Influence of low dietary lipid content on anorexia and [^{14}C]glucose uptake in the intestine of zinc-deficient mice

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Zinc deficiency was induced in adult male mice by feeding them for 8 weeks on a purified semi-synthetic Zn-deficient diet (ZD) containing 90 g lipid/kg (60 g maize oil plus 30 g cod-liver oil). One group was then fed on a low-lipid Zn-deficient diet (ZDLR) containing 30 g cod-liver oil/kg as the sole lipid source for a further 8 weeks. At the end of the experiment the stomach clearance rate, daily food intake, body-weight gain and [^{14}C]glucose uptake in the intestine were significantly higher in group ZDLR than in mice that continued eating the Zn-deficient lipid-adequate diet ZD, and were comparable to results for a group given a Zn-supplemented diet. These results suggest that the pathogenesis of anorexia, nutrient malabsorption and growth retardation are secondary to lipid malabsorption resulting from Zn deficiency.

Dietary lipid: Anorexia: Glucose uptake: Zinc deficiency: Mice

Anorexia and growth retardation are the early manifestations of the clinical symptoms of zinc-deficiency syndrome. Changes in taste acuity (Henkin *et al.* 1969), the molar ratio of tryptophan (Ashley & Anderson, 1975) or tyrosine (Wallwork *et al.* 1979) to the sum of the neutral amino acids in blood plasma, reduction in catecholamine level in the hypothalamus (Halas *et al.* 1982) and membrane fluidity in receptors to neurotransmitters (Essatara *et al.* 1984; McClain *et al.* 1985) have been suggested as the possible causes of Zn-deficiency-linked anorexia. Earlier growth retardation was linked initially with low food intake, but recent studies (Koo & Turk, 1977; Moran & Lyerly, 1985), based on diet control and ^{14}C uptake of triolein and amino-isobutyric acid in the intestine, suggest nutrient malabsorption as its possible cause. The pathogenesis of both anorexia and malabsorption is unclear.

Table 1. *Composition of low-zinc basal diet (g/kg)*

Casein (EDTA-treated)	300
Sucrose	510
Maize oil	60
Cod-liver oil	30
Mineral mixture*	35·5
Vitamin mixture†	50
Choline chloride	2·0
Agar agar	12·5

* Mineral mixture provided (g/kg diet): $CaHPO_4$ 2·58, KCl 3·43, Na_2CO_3 1·15, $MgSO_4.7H_2O$ 4·05, $FeSO_4.7H_2O$ 0·60, $MnSO_4.H_2O$ 0·31, $CoCl_2.6H_2O$ 0·04, $CuSO_4.6H_2O$ 0·06, KI 0·004, NaF 0·008.

† Vitamin mixture provided (mg/kg diet): thiamin hydrochloride 200, riboflavin 120, pyridoxine hydrochloride 80, calcium pantothenate 320, biotin 4, nicotinic acid 300, folic acid 10, cyanocobalamin 0·40, α-tocopherol 60, retinol 0·3, ergocalciferol 0·0031.

It has been observed that triacylglycerol accumulates in the mucosal epithelial cells of the intestine and its transport to the lacteals is relatively slow in Zn-deficient animals compared with Zn-supplemented animals (Koo & Turk, 1977; Taneja & Kaur, 1988). This prompted us to consider (1) the accumulation of lipid in mucosal epithelium as a possible cause of anorexia under Zn-deficiency conditions because of its reported inhibitory action on gastric secretion and the stomach-emptying process (Isselbacher & Budz, 1963; Long & Brooks, 1965) and (2) that this accumulation may be responsible for the relatively low absorption rate of the end-products of digestion leading to their losses.

We have examined these two aspects in mice by restricting the dietary lipid content to 30 g/kg semi-synthetic diet after the induction of Zn deficiency, and the results were compared with those for Zn-supplemented, Zn-deficient and pair-fed groups given diets containing 90 g dietary lipid/kg.

MATERIALS AND METHODS

Dietary treatment

Adult male mice (120) of the Lacca strain weighing 23–28 g were divided into three groups and were housed in plastic cages. Sixty animals in one group (ZD) were fed *ad lib.* on a Zn-deficient diet (Table 1) containing 0·5–1 mg Zn/kg, and thirty animals in a second group were fed on a Zn-supplemented (ZS) diet identical to diet ZD except that $ZnSO_4 . 7H_2O$ was added to the mineral mixture in order to raise its zinc content to 100 mg/kg. A third group (PF) of thirty animals was pair fed on diet ZS in amounts equal to the average intake of group ZD during the preceding 24 h. Double-distilled deionized water was given *ad lib.* to the animals of all groups. After 8 weeks dietary treatment, thirty ZD animals were separated and were fed on the modified lipid-restricted diet (ZDLR) containing 30 g cod-liver oil/kg as the sole lipid source (i.e. 60 g maize oil/kg was omitted from the diet) while ZS and PF groups continued to receive the previous lipid-adequate diets for another 8 weeks. Their food intake and body-weights were recorded weekly.

