Lipid–zinc interaction: its effect on the testes of mice

BY S. K. TANEJA, S. CHADHA AND P. ARYA

Department of Zoology, Panjab University, Chandigarh – 160 014 India

Lipid–Zn interaction in the testes of mice was studied by feeding them low-fat (30 g maize oil/kg; group LFZD) and high-fat (90 g maize oil/kg; group HFZD) Zn-deficient diets for 6 weeks. The results were compared with those of corresponding Zn-supplemented-diet-fed controls (groups LFZS and HFZS). The integument-related Zn-deficiency symptoms appeared in group HFZD and not in group LFZD mice despite lack of Zn in their ration and an equal level of Zn in their blood serum. The feed intake, gain in body weight and testes weight of the LFZD group were comparable with those of the LFZS and HFZS groups ($P > 0.05$) but were higher than those of the group HFZD ($P < 0.05$). The testes of group HFZD displayed necrotic changes marked by the presence of giant cells, lower RNA, DNA and protein concentrations and higher phospholipid and cholesterol levels than those of mice in the LFZD group. The concentrations of these fractions were not significantly different between LFZD and HFZS. The results do not support the hypothesis that Zn is essential either for testicular function or for nucleic acid and protein synthesis in animals fed on a low-fat diet; however, it appears to be essential for animals fed on a high-fat diet. The changes observed in the testes of the HFZD animals suggest the excess intake of fat as their cause in Zn-deficient animals.

Lipid: Zinc: Lipid–zinc interaction: Testes: Mice

Sterility as one of the symptoms of Zn deficiency has been reported in all mammalian species including man (Millar *et al.* 1958; Prasad *et al.* 1961; Bieri & Prival, 1966; Sandstead *et al.* 1967; Halstead *et al.* 1972; Abbasi *et al.* 1976; Lee *et al.* 1976; Underwood, 1977; Taneja & Nirmal, 1980; Golden & Golden, 1981; Walravens *et al.* 1983; Prasad & Cossack, 1984; Haschke *et al.* 1985; McClain *et al.* 1985; Reeves, 1990). Smaller testes, loss of tubular size, nuclear chromatolysis in germ cells, arrest of spermatozoa and depletion of Leydig cells are the pathological changes that have been reported in the testes of severely Zn-deficient mammals (Perizek *et al.* 1966; Swenerton & Leucille, 1968; Somers & Underwood, 1969; Underwood & Somers, 1969; Taneja & Nirmal, 1980). These degenerative changes have been correlated either with the defects in nucleic acid, protein (Macapinlac *et al.* 1968; Somers & Underwood, 1969; Terhune & Sandstead, 1972) and fat metabolism (Bieri & Prival, 1966; Clejan *et al.* 1982; Chanmugam *et al.* 1984) or with the hormonal disorders involving pituitary gonadotropins and testosterone (Lei *et al.* 1976; Underwood, 1977; McClain *et al.* 1984). Underwood (1977) suggested that the hormonal changes might be secondary to inanition and growth retardation effects caused by Zn deficiency. Taneja & Arya (1992) observed that the low feed intake and growth retardation effects caused by Zn deficiency were ameliorated by restricting the fat content to 30 g/kg in a Zn-deficient basal diet. This suggests that a high lipid content in a Zn-deficient diet is responsible for the induction of these symptoms and possibly may influence the testicular function so widely reported in Zn-deficient mammals. This led us to investigate the effect of high- and low-fat Zn-deficient and Zn-supplemented diets on the testes of mice.

MATERIALS AND METHODS
Dietary treatment

Adult male mice of the lacca strain (n 40) weighing 21–25 g were obtained from the Central Animal House of the Panjab University, Chandigarh, India and maintained according to the guidelines approved by the Ethics Committee of the Panjab University, Chandigarh. They were divided randomly into four groups, ten in each group, and housed in plastic cages in a room maintained at 22–27°. Group LFZD was fed on a purified semi-synthetic (Table 1) low-lipid, Zn-deficient (30 g maize oil/kg, 0·5–1·0 μg Zn/g) diet, group LFZS was given a low-lipid Zn-supplemented (30 g maize oil/kg, 20 μg Zn/g) diet, group HFZD was given a high-lipid, Zn-deficient (90 g maize oil/kg, 0·5–1·0 μg Zn/g) diet and group HFZS was given a high-lipid, Zn-supplemented (90 g maize oil/kg, 20 μg Zn/g) diet. All the animals were fed *ad lib.* on their respective diets for 6 weeks and double-distilled deionized water was made freely available to them.

