

Relationship between abnormal sperm morphology induced by dietary zinc deficiency and lipid composition in testes of growing rats

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The present study investigated the effect of dietary Zn deficiency during sexual maturation on sperm integrity and testis phospholipid fatty acid composition. Male weanling Sprague–Dawley rats were randomised into four dietary groups for 3 weeks: Zn control (ZC; 30 mg Zn/kg); Zn marginally deficient (ZMD; 9 mg Zn/kg); Zn deficient (ZD; <1 mg Zn/kg); pair fed (PF; 30 mg Zn/kg) to the ZD group. Morphology of cauda epididymal sperm and lipid profiles of testis phospholipids were analysed. The rats fed the ZD diet had a lower testis weight ($P<0.02$). Seminal vesicles and prostate weight were also lower in the ZD and PF groups. Rats fed the ZD diet, but not the ZMD diet, had 34–35% more abnormal spermatozoa and 24% shorter sperm tail length than the ZC and PF rats ($P<0.001$). Testis cholesterol concentration was higher in the ZD rats compared with the ZC and PF rats ($P<0.04$). Testes were highly enriched with $n-6$ fatty acids by showing $n-6:n-3$ fatty acid ratios of 27:1 in phosphatidylcholine (PC) and 23:1 in phosphatidylethanolamine (PE). The dominant fatty acid in testes was docosapentaenoic acid (22:5 $n-6$), comprising 15 and 24% of PC and PE, respectively. This fatty acid was significantly lower in the ZD rats, whereas 18:2 $n-6$ was higher compared with the rats in the other diet groups. These results demonstrate that severe Zn deficiency adversely affects sperm integrity and modulates testis fatty acid composition by interrupting essential fatty acid metabolism. This suggests that Zn deficiency-associated abnormal testicular function is perhaps preceded by altered membrane fatty acid composition, especially of a major fatty acid, 22:5 $n-6$.

Zinc deficiency: Rats: Sperm morphology: Fatty acids: Docosapentaenoic acid: Elongation and desaturation

Zn is abundant in the male reproductive system. The adult prostate followed by testes have the highest Zn concentrations of all other organs in the body⁽¹⁾. The role of this element in testicular function is to promote the maturation of testis germinal cells into spermatozoa, and Zn is believed to prolong the life of spermatozoa once released into the external environment^(1,2). Zn deficiency induces testicular atrophy, spermatocyte loss, an increase in spermatogenic cell apoptosis and significant decreases in seminiferous tubule diameter^(3–5). Adult males who were on a Zn-restricted diet for 24–40 weeks experienced oligospermia⁽⁶⁾, defined as a sperm count of less than 20 million per ml ejaculate in humans. It is therefore evident that Zn is an essential element in male fertility.

Male reproductive organs have a unique biomembrane lipid composition, especially in the testes and sperm. Mammalian testes and spermatozoa are enriched in long-chain PUFA of up to twenty-two carbons^(7,8). In the testes, up to 65% of long-chain PUFA are composed of 20:4 $n-6$ (arachidonic acid), 22:5 $n-6$ (docosapentaenoic acid) and 22:6 $n-3$ (DHA). In rat testes and spermatozoa, 22:5 $n-6$ is the dominant long-chain PUFA, whereas in humans it is 22:6 $n-3$ ⁽⁸⁾. Both have similar roles, increasing in concentration in testis germinal cells during the later stages of their maturation into spermatozoa^(7,8). When the level of testis long-chain PUFA is altered, spermatogenesis is impaired, and this is observed in

all mammals⁽⁷⁾. This suggests that in addition to Zn, long-chain PUFA are also important for proper sperm production and testis function.

Long-chain PUFA in the tissues and organs of the body are derived from dietary essential fatty acids, 18:2 $n-6$ (linoleic acid) and 18:3 $n-3$ (α -linolenic acid) by elongation and desaturation systems. Since Zn plays a role in the desaturation of $n-3$ and $n-6$ long-chain PUFA⁽⁹⁾, a deficiency of Zn may influence the long-chain PUFA content and composition, thereby affecting overall testicular function. Animals on Zn-deficient diets for more than 6 weeks have shown decreased activity of $\Delta 5$ and $\Delta 6$ desaturases in the testes^(10–12). This condition may affect 22:5 $n-6$, the dominant long-chain PUFA in rat testes. Thus, the present study investigates the effects of severe and marginal Zn deficiency during sexual maturation on the level and composition of long-chain PUFA in testes as well as sperm morphology during sexual maturation.

Materials and methods

Animals and diets

Male weanling Sprague–Dawley rats, aged 3 weeks (Charles River Laboratories, St Constant, PQ, Canada), were acclimatised for 5 d to a controlled environment of 21–23°C, 55%

Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PF, pair fed; ZC, Zn control; ZD, Zn deficient; ZMD, Zn marginally deficient.