Estimation of stomach clearance rate

Seventy-two animals, eighteen from each group were separated after 16 weeks feeding on their respective diets and starved for 24 h. They were then fed *ad lib.* with their respective diet for 1 h. Their gastrosomatic indices (GSI) and stomach clearance rates (SCR) at 0, 3 and 6 h after suspending their feeding were calculated as

$$\text{GSI} = \frac{\text{Stomach weight}}{\text{Body-weight}} \times 100,$$

$$\text{SCR} = \frac{\text{GSI}_0 - \text{GSI}_t}{\text{GSI}_0 \times t} \times 100,$$

where GSI_0 is the gastrosomatic index at 0 h, GSI_t the gastrosomatic index at time-interval t, and t is the period of starvation (h).

Localization of lipids

The lipids were localized in the intestine cytochemically. The jejunum of each group at 0, 3 and 6 h after feeding was fixed in formalin calcium and processed for cold gelatin sections. Gelatin sections (7 μm) were stained in Sudan black B (SBB; Baker, 1956) and Nile blue sulphate (NBS; Cain, 1947) for general lipids and neutral lipids respectively. Relative densities of the reactions were estimated on a microdensitometer (Vickers M-85). A 30 μm^2

area of enterocytes was scanned each time at a magnification of 10×100 and wavelength 590 nm.

Measurement of intestinal uptake

Intestinal uptake of glucose was measured in a portion of jejunum by the tissue accumulation method of Crane & Mandelstain (1960). A 30 mm section of the intestine of six mice from each of the four groups at 0 h after feeding was removed from the Treitz ligament. It was flushed with chilled saline (9 g sodium chloride/l) and everted using a thin stainless-steel rod. Small rings of the everted intestine were incubated in 5 ml oxygenated Krebs–Ringer bicarbonate (KRB) buffer, pH 7·4 with 5 ml 50 mM-D-glucose containing 50 μCi [^{14}C]glucose/l KRB at 37° for 5 min. The accumulated radioactivity in tissue was determined by digesting the tissue in potassium hydroxide (200 g/l) (Robinson & Alvardo, 1971), dissolving in a dioxan-based scintillation cocktail (Butler, 1961) and counting on a KLB 1215 Rackbeta Liquid Scintillation Counter with more than 90% counting efficiency for ^{14}C isotopes. Extracellular space was separately measured by incubating the tissue in [^{3}H]inulin following the procedure of Alvardo & Mahmood (1974). After making the necessary correction for extracellular space, the tissue uptake was calculated and expressed as units/g tissue where one unit represented 1 mol of the substrate taken up per 5 min at 37°.

Estimation of Zn

The Zn concentration in the intestine of each group was estimated by atomic absorption spectrophotometry after digesting 100 mg fresh tissue in nitric acid–perchloric acid (3:1 v/v).

Statistics

The results were analysed using Student's t test (Ipsen & Feigl, 1970).

RESULTS

The daily food intake in groups ZD and ZS remained identical during the first 2 weeks dietary treatment. It started declining gradually in ZD animals and stabilized to 50 g/kg body-weight *v.* 90 g/kg body-weight in group ZS after 6 weeks (Table 2). Their mean body-weight (Fig. 1) gradually fell during the first 3 weeks and remained almost constant in the following weeks, but was significantly less than body-weights in groups PF ($P < 0{\cdot}05$) and ZS ($P < 0{\cdot}001$). Mean Zn concentrations in the intestine after 8 weeks dietary treatment were 22·66 (SE 0·17), 14·39 (SE 0·44) and 10·73 (SE 0·12) μg/g fresh tissue weight in groups ZS, PF and ZD respectively.

Table 3 summarizes GSI and SCR of groups ZD, PF and ZS after 16 weeks of dietary treatment. The higher GSI in group ZD at 0, 3 and 6 h compared with groups ZS and PF was a result of the loss of body-weight as a consequence of Zn deficiency. However, the SCR in group ZD was 7·4%/h compared with 11 and 11·2%/h for ZS and PF animals respectively. This suggests that the stomach contents of ZD animals moved to the intestine at a lower rate and it took relatively longer than for ZS and PF animals to clear the equivalent amount of food. These values for SCR indicate that ZD mice took 0·5-fold longer than ZS mice to clear an equivalent amount of food, or that they would consume 0·5-fold less food than ZS mice. This approximates to the actual food intakes in the two groups, 30 g/kg for ZS mice and 21 g/kg body-weight for ZD animals during 1 h feeding, the recorded daily food intake during the 8th week of the experiment.