A record of feed intake and body weights was maintained on a weekly basis.

After the 6 weeks of dietary treatment, six animals of each group were lightly anaesthetized with diethyl ether and the testes and blood were removed and processed for the bioassay.

Estimation of zinc

Zn concentrations in the serum of six animals of each group were estimated separately by atomic absorption spectrophotometry (Model 306, Perkin Elmer; Norwalk, CT, USA) after digesting 0·1 ml serum in nitric acid–perchloric acid (3:1, v/v).

Histology

The testes of four mice from each group were cut into small pieces. They were fixed in Zenker's and formalin–calcium fluids. The Zenker's-fluid-fixed tissue was processed for paraffin sectioning and stained in delafield haematoxylin and eosin. The formalin–calcium-fixed tissue was postchromed and processed for gelatin sections on a cold microtome. Thin (7 μm) gelatin sections were stained in Sudan Black B and acid haematein for the localization of total lipids and phospholipids respectively (Pearse, 1985).

Table 1. *Compositions of the high- and low-fat diets for mice (g/kg)**†

Ingredient	High-fat	Low-fat
EDTA-washed casein	300	300
Agar	15	15
Maize oil	90	30
Cellulose	80	140
Sucrose	430	430
Vitamin mixture‡	50	50
Mineral mixture§	35	35

* Each diet was modified to be zinc-deficient or zinc-supplemented by the addition of $ZnSO_4.7H_2O$ as given on p. 724.

† The diets were stored in separate sealed sterilized glass bottles in a deep freezer at −8° until use.

‡ Contained (mg/g vitamin mixture): ascorbic acid 100, biotin 0·5, calcium pantothenate 50, choline chloride 500, pteroylglutamic acid 0·15, inositol 200, menadione 4, nicotinic acid 65, pyridoxine hydrochloride 5, riboflavin 20, thiamine hydrochloride 50, tocopherol acetate 6, cyanocobalamin 0·01, ergocalciferol 0·0031, retinol 0·03.

§ Contained (g/kg diet): $CaHPO_4$ 25·8, $CoCl_2.6H_2O$ 0·04, $CuCl_2$ 0·06, $FeSO_4.7H_2O$ 0·06, $MnSO_4.H_2O$ 0·4, $MgSO_4.H_2O$ 4·05, KCl 3·43, KI 0·004, Na_2CO_3 1·16, NaF 0·006.

Estimation of DNA, RNA and proteins

A 100 mg portion of testes in each case was homogenized for the extraction of DNA, RNA and proteins by Schneider's method (1945). Protein was estimated by the method of Lowry *et al.* (1951). DNA was estimated by the method of Bradshaw (1966) and RNA was estimated by Mejbaum's (1939) modified method based on the orcinol reaction.

Estimation of total lipids, phospholipids and cholesterol

The total lipids were extracted following the method of Folch *et al.* (1957) and the colorimetric method of Frings & Dunn (1970) was employed for the estimation of total lipids. Phospholipids were assayed by the method of Zilversmit & Davis (1950). The ferric chloride method of Chiamori & Henry (1959) was employed for the estimation of cholesterol.

Biostatistics

The data collected were subjected to statistical analysis by a two-way ANOVA test (Daniel, 1983). The significance between the variants was determined by F test and was considered significant when the P values were < 0.05.

RESULTS

Morphological changes

The integument-related Zn-deficiency symptoms started appearing in HFZD mice after 3 weeks of dietary treatment and became more prominent as the duration of feeding was prolonged. The symptoms included the greying of hair, appearance of spiny hair coat, loss of hair, dermatitis near the toes, nails and anal region. These symptoms were lacking in the animals of the LFZD group and the LFZS and HFZS groups.