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humidity and 14 h light–10 h dark cycles while being fed the control diet. The rats were then randomised into four dietary groups with a varied Zn level: Zn control (ZC; 30 mg Zn/kg), Zn marginally deficient (ZMD; 9 mg Zn/kg), Zn deficient (ZD; <1 mg Zn/kg), or pair fed to the ZD group (PF; 30 mg Zn/kg). Since Zn deficiency can induce anorexia within 3 d, PF rats were fed the same nutritionally complete diet as the ZC rats; however, each PF rat received only the amount of feed as consumed on the previous day by the individual ZD rat paired to it. The PF group controls for the effects of under-nutrition commonly seen in Zn deficiency. Basal experimental diets were semi-synthetic, nutritionally complete diets based on AIN-93G with slight modifications⁽¹³⁾. The composition (per kg diet) was as follows: dextrose, 594.6 g; egg albumin, 212.5 g; cellulose, 50 g; mineral mix, 35 g (AIN-93M, Zn free); vitamin mix, 10 g (AIN-93); soyabean oil, 70 g; biotin premix, 10 g (contained 200 mg biotin/kg); potassium phosphate, 5.4 g; choline, 2.5 g. Zn in the premix was added as ZnCO₃ in the necessary concentrations for each diet group. With the exception of the PF group, animals were allowed access to food and water *ad libitum*. After a 3-week feeding period, animals were killed by CO₂ asphyxiation and decapitation. Trunk blood was collected to obtain serum. Body weight was recorded, and the epididymis, seminal vesicles and prostate were removed. Excised testes were weighed, immediately frozen in liquid N₂, and stored at –80°C. Animal care was in accordance with a protocol approved by the University of Manitoba Protocol Management and Review Committee and followed the guidelines of the Canadian Council on Animal Care⁽¹⁴⁾.

Mineral analysis

Zn concentration was measured in serum and femurs as described by Hosea *et al.*⁽¹⁵⁾. Femurs were excised and cleaned of soft tissue. After wet and dry weights were obtained, whole femurs were wet-washed using nitric acid as previously described⁽¹⁵⁾. All glassware was acid-washed to prevent contamination. After appropriate dilution of digests, Zn concentration was determined in triplicate by atomic absorption spectroscopy (Spectra AA-30 Spectrophotometer; Varian Canada, Georgetown, Canada). Quality control was monitored using bovine liver standard reference material 1577b (US Department of Commerce, National Institute of Standards and Technology, Gaithersburg, MD, USA).

Sperm morphology

Sperm morphology was measured according to Sprando *et al.*⁽¹⁶⁾ and the *Rat Sperm Morphological Assessment Guideline Document*⁽¹⁷⁾. Briefly, the cauda epididymis was removed, weighed and minced in 2 ml of 0.9 % saline solution. After 5 min, a sample of minced epididymis was fixed in 10 % buffered formalin for 5 min and then further diluted with deionised water. Staining was obtained with 1 % eosin Y and the sample was smeared onto microscope slides and air-dried. Slides were analysed using a bright field optical microscope at 400X (Carsen, Olympus, Tokyo, Japan). A total of 200 spermatozoa were counted per animal and were divided into four categories based on their morphology: normal, abnormal head, abnormal tail and both abnormal head and tail.

Normal morphology was defined as a hooked head and smooth tail⁽¹⁷⁾. An abnormal head was defined as a missing head, flattened hook (banana) or pinhead. An abnormal tail was characterised with a bent or coiled tail. Sperm tail length was also measured using Image Pro Plus 5.0 computer software (Media Cybernetics Inc., Silver Spring, MD, USA). Measurements were taken by tracing the tail starting from the bottom of the dense nucleus (of the head) down to the tip of the tail.

Lipid and fatty acid analysis

Testes were decapsulated, weighed and total lipids were extracted using the Folch method⁽¹⁸⁾. Individual phospholipids were separated on silica gel H plates with a chloroform–methanol–2-propanol–0.25 % KCl (w/v)–triethylamine (30:9:25:6:18, by vol.) developing system. Phospholipids were visualised with 0.1 % aniline naphthalene sulfonic acid in water (w/v) under UV light. Fatty acids in individual phospholipids were transformed into fatty acid methyl esters (FAME) by boiling in 1.5 ml 14 % boron trifluoride in methanol and 2 ml hexane. FAME were analysed by GC using a GC-17A Shimadzu gas chromatograph (Mandel Scientific Co. Ltd, Guelph, ON, Canada). An SGE BPX70 capillary column (30 m × 0.25 μm internal diameter) was used⁽¹⁹⁾. H₂ was the carrier gas. The initial temperature was set at 130°C and increased to 240°C at the rate of 10°C/min (0 min holding time), 1°C/min (0 min holding time) and 3°C/min (15 min holding time). All fatty acids were compared with a commercial standard (NuChek Prep 461; Elysian, MN, USA).