The ZD animals, when allowed to feed on a Zn-deficient lipid-restricted diet (ZDLR) following the lipid-adequate diet ZD, showed a gradual improvement in food intake. At

Table 2. *Mean body-weight and mean daily dry food intake (DFI) (as g and g/kg body-weight) for zinc-supplemented (ZS), Zn-deficient (ZD) and pair-fed (PF) groups of mice*†

(Mean values with their standard errors for thirty mice)

Dietary treatment...	ZS			ZD			PF		
		Daily DFI			Daily DFI			Daily DFI	
Period of treatment (weeks)	Body-wt (g)	(g)	(g/kg body-wt)	(g)	(g)	(g/kg body-wt)	(g)	(g)	(g/kg body-wt)
1 Mean	23·66	2·32	98·1	28·75	2·55	88·9	27·05	2·18	80·6
SE	0·05	0·06	2·5	0·09	0·03	1·0	0·12	0·01	0·5
2 Mean	24·58	2·44	99·3	27·38	2·49	90·9	27·41	2·25	82·2
SE	0·06	0·02	1·2	0·09	0·03	1·3	0·12	0·00	0·3
3 Mean	26·38	2·44	91·5	26·27	2·48	94·4	27·80	2·36	85·0
SE	0·07	0·02	1·0	0·10	0·02	1·1	0·11	0·02	0·7
4 Mean	30·30	2·49	82·3	26·47	2·33	88·3	28·66	2·20	76·7
SE	0·06	0·01	0·5	0·07	0·02	1·0	0·11	0·00	0·1
5 Mean	31·36	2·60	82·9	27·05	2·18	80·7	28·80	2·03	70·6
SE	0·08	0·01	0·3	0·08	0·02	1·0	0·11	0·01	0·3
6 Mean	31·69	2·78	87·8	27·30	2·06	75·4	29·77	2·03	68·2
SE	0·06	0·02	0·6	0·08	0·01	0·4	0·09	0·00	0·1
7 Mean	32·19	2·82	87·6	27·32	1·62	59·3	30·72	1·69	55·1
SE	0·07	0·03	1·0	0·07	0·05	2·0	0·09	0·00	0·0
8 Mean	34·63***	3·15***	91·2***	27·38	1·63	59·6	31·52*	1·62*	51·4*
SE	0·06	0·02	0·6	0·07	0·03	1·3	0·03	0·01	0·1
Percentage wt change (weeks 1–8)	+46·49			−4·7			+16·5		

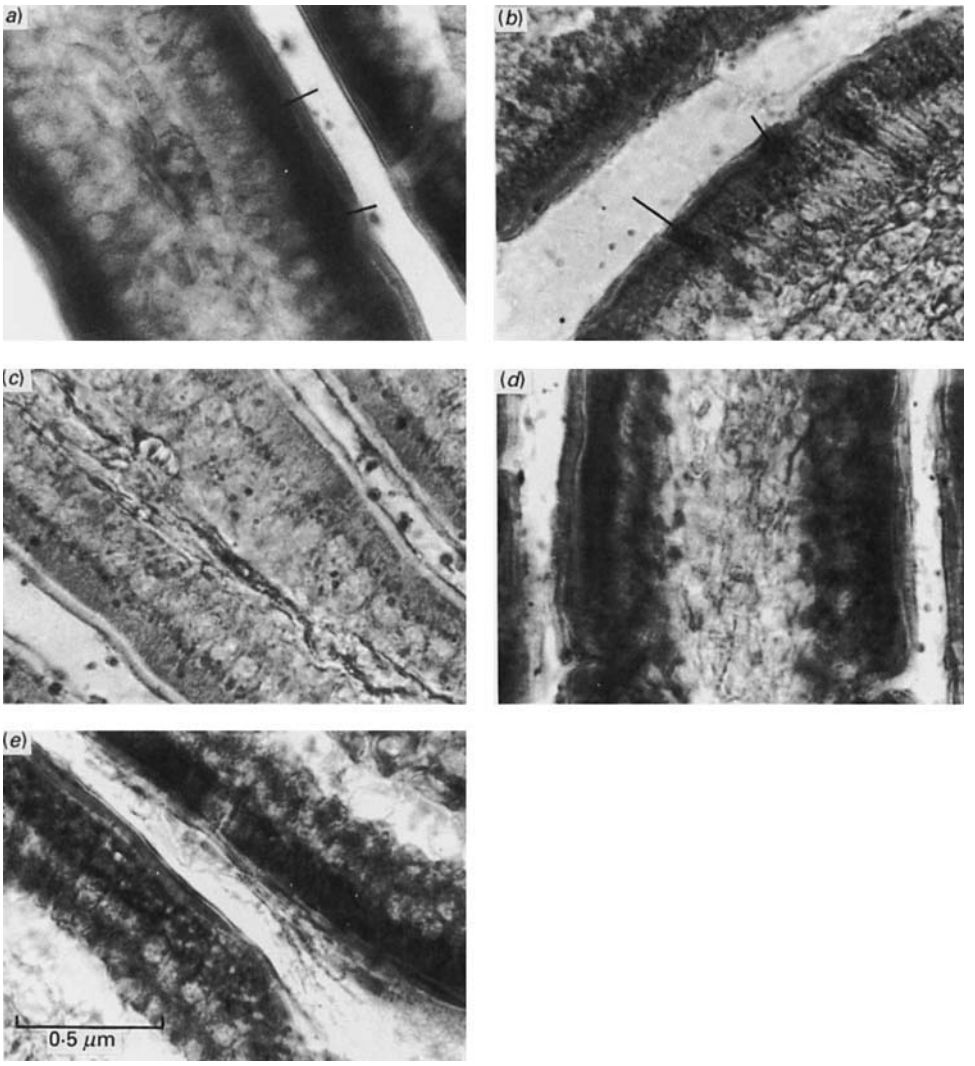
Mean values were significantly different from those for group ZD: * $P < 0·05$, *** $P < 0·001$.