The feed intake in group HFZD was significantly lower compared with those of groups LFZD, LFZS and HFZS. The animals of group HFZD consumed 20–25% less diet than those of groups LFZD, LFZS and HFZS (see Table 2). The total feed intake per week was identical in groups LFZD and LFZS but was slightly higher than that of group HFZS.

Gain in body weight

The gain in body weight recorded at the end of each week has been depicted in Fig. 1. A steep growth curve up to week 3 followed by a plateau was observed in group LFZS. Groups LFZD and HFZS showed a rather gradual increase in their body weights over weeks 1–6 although the percentage gain in body weight was less in comparison with that of group LFZS (Table 2). In contrast, the body weight of group HFZD remained almost constant during weeks 1–3 and started declining gradually during the following weeks.

Weight of testes

The testicular weight per kg body weight between groups LFZD and HFZD differed significantly ($P < 0.05$), being lower in the latter group (Table 2), while it was not significantly different between groups LFZD, LFZS and HFZS ($P > 0.05$).

Histology

The testes of groups LFZD, LFZS and HFZS did not display any abnormality in the germ-cell population of the seminiferous tubules. They were healthy and the stages of spermatogenesis were distinctly marked in the sections stained with delafield haematoxylin and eosin. However, the testes of group HFZD mice exhibited some degenerative changes marked by the presence of giant cells, lying in the lumen and in between the germ cells, wavy theca albuginea and the presence of fatty deposits, positive to Sudan Black B and acid
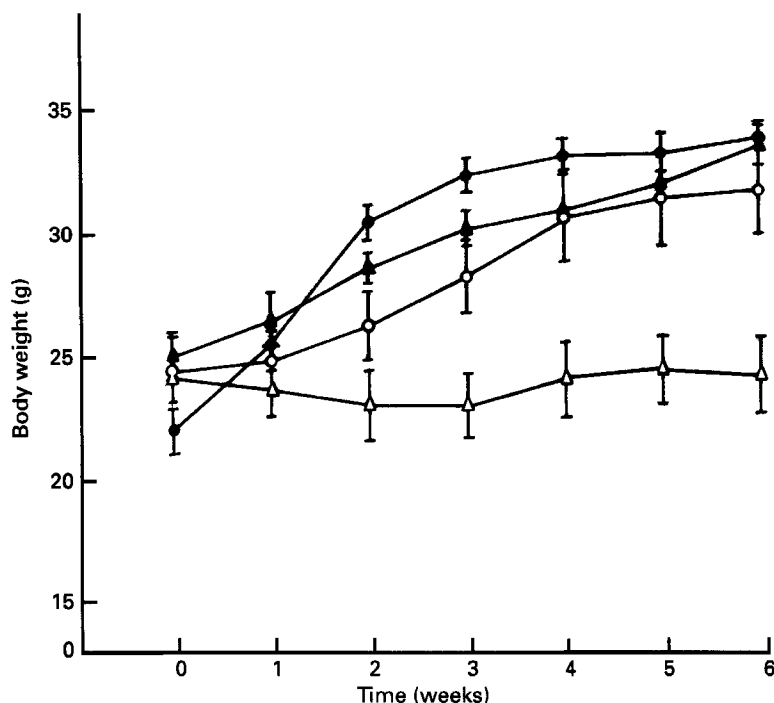
Fig. 1. Change over time in the body weight of mice fed on low-lipid, zinc-deficient (○), low-lipid, zinc-supplemented (●), high-lipid, zinc-deficient (△) and high-lipid, zinc-supplemented (▲) diets. Values are means for ten mice with standard deviations indicated by vertical bars. For details of diets, see Table 1.

