, suggest that these mechanisms are altered in response to dietary Zn-depletion (Southon *et al.* 1984). There also appeared to be some response to prolonged feed restriction in the control animals, in that rates of galactose transport per unit dry weight and per unit length of jejunum tended to be higher in this group of rats compared with *ad lib.*-fed controls. This agrees with earlier kinetic studies where we observed a significant increase in maximum transport rate in feed-restricted animals compared with *ad lib.*-fed controls (Southon *et al.* 1984).

Fluid absorption in the jejunum is generally regarded as a passive consequence of glucose-stimulated electrolyte movement. It may be expected, therefore, that an increased rate of hexose absorption would be accompanied by an increase in the net transport of sodium and hence water and, indeed, lumenal loss of water from jejunal loops of Zn-deficient rats was significantly higher than that of controls irrespective of the basis of expressing the

values. It is surprising that Ghishan (1984), in a single-pass perfusion study, observed net secretion of both Na and water, accompanied by net absorption of glucose, in Zn-deficient rats. It should be noted, however, that the animals used by Ghishan (1984) suffered from diarrhoea, which is a feature we have never observed in uncomplicated Zn-deficiency. The possibility exists therefore that the fluid secretion observed by Ghishan (1984) might reflect secondary bacterial infection.

Our findings suggest that Zn-deficiency is associated with an intestinal adaptation which permits the maintenance of normal, and possibly increased, rates of galactose and glucose transport into the body despite a reduction in total absorptive surface. The implication of this adaptation in animals exhibiting lower body-weight, size and hence blood volume compared with Zn-supplemented animals has yet to be determined. Measurements of radiolabelled galactose in whole blood at the end of the 8 min incubation period indicated that, although total hexose transfer into the body was only slightly greater in Zn-depleted rats compared with controls, the concentration of galactose in the blood of Zn-deficient rats was significantly higher. This may be a consequence of reduced blood volume in these animals, or the result of differences in galactose clearance from the blood. Investigations of the effect of dietary Zn-depletion on glucose homeostasis have provided conflicting results. Hendricks & Mahoney (1972) demonstrated that although glucose tolerance was impaired when rats were dosed intraperitoneally, this was not the case when the dose was administered orally. Boquist & Lernmark (1969) and Brown *et al.* (1975) found no differences in serum insulin between Zn-deficient and Zn-supplemented animals, whereas the work of Quarterman *et al.* (1966), Quarterman & Florence (1972) and Huber & Gershoff (1973) suggests that there are decreased levels of circulating insulin in Zn-deficient rats, coupled with a resistance to hypoglycaemic coma after insulin dosing. Both glucose homeostasis and metabolism are notoriously difficult to study in Zn-deficiency because of the effect of poor Zn nutrition on food intake, feeding patterns and growth. The findings of Reeves & O'Dell (1983), however, provide evidence of alterations in glucose incorporation into adipose free fatty acids and liver glycogen in meal-fed Zn-deficient rats compared with meal-fed feed-restricted controls, which suggests a regulatory role for Zn in carbohydrate metabolism.

The importance of the changes in intestinal structure and function described in the present paper, in relation to whole-body nutrient utilization, requires further investigation. In particular, both the magnitude and dynamics of insulin response, rates of glucose entry into tissues and glucose utilization by tissues in Zn-depleted and feed-restricted animals with comparable patterns of food intake should be considered in more detail.

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