Lipid deposition in intestine as a possible cause of malabsorption of nutrients in zinc-deficient common carp (*Cyprinus carpio*)

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An experiment was performed to examine the interaction between Zn deficiency and lipid intake in carp. The carp were given a high-lipid diet that was either Zn-deficient (ZD) or Zn-supplemented (ZS), or were pair-fed (PF) the ZS diet to the intake of the ZD group. After 8 weeks the carp were killed and measurements were made of intestinal glucose uptake, levels of DNA, RNA and triacylglycerol, and alkaline phosphatase (*EC* 3.1.3.1) activity in liver and intestine samples. A further group of similar carp were given the same diets but at week 8 were transferred to low-lipid diets, with the exception of half the ZD group. After a further 8 weeks of treatment, carps were killed for biochemical studies. Intestinal [^{14}C]glucose uptake, levels of DNA, RNA and alkaline phosphatase activity in intestine and liver were significantly ($P < 0.05$) lower in the high-lipid ZD group than in the high-lipid ZS and PF diet groups. The triacylglycerol concentration in the intestine was higher in the high-lipid ZD group than in the other two groups. When the carp were given the corresponding low-lipid diets, the variables measured in intestine and liver of the ZD group were close to those of the other groups. The results of this study demonstrate that lipid, when present in excess in the diet, accumulates in the intestine under Zn-deficient conditions and may reduce the absorption of glucose in carp. The reduced RNA and DNA levels and alkaline phosphatase activity in liver and intestine of ZD fish compared with those of ZS fish given high-lipid diets is proposed to be due to the malabsorption of nutrients linked with lipid deposition in the intestine, rather than their dependence on the level of Zn in the diet.

Lipid metabolism: Zinc: Malabsorption: *Cyprinus carpio*

Low feed intake, growth retardation and cataract formation have been reported frequently in fish as consequences of Zn deficiency (Ogino & Yang, 1978; Satoh *et al.* 1983; Richardson *et al.* 1985). Growth retardation is associated with low feed intake but recent experiments on rats (Koo & Turk, 1971; Moran & Lyerly, 1985) have recognized malabsorption as an important factor in the pathogenesis of this disorder.

In one of our recent communications (Taneja *et al.* 1990) we have reported that a reduction in the lipid content of the basal diet of Zn-deficient fish eliminates anorexia; their feed intake and body weight increase, becoming close to those of the control group. This contradicts earlier reports of the essentiality of Zn in the regulation of feed intake and subsequent absorption of nutrients and suggests that dietary lipid, rather than the lack of Zn in diet, contributes to the malabsorption of nutrients under Zn-deficient conditions. We report a study examining [^{14}C]glucose uptake in isolated intestinal segments and the levels of DNA, RNA and alkaline phosphatase (*EC* 3.1.3.1) activity in intestine and liver of juvenile carp (*Cyprinus carpio*) after feeding a low-lipid diet for a period of 8 weeks following the induction of Zn deficiency.

MATERIALS AND METHODS

A group of 120 juvenile common carp were separated from the main stock and divided into three dietary groups. Each group was further divided into three subgroups to allow them

Table 1. *Composition of diet for carp*

Component	g
Casein*	38
Gelatin	12
Maize oil	6
Cod-liver oil	3
Cellulose	8
Sucrose	28
Vitamin mixture†	1
Mineral mixture‡	4
Water	200
Total diet	300

* Commercial vitamin-free casein was treated with 0·1 % EDTA to remove metal ions including Zn^{2+}, washed twice with water and dehydrated with acetone and ether, before drying overnight at 60°. The resulting granules were powdered.

† The vitamin mixture (Loba Chemie IndoAustrianal Co., Bombay, India) provided (mg/g): ascorbic acid 100, biotin 0·5, calcium pantothenate 50, choline chloride 500, folic acid 1·5, inositol 200, menadione 4, nicotinic acid 75, pyridoxine hydrochloride 5, riboflavin 20, thiamin hydrochloride 5, α-tocopherol acetate 4, cyanocobalamin 0·01.