† For details of dietary treatments, see p. 506 and Table 1.

week 14 and onwards (Table 4) their mean food intake approached that of group ZS. At week 16 their final mean-body weight was identical to that of the ZS mice recorded at week 8 (Fig. 2), despite Zn deficiency in these mice. However, their weight gain per kg body mass was less than that of group ZS. Their percentage weight gain exceeded those of groups ZS and PF during weeks 8–16. However, over 16 weeks the total percentage weight gain was less than those of groups ZS and PF. The reduction in food intake, malabsorption of nutrients during weeks 1–8, and the time-interval between anorexia and recovery from it after the change from the lipid-adequate diet ZD to the lipid-restricted diet ZDLR were evidently possible factors contributing to the difference in weight gain, and not the lack of dietary Zn.

The recovery of food intake from week 14 in group ZDLR, equal to that in group ZS, correlated with the SCR which was identical to those of groups ZS and PF at the end of 16 weeks (Table 3). This suggests that the low SCR resulting from the high dietary lipid was the basic cause of anorexia and growth retardation, rather than the lack of Zn in diet ZD.

The cytochemical investigations of ZD, ZS, PF and ZDLR animals revealed intense homogeneous reactions in the cytoplasmic area of the absorptive epithelial cells of the jejunum with SBB and NBS at the 0 h stage (Plate 1(*a*)). These reactions changed to moderate intensity in the form of fine granules at the 3 h stage (Plate 1(*b*)) and were almost negative at the 6 h stage in groups ZS, PF and ZDLR (Plate 1(*c*)). In ZD animals, however,



EXPLANATION OF PLATE

Plate 1. Transverse sections stained with formalin calcium–Sudan black B (Baker, 1956) of the intestines of mice fed on zinc-supplemented (ZS) or Zn-deficient (ZD) diets at intervals after feeding: ZS at (*a*) 0 h, (*b*) 3 h and (*c*) 6 h, and ZD at (*d*) 3 h and (*e*) 6 h after feeding. ↑, Concentration of lipids in mucosal epithelial cells. For details of dietary treatments, see p. 506 and Table 1.