Table 2. *Initial body weight, final body weight, feed intake, percentage change in body weight and testis weight in mice fed on low-fat and high-fat diets that were deficient in, or supplemented with, zinc**

(Mean values with their standard errors for ten mice)

Groups	Initial body wt (g)	Final body wt (g)	Mean feed intake (g/kg body wt)	Change in body wt (%)	Mean testis wt (mg)	Mean wt of testis (mg/kg body wt)
LFZD	24·36	31·27	95·7[a]	+28·27[a]	97·64	3216·7[a]
SE	1·0	1·74	0·5	0·37	2·582	58·3
LFZS	23·90	33·27	95·4[a]	+38·10[b]	109·27	3296·5[a]
SE	0·732	0·681	0·009	0·31	1·432	39·0
HFZD	24·36	24·0	77·8[c]	−1·48[c]	66·09	2854·3[b]
SE	0·902	1·423	0·08	0·056	1·20	42·1
HFZS	25·0	33·73	89·6[b]	+34·96[b]	106·91	3184·4[a]
SE	0·761	0·834	0·13	0·21	0·99	57·9

LFZD, low-fat, zinc-deficient; LFZS, low-fat, zinc-supplemented; HFZD, high-fat, zinc-deficient; HFZS, high-fat, zinc-supplemented.

[a,b,c] 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 p. 724.

Table 3. *Serum zinc concentration, and protein, DNA and RNA concentrations in the testes of mice fed on low-fat and high-fat diets which were either deficient in, or supplemented with, zinc**

(Mean values with their standard errors for six mice)

Groups	Serum Zn (μg/ml)	Protein (mg/g tissue wt)	DNA (mg/mg protein)	RNA (mg/mg protein)	RNA/DNA
LFZD	0·624[a]	291·1[a]	0·13[b]	0·054[c]	0·42[c]
SE	0·027	4·85	0·004	0·002	0·002
LFZS	0·924[b]	301·1[a]	0·13[b]	0·064[b]	0·48[b]
SE	0·058	4·34	0·005	0·002	0·022
HFZD	0·623[a]	252·6[c]	0·11[a]	0·059[a]	0·57[a]
SE	0·018	9·18	0·011	0·003	0·044
HFZS	0·893[b]	292·4[a]	0·12[b]	0·057[a]	0·48[b]
SE	0·097	7·56	0·005	0·002	0·024

LFZD, low-fat, zinc-deficient; LFZS, low-fat, zinc-supplemented; HFZD, high-fat, zinc-deficient; HFZS, high-fat, zinc-supplemented.

[a,b,c] 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. 724–725.

haematein tests, concentrated mostly in spermatogonia of many of the seminiferous tubules. Some of these tubules appeared healthy.

Zinc concentration

The serum Zn concentration was not significantly different between groups LFZD and HFZD and between LFZS and HFZS ($P > 0.05$). Group LFZS showed an increase of 48·1 % compared with the LFZD group ($P < 0.05$); and group HFZD registered a decrease of 30·2 % compared with that of group HFZS ($P < 0.05$).

Extractable proteins

The protein fractions in groups HFZS, LFZD and LFZS were not significantly different amongst one another ($P > 0.05$) but were all significantly higher than that of group HFZD ($P < 0.01$; Table 3).

Nucleic acids

The DNA fractions of the testes in diet groups LFZD, LFZS and HFZS were almost identical (Table 3), but were significantly higher than that of group HFZD ($P < 0.001$).

The RNA concentration in group HFZD was slightly higher than those of groups LFZD and HFZS but lower than that of group LFZS. However the RNA:DNA ratio in group HFZD was the highest compared with those of the other three groups (Table 3).

Total lipid, phospholipids and cholesterol

The total lipid fraction in the testes of mice fed on LFZS, HFZD and HFZS diets did not differ significantly but it was significantly lower in the mice fed on the LFZD diet ($P < 0.05$) than in those of the other three groups (Table 4). The phospholipid and cholesterol fractions were also identical in groups LFZD and HFZS ($P > 0.05$) but were significantly lower than that of group HFZD ($P < 0.001$). Amongst the four dietary groups, the lowest concentrations were obtained in the group fed on the LFZS diet (Table 4). Similar results were found for the cholesterol:phospholipid molar ratio.