‡ The mineral mixture (Loba Chemie IndoAustrianal Co., Bombay, India) provided (g/4 g): CaH_2PO_4 2·58, $CoCl_3$ 0·004, $CuCl_2$ 0·01, $FeSO_4 \cdot 7H_2O$ 0·06, $MnSO_4 \cdot H_2O$ 0·08, $MgSO_4 \cdot H_2O$ 0·405, KCl 0·343, KI 0·015, Na_2CO_3 0·115, NaF 0·008, $AlCl_3$ 0·015.

to be accommodated in plastic portable rectangular tanks each measuring 0·6 × 0·3 × 0·5 m with a water depth of 0·4 m. The temperature of the water supply to the tanks varied between 20 and 30°; pH 7·8–8·0; dissolved O_2, 6–8 ppm; HCO_3^- ions, 3–5 ppm; total hardness ($CaCO_3$), 196 mg/l; Ca^{2+} and Mg^{2+} ions, 3·93 mg/l and Zn, 0·16 μg/l. The water of the tank was aerated and circulated continuously throughout the experiment that lasted for 16 weeks. The tank water was changed each week.

Dietary treatment

Carp were given a high level of dietary lipid (90 g/kg, Table 1) for 8 weeks followed by a low level of dietary lipid (30 g/kg; maize oil was omitted) for a further 8 weeks. Within each lipid group carp were divided into three further dietary treatments.

Sixty carp were fed *ad lib.* on a pelletized Zn-deficient diet (ZD; Table 1) containing 0·5–1·0 ppm Zn; 30 fish were fed *ad lib.* on a Zn-supplemented diet (ZS) containing 1 g $ZnSO_4 \cdot 7H_2O$/kg dry diet; the remaining thirty fish were pair-fed the ZS diet in an amount equal to the average intake of the ZD group during the preceding 24 h. Each group of fish was fed twice a day at 08·00 and 18·00 hours for 2 h on each occasion. The record of the feed intake was maintained by providing a known amount of the diet to each group in a 100 mm diameter glass Petri dish. The left over feed was blotted dry and weighed each time before adding fresh diet in the same container. The loss of feed in water, if any, was calculated by keeping the same amount of feed in a water-filled tank without fish for a duration equal to the feeding period.

To ensure that the different ages of the carp fed on the high- and low-lipid diets did not confound the experimental results, thirty fish of the ZD group remained on the high-lipid diet during the second period when the other groups were switched to the low-lipid diet. The 120 carp used in this part of the experiment were reserved exclusively for food intake and growth studies.

Another similar set of 240 carp was reared under the same environmental and feeding

conditions for biochemical investigations. At the end of the high- and low-lipid treatment periods the carp were starved overnight and killed after feeding for 1 h the next morning before performing the following tests.

Glucose uptake in intestine

Intestinal uptake of glucose was measured in the midgut portion by the tissue accumulation method of Crane & Mandelstam (1960) after the end of the high- and low-lipid dietary treatment periods. A 30 mm length of the duodenum adjacent to the pylorus was removed from each of six fish from each group after 1 h of feeding, flushed with 1 M-NaCl and everted using a thin stainless steel rod. Small rings of the everted intestine were cut and incubated in 5 ml oxygenated Kreb's Ringer bicarbonate (KRB) buffer, pH 7·4, fortified with glucose (5 mM-D-glucose with 0·18 MBq [^{14}C]glucose/100 ml KRB) at 37° for 5 min. Since the tropical carp flourishes at 20–30° and can withstand slightly higher water temperatures, the mammalian conditions for [^{14}C]glucose uptake studies were preferred to fish saline 7 g NaCl/l. The accumulated radioactivity in the tissue was determined by digesting 50 mg tissue in 0·2 ml 3·5 M-KOH (Robinson & Alvarado, 1971) and dissolving in dioxan-based scintillation cocktail (Butler, 1961) before counting on a KLB 1215 Rockbeta Liquid Scintillation Counter with more than 90% counting efficiency for ^{14}C-isotopes. Extracellular space was measured separately by incubating the tissue in [^{3}H]inulin following Alvarado & 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 μmol of the substrate taken up per 5 ml at 37°.

Analytical procedures

Six fish from each of the high- and low-lipid diet subgroups were separated and deprived of feed overnight, then fed for 1 h on their respective diets the next morning. They were then killed after 1 h of feeding; liver and small intestine were removed, rinsed in saline 7 g NaCl/l and blotted dry. The tissues of each subgroup were homogenized separately in a glass homogenizer; DNA, RNA and alkaline phosphatase activity were measured in their homogenates. Nucleic acids and protein were extracted by the method of Schneider (1945). DNA, RNA and protein were determined following the procedures of Bradshaw (1966), Schneider (1957) and Lowry *et al.* (1951) respectively.