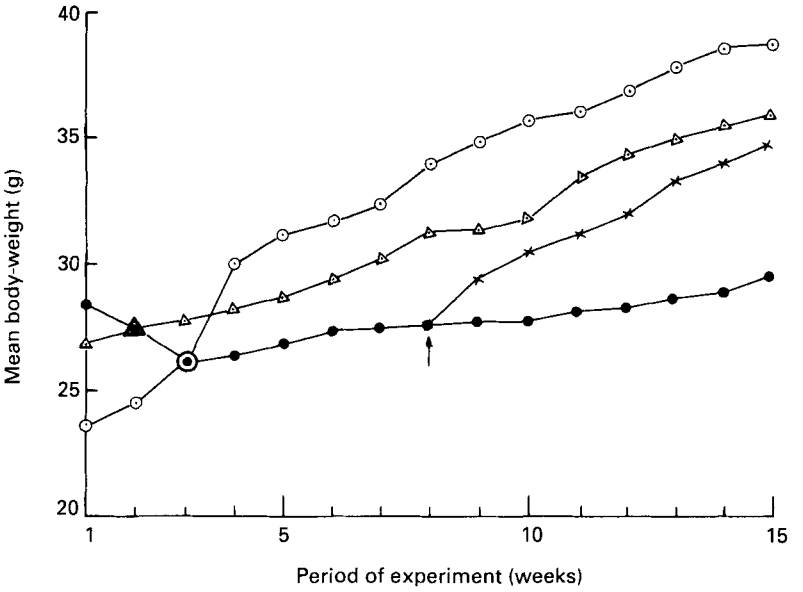


Fig. 1. Mean body-weights of mice fed on zinc-supplemented (○), Zn-deficient (●) or pair-fed (△) diets during the experiment. ↑, Point at which mice fed on the lipid-adequate Zn-deficient diet were changed to a lipid-restricted Zn-deficient diet (×). For details of dietary treatments, see p. 506 and Table 1.

Table 3. *Gastrosomatic indices (GSI) and stomach clearance rate (SCR; % of food cleared/h) in mice fed on zinc-supplemented (ZS), Zn-deficient (ZD), pair-fed (PF) and Zn-deficient lipid-restricted (ZDLR) diets, at 0, 3, and 6 h after feeding them for 1 h*†

(Mean values with their standard errors for six animals per group)

Dietary treatment...	ZS		ZD		PF		ZDLR	
Time-interval after feeding (h)	GSI	SCR	GSI	SCR	GSI	SCR	GSI	SCR
0 Mean	3·25	—	6·89***	—	3·55	—	4·27	—
SE	0·56	—	0·73	—	0·45	—	0·13	—
3 Mean	2·34	8·74	6·57***	1·85	2·69	8·04	2·02	18·85
SE	0·50	2·09	0·39	1·24	0·40	1·14	0·06	0·23
6 Mean	1·31	18·09	3·82***	12·47	1·17	18·62	1·01	12·54
SE	0·11	1·82	0·44	0·55	0·10	1·21	1·21	0·09
Average SCR...		10·97	—	7·43	—	11·17	—	11·78

Mean values were significantly different from group PF: *** $P < 0.001$.
Mean values for groups ZS, PF and ZDLR were not significantly different: $P > 0.05$.
† For details of dietary treatment, see p. 506 and Table 1.

they appeared unchanged at the 3 h stage (Plate 1(*d*)) and slightly less intense at the 6 h stage (Plate 1(*e*)). The relative density of the SBB reaction at each stage in different animal groups has been summarized in Table 5. The differential response to SBB in ZS, PF and ZDLR animals, compared with group ZD, indicates that the dietary lipid was cleared from the epithelial cells in less than 6 h feeding in the former groups while it remained in them and took relatively longer for its clearance to the lacteals in the last group.

Table 4. *Mean body-weight and mean daily dry food intake (DFI) (as g and g/kg body-weight) for zinc-supplemented (ZS), Zn-deficient lipid-restricted (ZDLR) and pair-fed (PF) groups of mice from weeks 8 to 16 of treatment*†

(Mean values with their standard errors for thirty mice)

Period of treatment (weeks)		ZS			ZDLR			PF		
			Daily DFI			Daily DFI			Daily DFI	
		(g)	(g)	(g/kg body-wt)	(g)	(g)	(g/kg body-wt)	(g)	(g)	(g/kg body-wt)
9	Mean	34·88	3·51	100·6	27·16	1·57	57·8	31·22	1·54	50·2
	SE	0·72	0·07	2·1	0·85	0·04	1·4	0·81	0·04	1·3
10	Mean	35·88	3·56	96·1	29·94	1·82	60·8	31·38	1·86	58·6
	SE	0·73	0·03	0·9	0·70	0·04	1·4	0·82	0·02	1·4
11	Mean	36·0	3·48	96·8	30·61	2·11	69·1	32·11	1·97	61·4
	SE	0·74	0·02	0·8	0·70	0·04	1·4	0·78	0·04	1·3
12	Mean	37·0	3·24	87·6	31·44	2·38	75·7	33·83	2·21	65·0
	SE	0·64	0·01	1·0	0·65	0·02	0·9	0·86	0·04	0·8
13	Mean	38·0	3·39	89·2	32·05	2·61	81·4	34·55	2·60	75·2
	SE	0·49	0·03	0·8	0·65	0·03	1·2	0·88	0·03	1·1
14	Mean	38·11	3·47	91·0	33·55	3·01	89·8	35·33	2·90	82·2
	SE	0·48	0·02	0·5	0·67	0·50	1·3	0·83	0·04	1·2
15	Mean	38·88	3·65	93·9	34·0	3·19	93·8	35·88	3·05	84·8
	SE	0·44	0·02	0·6	0·66	0·01	0·4	0·86	0·02	0·3
16	Mean	38·88*	3·82*	98·4*	35·07	3·39	96·8	36·02	3·30	92·2
	SE	0·38	0·02	0·5	0·61	0·02	0·7	0·82	0·03	0·7
Percentage wt change										
Weeks 9–16		+11·46			+29·09			+15·36		
Weeks 1–16		+64·36			+22·0			+29·31		

Mean values were significantly different from groups ZDLR and PF: * $P < 0·05$.
† For details of dietary treatment, see p. 506 and Table 1.