Table 4. *Concentrations of total lipid, phospholipid and cholesterol in the testes of mice fed on low-fat and high-fat diets that were either deficient in, or supplemented with, zinc**

(Mean values with their standard errors for six mice)

Groups	Total lipids (mg/mg protein)	Phospholipids (mg/mg protein)	Cholesterol (mg/mg protein)	Cholesterol: phospholipid molar ratio
LFZD	6·26[a]	0·235[b]	0·019[b]	0·156[b]
SE	0·231	0·009	0·001	0·003
LFZS	7·56[b]	0·066[c]	0·004[c]	0·114[c]
SE	0·308	0·003	0·001	0·01
HFZD	7·67[b]	0·345[a]	0·030[a]	0·178[a]
SE	0·721	0·015	0·002	0·007
HFZS	7·55[b]	0·222[d]	0·017[b]	0·158[b]
SE	0·358	0·008	0·001	0·015

LFZD, low-fat, zinc-deficient; LFZS, low-fat, zinc-supplemented; HFZD, high-fat, zinc-deficient; HFZS, high-fat, zinc-supplemented.

[a,b,c,d] 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. 724–725.

DISCUSSION

Alopecia, dermatitis, anorexia and growth retardation as Zn-deficiency symptoms appeared in the mice fed on the high-lipid Zn-deficient diet (group HFZD) in accordance with the observations of Prasad *et al.* (1961); Sandstead *et al.* (1967); Hurley (1969); Koo & Williams (1981); Petering *et al.* (1982); Essatara *et al.* (1984*a, b*); Giugliano & Millward (1984); McClain *et al.* (1984); Moran & Lyerly (1985) and Taneja *et al.* (1990). However the mice fed on the low-lipid, Zn-deficient diet (group LFZD) did not exhibit these symptoms. Their fur coat, growth and feed intake were similar to those fed on LFZS and HFZS diets. The close similarity between the mice of groups LFZD, LFZS and HFZS does not support the idea that the induction of symptoms is primarily related to Zn deficiency. Had Zn been the sole factor responsible for the induction of symptoms in the mice fed on Zn-deficient diets, then symptoms should have appeared in both high- and low-lipid Zn-deficient groups (i.e. LFZD and HFZD diet groups) and not in group HFZD alone. The difference between the two groups was in the level of fat and not in the level of Zn in the diet. Their Zn concentration in serum being identical, the excess lipid in diet group HFZD appears to have been responsible for the induction of Zn-deficiency-related symptoms. This is in accordance with our earlier report (Taneja & Arya, 1992). We reported that the symptoms appeared in mice when fed on a Zn-deficient diet containing cod-liver oil (30 g/kg) and maize oil (60 g/kg) as the lipid source and they disappeared, despite Zn deficiency, when the maize oil was withdrawn from the Zn-deficient diet. The similarity of the results between the present study and the one reported previously (Taneja & Arya, 1992) clarifies that the quantity rather than quality of the fat induces the symptoms in Zn-deficient mice.

Associated with the reduction of body weight, significantly lower testes weights in the animals of group HFZD are in conformity with the observations of previous investigators who fed fat at 50 g/kg or above in the basal diet (Millar *et al.* 1958; Bieri & Prival, 1966; Sandstead *et al.* 1967; Abbasi *et al.* 1976; Lei *et al.* 1976; Underwood, 1977; Taneja & Nirmal, 1980; McClain *et al.* 1984; Reeves, 1990). The pathological changes in the testes of mice of group HFZD marked by the presence of giant cells and their absence in group

LFZD reflected the onset of the process of degeneration in group HFZD and not in group LFZD despite the identical level of Zn concentration in the blood serum. The severe damage in the testes *per se* as reported by many investigators who employed weanling rats was not witnessed in the mice in the present study. We employed mature mice that require 8 weeks of dietary treatment for complete testicular atrophy (Taneja & Nirmal, 1980). The present experiment was deliberately terminated after 6 weeks to ascertain the involvement of Zn in testicular function rather than to obtain the net pathological information.