The alkaline phosphatase activity was estimated following Bergmeyer (1963). Eighteen more fish from each of the high- and low-lipid diet groups were prepared in a similar manner to that described above. The intestines of six fish from each subgroup were removed each time at intervals of 0, 3 and 6 h of their feeding. Triacylglycerol content was estimated in the intestine by the method of Gottfried & Rosenberg (1973).

Estimation of Zn

The Zn level in the intestine was estimated on a Perkin Elmer atomic absorption spectrophotometer after digesting 100 mg fresh tissue in a 22·5 M-HNO_3–11·6 M-$HClO_4$ (3:1, v/v) mixture.

Statistical analysis

The data collected were subjected to two-way ANOVA (Daniel, 1983). The significance between the variants was determined by the F test and was considered significant when the P values were $< 0{\cdot}05$.

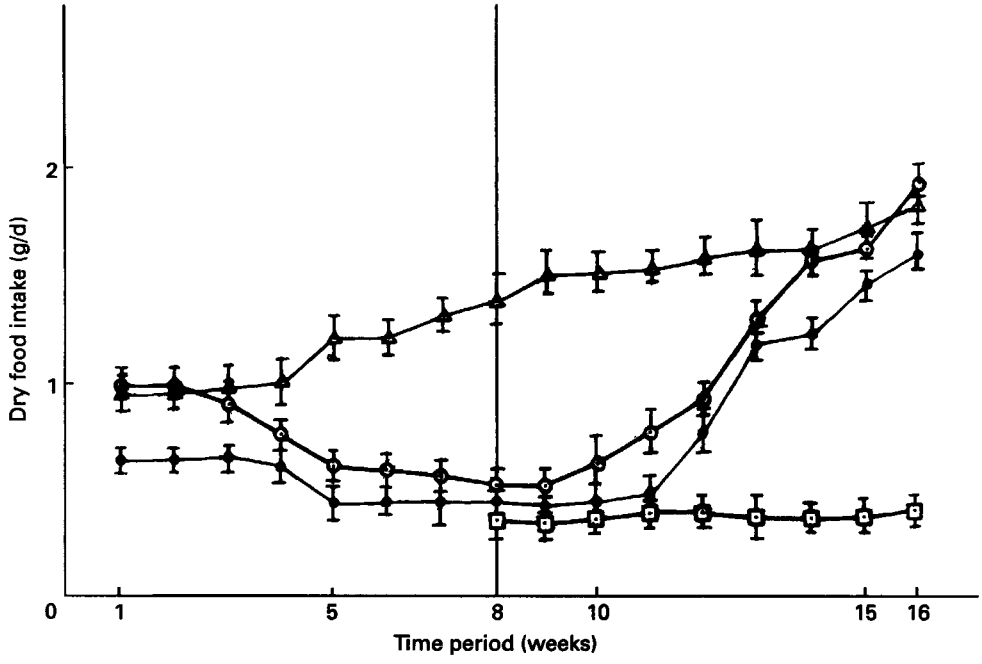

Fig. 1. Mean food intake of *Cyprinus carpio* fed on diets containing high or low levels of lipid and supplemented with zinc (ZS; △—△), deficient in zinc (ZD; ●—●) or pair-fed (PF; ⊙—⊙) on the ZS diet to the intake of the ZD group. The vertical line at week 8 represents the termination of high-lipid diet feeding and the start of low-lipid diet feeding. One half of the ZD group (⊡—⊡) continued to receive the high-lipid diet throughout the experiment.

RESULTS

Feed intake and body weight

The daily feed intake in ZD and ZS groups given the high-lipid diet remained similar during the first 2 weeks of their feeding. During this period, ZD carp consumed 30 g/kg body weight per d. In week 3 they could consume only the morning ration and the evening ration was left unconsumed. In week 4, their feed intake reduced further, and stabilized at 20 g/kg body weight, while ZS fish continued to utilize their usual ration (Fig. 1). The Zn concentration in the intestine was estimated as 178·9 (SE 6·6), 101·7 (SE 5·5) and 48·2 (SE 3·6) μg/g fresh tissue in ZS, PF and ZD groups respectively, after 8 weeks of the dietary treatment (Fig. 1).

Fig. 2 depicts body weights at weekly intervals. After 8 weeks of the high-lipid diet the ZD group had lost 12·18% of their body weight in contrast to a negligible loss (2·15%) in the PF group and a gain of 19·70% in the ZS group.