Table 6 depicts relative [^{14}C]glucose uptake in groups ZS, ZD, PF and ZDLR in an isolated intestinal segment in vitro estimated 0 h after the suspension of feeding. These results showed that ^{14}C uptake in ZD at 0 h was less by 2·7- and 2·1-fold in groups ZS and PF respectively. However, in group ZDLR uptake was not significantly different from those for ZS and PF animals, but was significantly higher than that for group ZD.

DISCUSSION

The pathogenesis of growth retardation in Zn deficiency has been reported as a consequence of poor food intake (Chesters & Quarterman, 1970), impaired protein synthesis (Prasad, 1984) and nutrient malabsorption (Moran & Lyerly, 1985). The results of the present studies also showed that the ZD animals gained less weight than the PF group in spite of the comparable food intake, and confirmed earlier reports of nutrient malabsorption associated with Zn deficiency. One such effect, evident from cytochemical pictures, showed that excess triacylglycerol accumulated in the absorptive epithelial cells and remained for a relatively longer time in ZD animals than in ZS and PF animals, although the diet of each group contained equal amounts of dietary lipid. Similar results

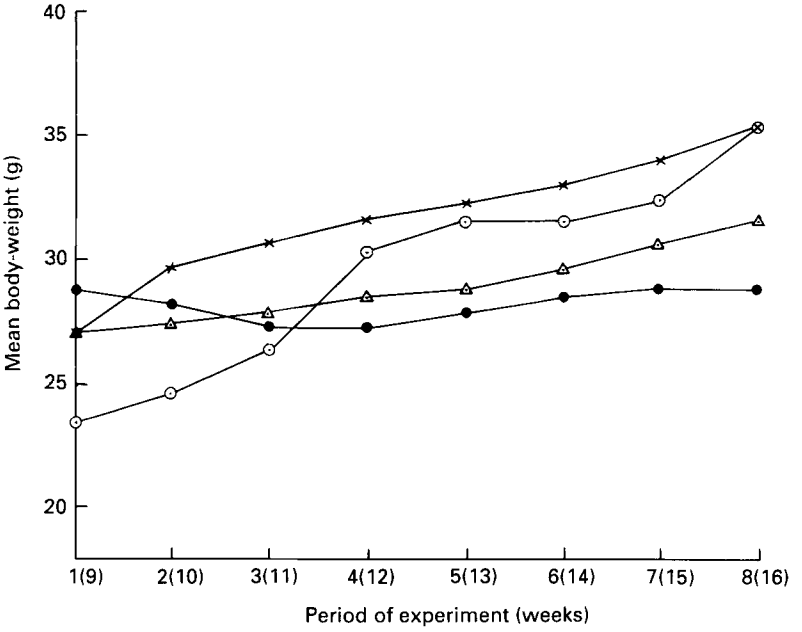


Fig. 2. Mean body-weights of mice fed on zinc-supplemented (○), Zn-deficient (●) or pair-fed (△) diets for 8 weeks; shown on the same scale are the mean body-weights for the mice changed from a lipid-adequate Zn-deficient diet to a lipid-restricted Zn-deficient diet (×) during weeks 9–16. For details of dietary treatments, see p. 506 and Table 1.

Table 5. *Mean density of Sudan black B (SBB; Baker, 1956) reaction in enterocytes of mice fed on zinc-supplemented (ZS), pair-fed (PF), Zn-deficient (ZD) and Zn-deficient lipid-restricted (ZDLR) diets at 0, 3 and 6 h after feeding*†

(Mean values with their standard errors for sixty observations: ten on each rat for 6 rats per group)

Dietary treatment... Time-interval after feeding (h)		ZS	ZD	PF	ZDLR
0	Mean	0·479***	0·581	0·465	0·309***
	SE	0·011	0·006	0·011	0·014
3	Mean	0·253***	0·485	0·250	0·207***
	SE	0·012	0·007	0·005	0·002
6	Mean	0·186***	0·343	0·189	0·115***
	SE	0·009	0·013	0·010	0·002

Mean values were significantly different from those for group ZD: *** $P < 0·001$.
Mean values for groups ZS and PF were not significantly different: $P > 0·05$.
† For details of dietary treatments, see p. 506 and Table 1.

have also been reported by Koo & Turk (1977) in rats. From electron microscopic and chromatographic studies, they concluded that the movement of lipid droplets out of the mucosal cells is blocked owing to the failure of chylomicron formation within them.