The testicular degenerative changes in Zn-deficient animals have been correlated with the reductions in DNA, RNA and protein concentrations (Macapinlac *et al.* 1968; Somers & Underwood, 1969; Underwood & Somers, 1969; Terhune & Sandstead, 1972) attributing the defects in the nucleic acids and protein metabolism as their cause. The lower concentrations of DNA and protein in the testes of the mice fed on the HFZD diet than those fed on the HFZS diet were also observed by us. Almost identical concentrations of DNA and protein in groups LFZD and HFZS, despite a lower Zn concentration in the serum of the former than the latter group but equal to that of group HFZD, do not support the possibility of the involvement of Zn in the DNA and protein metabolism leading to the arrest of spermatogenesis.

Further, the concentration of total lipid did not change significantly in the testes of groups LFZS, HFZD and HFZS. Bieri & Prival (1966) and Clejan *et al.* (1981), however, reported a higher concentration of total lipid along with phospholipid and cholesterol in Zn-deficient than in Zn-supplemented rats. Higher concentrations of cholesterol and phospholipid were also observed by us in group HFZD compared with those of group LFZD. Their concentrations in the LFZD group were almost identical to those in group HFZS. Since the rise of phospholipid and cholesterol concentrations in the testes has been reported to be associated with the impairment of testicular function (Bieri & Prival, 1966; Gambal & Ackerman, 1967; Nakamura *et al.* 1968; Johnson, 1970), the present results suggest that the defects in testicular function occurred only in group HFZD and not in group LFZD despite both groups having equal concentrations of Zn in their blood serum. This is contrary to earlier reports suggesting essentiality of Zn for testicular functions. (Millar *et al.* 1958; Bieri & Prival, 1966; Abbasi *et al.* 1976; Lei *et al.* 1976; Taneja & Nirmal, 1980; McClain *et al.* 1985; Reeves, 1990). The lower concentrations of total lipid, phospholipids and cholesterol in the testes of LFZS than in HFZS and LFZD groups might be due to their faster utilization in the former than in the latter two groups which, in fact, was evidenced by the highest testicular weight in group LFZS.

The low feed intake, loss of body weight and impairment of testicular function appeared only in response to Zn-deficiency coupled with a high fat concentration in the diet and not in response to a low fat concentration in the diet. In the absence of Zn in the diet, lipid has been observed to accumulate in the mucosal epithelial cells of the intestine of rats (Koo & Turk, 1977), mice (Taneja & Arya, 1992) and carp (*Cyprinus carpio*; Taneja *et al.* 1990). Based on electron microscopic studies, Koo & Turk (1977) reported that the nascent chylomicrons in the Zn-deficient rats lost their stability and coalesced to form bigger droplets that were unable to pass to the lacteals. Their transport from the intestine to the lacteals was reduced drastically as estimated from olive oil uptake by the intestine. Lipid deposition in the intestinal mucosal epithelial cells causes an inhibition of stomach emptying and gastric secretion through a feedback mechanism in rats (Konturek & Grossman, 1965; Long & Brooks, 1965). A similar mechanism appears to operate in mice as well which we determined by estimating the feed intake and stomach clearance in mice fed on a high-fat Zn-deficient diet (Taneja & Arya, 1992). It also exacerbated the malabsorption of nutrients by interfering with the intestinal uptake mechanism which we concluded by estimating [^{14}C]glucose uptake (Taneja & Arya, 1992). The anorexia and

malabsorption of [^{14}C]glucose were not evident when the mice were given a low-lipid Zn-deficient diet or a high-fat, Zn-supplemented diet. The feed intake and growth rate in them were identical, as was the case in the present study.

This implies that Zn in the diet promotes the transport of lipid from enterocytes to the lacteals facilitating the movement of other nutrients from the lumen of the intestine to the blood, and explains the higher body weight and testes weight in group LFZS than group LFZD. The lower growth rate and lower testes weight in mice fed on the HFZS diet than those fed on the LFZS diet may be the result of the suboptimal Zn:fat ratio in their diet.

The results of the present study, therefore, suggest that Zn is not essential for testicular function in mice fed on a diet containing 30 g fat/kg but is of prime importance when the diet is rich in fat. The requirement for Zn in the diet seems to depend upon the level of dietary fat. The malfunctioning of testes in mice fed on the HFZD diet was due to the excess fat intake which required Zn for its utilization.

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