On feeding the low-lipid diet to the respective groups during the second period, the feed intake improved in the ZD group. ZD fish consumed 20 g diet/kg body weight per d from weeks 1 to 3 of the low-lipid period; this increased to 30 g/kg in week 4 and to 40 g/kg from week 5 onward (Fig. 1). Consequently their body weights increased and approached the level of the ZS group (Fig. 2) after 8 weeks on the low-lipid diet. However, the ZD group maintained on the high-lipid diet for 16 weeks did not exhibit a rise in body weight during the second period of the experiment.

Zn concentrations recorded in the intestine at the end of the low-lipid dietary period were 175·3 (SE 7·3), 100·8 (SE 8·4) and 50·1 (SE 5·6) μg/g fresh tissue in ZS, PF and ZD groups respectively.

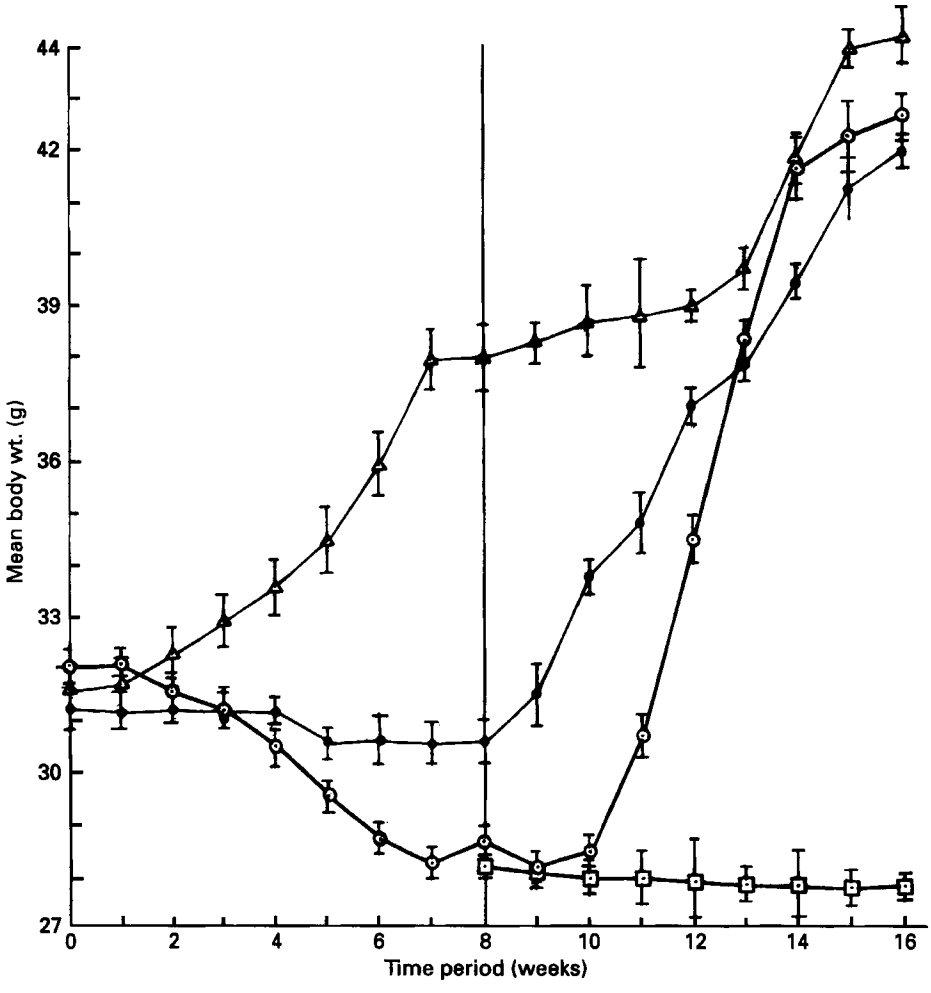

Fig. 2. Mean body weight (g) of carp (*Cyprinus carpio*) fed on diets containing high or low levels of lipid and supplemented with zinc (ZS; △—△), deficient in zinc (ZD; ⊙—⊙) or pair fed (PF; ●—●) the ZS diet to the intake of the ZD group. The vertical line at week 8 represents the termination of high-lipid diet feeding and the start of low-lipid diet feeding. One half of the ZD group (⊡—⊡) continued to receive the high-lipid diet throughout the experiment.