Konturek & Grossman (1965) and Long & Brooks (1965) demonstrated the inhibitory action of intestinal lipids on gastric secretion and the stomach-emptying process through a feedback mechanism. The excess lipid accumulation in the intestinal mucosal epithelium

Table 6. *Mean [^{14}C]glucose concentration (μmol/g per 5 min) in the isolated jejunum of mice fed on zinc-supplemented (ZS), pair-fed (PF), Zn-deficient (ZD) and Zn-deficient lipid-restricted (ZDLR) diets immediately after feeding* †

(Mean values with their standard errors for six mice)

Dietary treatment...		ZS	PF	ZD	ZDLR
Mean [^{14}C]glucose concentration	Mean	9·966	8·175	2·264***	9·381
	SE	0·638	0·920	0·600	0·671

Mean value was significantly different from group ZS: ***$P < 0{\cdot}001$.
Mean values for groups ZS, PF and ZDLR were not significantly different: $P > 0{\cdot}05$.
† For details of dietary treatment, see p. 506 and Table 1.

observed in ZD animals, even after 6 h of feeding, accordingly seems to impose a relatively stronger inhibitory action of a longer duration than in groups ZS and PF, causing a slower movement of stomach contents to the intestine. This explains the lower SCR in ZD than in ZS and PF animals. As a consequence, their stomachs emptied more slowly and remained in a state of satiety for a longer period, leading to lower food intake in ZD than ZS animals. Further evidence is provided by the SCR and food intakes of ZDLR animals, in which the lower dietary lipid content was associated with values equivalent to those of ZS animals, despite the absence of dietary Zn.

Moreover, the excess lipid accumulation in the intestinal epithelial cells interfered with the uptake mechanism for nutrients from the intestinal lumen, which was reflected in the [^{14}C]glucose concentrations in isolated intestine of the four groups. The lower [^{14}C]glucose concentration in group ZD compared with groups ZS and PF reflected the decreased capacity of the intestinal epithelium to absorb nutrients in the ZD compared with ZS and PF animals. The higher [^{14}C]glucose concentration in group ZDLR than in ZD establishes a link between ^{14}C concentrations and dietary lipid levels, but not dietary Zn levels. The similarities between ZDLR and ZS animals in SBB and NBS reactions, ^{14}C concentrations in intestine and gain in body-weight suggests that the accumulation of lipids may plausibly be involved in the lower uptake of nutrients in ZD animals, and accounts for their lower body-weight. Lower concentrations of dietary lipids in ZDLR animals evidently eliminate the interfering intestinal lipoidal factor, thus promoting uptake of nutrients to the level of ZS animals, leading to an increase in body-weight equal to that of ZS animals, despite Zn deficiency.

However, Southon *et al.* (1986) did not observe a significant difference between ZD and ZS rats in intestinal [^{3}H]galactose concentration. This contradiction between the present studies and those of Southon *et al.* (1986) can be attributed to the lack of an interfering intestinal lipoidal factor in ZD animals of the latter study, since Southon *et al.* (1986) worked with fasted rats while we studied fed mice. The elimination of the interfering intestinal lipoidal factor in group ZD (i.e. our group ZDLR) gave results close to those of Southon *et al.* (1986), despite the difference in the nutritional status of the animals employed in the two studies. However, the kinetic studies performed by Southon *et al.* (1986) suggest higher intestinal hexose uptake in ZD rats compared with ZS rats. We did not extend our investigations to kinetic studies and are therefore unable to comment further.

Moreover, an analysis of Table 3 reveals that GSI was higher in lipid-adequate ZD animals compared with ZS and PF animals. Stomach weights were about 50% higher in ZD mice than in ZS mice, even though food consumption was lower in ZD than ZS

animals. Such a significant rise in stomach weight cannot be the result of their lower stomach clearance rate. The higher stomach weights of ZD mice compared with ZS mice could result from excess fluid transudation from stomach wall. At present, there is no conclusive evidence to indicate the operative mechanism; however, circumstantial evidence such as lower rate of food consumption, malabsorption of nutrients and impaired protein synthesis reflected as weight loss point towards the induction of hypoproteinaemia in general, but specifically in blood, leading to lower colloidal osmotic pressure of plasma. This situation could be responsible for the passive transfer of fluid from the capillaries of the stomach wall to the lumen during 24 h starvation, contributing to the additional stomach weight. A reduction in the dietary lipid content of group ZD (in group ZDLR) seemed to eliminate hypoproteinaemia by improving food intake, absorption of nutrients and protein synthesis in terms of weight gain. The lower GSI in the ZDLR mice compared with the ZD mice (almost equal to the values for ZS and PF animals) supports this contention.