[^{14}C]*glucose uptake in the intestine*

The rate of intestinal glucose uptake was significantly lower in the fish fed on the high-lipid ZD diet (Table 2) compared with fish from the ZS and PF diet groups ($P < 0{\cdot}001$). However, there was no significant difference between these groups in [^{14}C]glucose uptake in the intestine when a low-lipid diet was fed during the second period of the experiment.

The [^{14}C]glucose uptake by the intestinal segment was not significantly different in the ZD group at 8 and 16 weeks of feeding a high-lipid diet.

Nucleic acids

Within the ZD group, changes in DNA and RNA concentrations were different for liver and intestine. During the high-lipid period the DNA level in liver of the ZD fish was not

Table 2. *Mean glucose uptake by the intestine, DNA and RNA concentrations and alkaline phosphatase (AP; EC 3.1.3.1) activities in intestine and liver of* Cyprinus carpio *fed on diets containing high or low lipid and supplemented with zinc (ZS), deficient in zinc (ZD) or pair-fed (PF) the ZS diet to the intake of the ZD group**

(Values are means with their standard errors for six fish)

		Intestine				Liver		
		Glucose uptake (units/mg DNA)	DNA (mg/100 mg protein)	RNA (mg/mg DNA)	AP (g phenol released/h per 100 mg protein)	DNA (mg/100 mg protein)	RNA (mg/mg DNA)	AP (g phenol released/h per 100 mg protein)
High lipid (period 1)								
ZS		1·89^{c}	16·07^{c}	1·15^{c}	10·35^{c}	16·22^{c}	0·95^{c}	6·13^{c}
	SE	0·02	0·04	0·03	0·34	0·24	0·04	0·16
PF		1·90^{c}	15·74^{c}	0·98^{c}	11·38^{c}	17·42^{c}	0·88^{d}	6·39^{c}
	SE	0·01	0·35	0·01	0·34	0·48	0·13	0·10
ZD		1·60^{a}	14·34^{a}	0·93^{a}	4·07^{a}	18·22^{c}	0·75^{a}	4·95^{a}
	SE	0·06	0·48	0·20	0·11	1·01	0·03	0·17
Low lipid (period 2)								
ZS		1·33^{b}	27·55^{b}	0·67^{b}	14·15^{b}	21·36^{b}	0·84^{b}	8·60^{b}
	SE	0·16	0·70	0·02	0·50	0·35	0·01	0·34
PF		1·34^{b}	27·88^{b}	0·65^{b}	14·48^{b}	20·94^{b}	0·85^{b}	7·10^{d}
	SE	0·01	0·56	0·01	0·27	0·42	0·01	0·28
ZD		1·36^{b}	29·20^{d}	0·65^{b}	12·99^{d}	20·20^{b}	0·79^{e}	8·30^{b}
	SE	0·08	0·46	0·01	0·23	0·38	0·02	0·29
High lipid (fed throughout)								
ZD		1·66^{a}	13·53^{a}	0·88^{a}	4·72^{a}	17·56^{a}	0·73^{a}	4·78^{a}
	SE	0·04	0·58	0·03	0·01	0·72	0·25	0·38

^{a,b,c,d,e} Mean values within a column not sharing a common superscript letter were significantly different ($P < 0·05$).

* For details of diets and procedures, see Table 1 and pp. 754–755.

significantly different from that of the ZS and PF groups (Table 2). In contrast, a reduction of DNA concentration in the intestine of ZD fish fed on the high-lipid diet was observed ($P < 0·001$) compared with ZS and PF diet groups. However, during the low-lipid period the level of intestinal DNA in ZD fish was similar to that in the ZS and PF groups (Table 2).

RNA in the intestine followed the same trend as observed for DNA. Its level decreased in ZD compared with ZS and PF groups during high-lipid intake ($P < 0·05$) and rose in the ZD group during low-lipid intake, approaching the level found in the ZS and PF groups ($P > 0·05$; Table 2).

In contrast, the liver RNA concentration in the ZD group fed on the high-lipid diet was lower than that of the ZS and PF groups ($P < 0·05$), and rose only slightly during the low-lipid period (Table 2).

DNA and RNA concentrations in the ZD group fed on the high-lipid diet throughout the experiment were not significantly different at weeks 8 and 16.

Table 3. *Mean concentration of triacylglycerol (mg/mg DNA) 0, 3 and 6 h after feeding, in homogenates of intestine of the carp* (Cyprinus carpio) *fed on high- and low-lipid diets supplemented with zinc (ZS) or deficient in zinc (ZD), or pair-fed (PF) on the ZS diet to the level of the ZD group**

(Values are means with their standard errors for six fish)

Time (h) after feeding...		0	3	6
High lipid (period 1)				
ZS		0·574[c]	1·340[c]	0·955[c]
	SE	0·002	0·021	0·043
PF		0·494[e]	1·116[e]	1·064[e]
	SE	0·007	0·034	0·080
ZD		0·688[a]	2·407[a]	2·296[a]
	SE	0·003	0·264	0·035
Low lipid (period 2)				
ZS		0·302[b]	0·687[b]	0·530[b]
	SE	0·004	0·019	0·017
PF		0·303[b]	0·722[b]	0·526[b]
	SE	0·008	0·017	0·033
ZD		0·366[d]	0·994[d]	0·605[d]
	SE	0·004	0·019	0·040
High lipid (fed throughout)				
ZD		0·620[a]	2·303[a]	2·270[a]
	SE	0·020	0·041	0·025

[a,b,c,d,e] Mean values within a column not sharing a common superscript letter were significantly different ($P < 0·05$).

* For details of diets and procedures, see Table 1 and pp. 754–755.

Alkaline phosphatase activity

Alkaline phosphatase enzyme activities recorded in intestine and liver are given in Table 2. Both intestine and liver displayed a fall in activity in ZD fish compared with ZS and PF groups fed on the high-lipid diet. However, in both liver and intestine of the ZD fish, alkaline phosphatase activity increased during the low-lipid diet period becoming much closer to the activity found in the ZS and PF groups.

The alkaline phosphatase activities in liver and intestine of the ZD fish fed on the high-lipid diet were not significantly different after 8 and 16 weeks.

Triacylglycerol

The triacylglycerol (TG) fraction in the homogenate of the intestine from the ZD group fed on the high-lipid diet was found to be higher by 34·8, 63·2 and 103·6% than in the ZS group, and higher by 45·9, 94·6 and 135·3% than in the PF group at 0, 3 and 6 h of starvation respectively ($P < 0·001$, Table 3). At 0 and 3 h the TG level was slightly less in PF compared with ZS carp ($P < 0·01$).

Intestinal TG concentration decreased significantly in the ZD group given the low-lipid diet compared with the ZD group given the high-lipid diet. It was still higher in ZD compared with ZS and PF groups but was less than that of ZS and PF groups fed on high-lipid diets at corresponding stages of starvation.

DISCUSSION

The amount of feed eaten by carp in the high-lipid ZD group was lower than that in the high-lipid ZS group, and this evidently resulted in a lower rate of weight gain in the ZD group. The carp in the high-lipid PF group gained more body weight than those of the ZD group despite equal feed intake. This differential gain in body weight of ZD and PF groups given high-lipid diets may reflect a difference in the level of intestinal absorption of nutrients between the two groups. The reduction of lipid in the diet during the second period stimulated an increase in feed consumption by the ZD carp and resulted in restoration of their body weight to a level close to that of the ZS group despite Zn deficiency. The almost identical body weights of the ZD, ZS and PF groups at the end of the second period, irrespective of Zn concentration in their diet/tissues, suggest that nutrient absorption was no longer impaired in the ZD carp given a low-lipid diet. This implies that defects in feed consumption, reduction in body weight and malabsorption of lipid in ZD carp during the first period of the experiment were interlinked and depended upon the dietary fat level.

The specific role of lipids in eliciting the defects becomes evident on examining the intestinal TG level which was higher in ZD carp given the high-lipid diet than in those fed on the low-lipid diet. This suggests that TG transport from the mucosal epithelium was relatively slow in ZD compared with ZS and PF carp fed on the high-lipid diet. This is consistent with the reports of Koo & Turk (1977) in rats and Taneja *et al.* (1990) in carp, who concluded that TG deposition in the mucosal epithelium was a result of its lower transformation to chylomicrons and their subsequent slower transport to extracellular spaces. Taneja *et al.* (1990) suggested that intestinal TG deposition might be the cause of anorexia because of its inhibitory effect on the stomach emptying process through a feed-back mechanism which they determined by estimating feed intake and stomach clearance rate in ZD carp fed on high- and low-fat diets.