The results of the present study thus indicate that dietary Zn is essential for the absorption of dietary lipids. Absence of Zn in the diet causes lipid to accumulate in the mucosal epithelium, which inhibits the stomach emptying process through a feedback mechanism and interferes through an unknown mechanism with the uptake of nutrients from the lumen of the intestine. Anorexia, malabsorption of nutrients and growth retardation are thus secondary to lipid malabsorption in Zn deficiency.

REFERENCES

Alvardo, F. & Mahmood, A. (1974). Co-transport of organic solutes in small intestine: A general model amino acid transport. *Biochemistry* **13**, 2882–2890.

Ashley, D. V. M. & Anderson, G. H. (1975). Correlation between the plasma tryptophan to neutral amino acid ratio and protein intake in the self-selecting weanling rat. *Journal of Nutrition* **105**, 1412–1421.

Baker, J. R. (1956). Improvements in the Sudan black B technique. *Quarterly Journal of Microscopical Science* **97**, 621.

Butler, F. E. (1961). Determination of tritium in water and urine. Liquid scintillation counter and rate of drift determination. *Annals of Chemistry* **33**, 409.

Cain, A. J. (1947). Use of Nile blue in the examination of lipids. *Quarterly Journal of Microscopical Science* **88**, 383.

Chesters, J. K. & Quarterman, J. (1970). Effects of Zn deficiency on food intake and feeding pattern of rats. *British Journal of Nutrition* **24**, 1061–1069.

Crane, R. K. & Mandelstain, P. (1960). The active transport of sugar by various preparation of hamster intestine. *Biochimica et Biophysica Acta* **45**, 460–476.

Essatara, M. B., McClain, C. J., Levin, E. S. & Morley, J. E. (1984). Zn-deficiency and anorexia in rats: The effect of central administration of norepinephrine, muscimol and bromergocryptine. *Physiology and Behavior* **32**, 479–482.

Halas, E. S., Wallwork, J. C. & Sandstead, H. H. (1982). Mild zinc deficiency and under nutrition during prenatal and postnatal periods in rats: Effects on weight, food consumption and brain catecholamine concentrations. *Journal of Nutrition* **112**, 542–551.

Henkin, R. I., Graziadei, P. P. G. & Bradley, D. E. (1969). The molecular basis of taste and its disorders. *Annals of Internal Medicine* **71**, 791–919.

Ipsen, J. & Feigl, P. (1970). *Bancrofts Introduction to Biostatistics*, 2nd ed. New York: Harper and Row.

Isselbacher, K. J. & Budz, D. (1963). Synthesis of lipoprotein by rat intestinal mucosa. *Nature* **200**, 363–365.

Konturek, S. & Grossman, M. I. (1965). Effect of perfusion of intestinal loops with acid, fat or dextrose on gastric secretion. *Gastroenterology* **49**, 481–489.

Koo, S. I. & Turk, D. E. (1977). Effect of zinc deficiency on intestinal transport of triglyceride in the rat. *Journal of Nutrition* **107**, 909–919.

Long, J. F. & Brooks, F. P. (1965). Relation between inhibition of gastric secretion and absorption of fatty acids. *American Journal of Physiology* **209**, 447–451.

McClain, C. J., Kasarkis, E. J. & Allen, J. J. (1985). Functional consequences of Zn-deficiency. *Progress in Food and Nutrition Science* **9**, 185–226.

Moran, J. C. & Lyerly, A. (1985). The effects of severe Zn-deficiency on intestinal amino acid losses in the rat. *Life Science* **36**, 2515–2521.

Prasad, A. S. (1984). Discovery and importance of zinc in human nutrition. *Federation Proceedings* **43**, 2829–2834.

Robinson, J. W. L. & Alvardo, F. (1971). Interaction between sugar and amino acid transport system at the small intestinal brush border. A comparative study. *Pflugers Archiv* **326**, 48–75.
Southon, S., Gee, J. M. & Johnson, I. T. (1986). Hexose absorption from jejunal loops *in situ* in zinc-deficient and Zn-supplemented rats. *British Journal of Nutrition* **55**, 193–200.
Taneja, S. K. & Kaur, B. (1988). Lipids in mucosal epithelium of the intestine of mice fed on Zn-deficient diet. *Current Science* **57**, 493–494.
Wallwork, J. C., Fosmire, G. J. & Sandstead, H. H. (1979). Cyclic feeding patterns and plasma amino acid concentrations in Zn-deficient rats. *Federation Proceedings* **38**, 606.

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