That TG deposition contributed to malabsorption is suggested by the data for [^{14}C]-glucose uptake in the intestine which reflect an inverse relationship between the two. High intestinal TG concentration during the high-lipid period caused a reduction of intestinal [^{14}C]glucose uptake in ZD carp, whereas the opposite was true during the low-lipid period. In contrast, Reeves & O'Dell (1983), Ghishan (1984) and Southon *et al.* (1984, 1986) did not find a significant difference in the uptake of radiolabelled hexose by the mucosa of ZD and ZS rats. The divergent results for hexose uptake obtained in our high-lipid-fed carp and those of others can be attributed to the difference in the degree of TG concentration in the mucosal epithelium. In the studies using starved rats there was a complete lack of intestinal TG accumulation in Zn-deficient animals whereas we studied hexose uptake in carp when TG accumulation was observed. The reduction of TG accumulation in ZD carp given the low-lipid diet during the second period elicited a rise in intestinal hexose uptake to a level close to that of the ZS carp. This result is in agreement with that reported by the above investigators in fasted rats, which thus lends support to our findings.

The idea that the malabsorption of nutrients associated with Zn deficiency is a consequence of TG deposition in the intestine is further supported by the observed lower concentrations of intestinal and liver DNA and RNA in ZD than in ZS and PF carp fed on a high-lipid diet, and their reversal in ZD carp fed on a low-lipid diet during the second period. The difference in the concentration of nucleic acids between the two ZD groups of carp demonstrates the impact of the level of feed intake and subsequent absorption of nutrients on nucleic acid synthesis and does not support the essentiality of Zn in their synthesis. The concept that Zn is involved in the synthesis of nucleic acids possibly emanated from the recorded lower levels of DNA and RNA (Mills *et al.* 1969; Somers &

Underwood, 1969; Sandstead *et al.* 1971; Prasad *et al.* 1971; Im *et al.* 1975) and lower activities of enzymes associated with their synthesis (Prasad & Oberleas, 1974; Im *et al.* 1975) which in fact may be a response to low nutrient concentrations in the tissues due to malabsorption, rather than to a low concentration of Zn.

The intestinal and liver alkaline phosphatase activities were also lower in ZD compared with ZS and PF carp fed on the high-lipid diet; however, when the low-lipid diet was fed during the second period the enzyme activity in the ZD carp rose and approached that of the ZS and PF groups. Leucke *et al.* (1968), Iqbal (1971), Williams (1972) and Adeniyi & Heaton (1980) observed a reduction in alkaline phosphatase activity in ZD rats and remarked that the reduction of enzyme activity in them was neither due to the lack of Zn^{2+} ions in the tissues nor to inanition but rather to the lowered concentration of the enzyme itself. This conclusion seems to be based upon the comparison of enzyme activity between ZD rats fed *ad lib.* and diet-restricted ZS rats using different substrates with and without Zn in the incubation medium. These investigators did not consider the level of nutrients in the tissues. Had Zn concentration been the sole rate-limiting factor as suggested by these workers then enzyme activity in the tissue would have remained unaltered in ZD carp when the lipid content of the diet was decreased. Instead, the enzyme activity was higher than that of ZD carp fed on a high-lipid diet and closer to that of the ZS and PF carp. The recovery of enzyme activity in the ZD fish fed on the low-lipid diet and not in the ZD carp fed on the high-lipid diet provides evidence that the rate of enzyme synthesis in the tissue depends on the availability of nutrients. The inverse relationship of enzyme activity to TG deposition and its direct relationship to intestinal [^{14}C]glucose uptake in ZD carp fed on high- and low-lipid diets provide strong evidence against the dependence of enzyme synthesis on the availability of Zn to the tissue, but support the idea that malabsorption of nutrients due to accumulation of intestinal TG is the cause of reduced enzyme activity in ZD carp fed on a high-lipid diet.

Our results, therefore, provide strong evidence in favour of the essentiality of Zn for the absorption of dietary lipids when they are present in excess in the diet. The lower uptake of nutrients in carp fed on a high-lipid ZD diet results from the accumulation of lipid in the intestine due to an unknown defect in their transport mechanism. The reported lower levels of DNA, RNA and alkaline phosphatase activity are a reflection of low absorption of nutrients linked with lipid deposition in the intestine rather than their dependence on the Zn level in the tissues.

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