Triacylglycerol and cholesterol determination in testes

TAG and cholesterol levels in testis lipid were determined using commercially available kits (Diagnostic Chemicals Ltd, Charlottetown, PEI, Canada). The DC-CAL SE-035 standard (Diagnostic Chemicals Ltd) was used for both assays. The analysis was performed by the protocol provided with the kit with samples dissolved in 2-propanol⁽²⁰⁾.

Statistical analysis

The effect of Zn treatment on sperm morphology, fatty acid composition and lipids (TAG, cholesterol) were tested by one-way ANOVA using the SAS Statistical package (version 9.0; SAS Institute, Inc., Cary, NC, USA). A probability of $P \leq 0.05$ was considered significant. Significant differences between diet treatments were determined by Duncan's multiple range test⁽²¹⁾. All data are expressed as mean values and standard deviations. For data representing $n = 2$ in Table 3, trend analysis (R^2) was performed on the two independent datasets.

Results

Body and organ weights and zinc status

There were significant differences in body and sex-organ weights among groups. The final body weight of the ZD rats was the lowest followed by the PF rats (53 and 62 % of ZC rats, respectively) (Table 1). Organ weights, both absolute (g) and relative organ weights (% of body weight), are shown in Table 1. The ZD rats, but not the PF rats, had

Table 1. Body and organ weight (g) and relative weight (% body weight) in response to dietary zinc treatment (Mean values and standard deviations for four or five rats per group)

Zn treatment...	ZC		ZMD		ZD		PF		P
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Body weight (g)	263.3 ^a	14.2	257.5 ^a	18.2	146.1 ^c	8.1	168.2 ^b	8.2	<0.0001
Organ weight (g)									
Testes	2.60 ^a	0.21	2.55 ^a	0.27	2.14 ^b	0.10	2.46 ^a	0.11	0.0173
Epididymis	0.40	0.06	0.41	0.08	0.28	0.05	0.34	0.06	NS
Prostate	0.37 ^a	0.04	0.37 ^a	0.07	0.18 ^b	0.06	0.19 ^b	0.05	0.0002
Seminal vesicles	0.59 ^a	0.12	0.62 ^a	0.07	0.21 ^b	0.09	0.30 ^b	0.06	<0.0001
Organ wt (% body weight)									
Testes	0.99 ^b	0.05	0.99 ^b	0.10	1.48 ^a	0.08	1.47 ^a	0.13	<0.0001
Epididymis	0.15 ^c	0.02	0.15 ^{b,c}	0.03	0.19 ^{a,b}	0.03	0.20 ^a	0.03	0.0346
Prostate	0.13	0.01	0.14	0.03	0.12	0.04	0.11	0.03	NS
Seminal vesicles	0.22 ^a	0.05	0.23 ^a	0.02	0.14 ^b	0.06	0.18 ^{a,b}	0.04	0.0428
Serum Zn (μmol/l)	30.6 ^a	1.8	15.3 ^b	4.6	4.9 ^c	0.7	26.6 ^a	4.0	<0.0001
Femur Zn (μmol/g dry wt)	4.82 ^a	0.20	2.23 ^c	0.78	1.06 ^d	0.16	3.93 ^b	0.11	<0.0001

ZC, Zn control; ZMD, Zn marginally deficient; ZD, Zn deficient; PF, pair fed.
^{a,b,c,d} Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).

significantly ($P < 0.02$) smaller testes in comparison with animals fed the ZC and ZMD diets. Seminal vesicle and prostate weights were lower in both the ZD and PF rats compared with the ZC and ZMD groups ($P < 0.0002$). Epididymis weight was lower in the ZD rats but was not significantly different from the other groups (Table 1). When these organs were calculated as a percentage of body weight, the ZD and PF rats had higher relative testis and epididymis weights in comparison with the ZC rats. Relative seminal vesicle weight was lowest in the ZD rats.

Serum and femur Zn concentrations reflected Zn treatment (Table 1). The ZD and ZMD rats had 84 and 50%, respectively, lower serum concentration compared with the ZC rats. Femur Zn concentration, an indicator of long-term Zn status, was 78 and 46% lower in the ZD and ZMD rats, respectively, compared with the ZC rats.

Sperm morphology and length

ZD rats had markedly low spermatozoa integrity. More than 80% of ZD rat spermatozoa were morphologically abnormal (Fig. 1). An abnormal head was the most common abnormality in the ZD rats, with an abundance of headless spermatozoa. A combined head and tail abnormality was also abundant in the ZD rats, where the head abnormality was again predominantly headless. Animals fed the ZC, ZMD and PF diet showed similar sperm morphology. The tail length of spermatozoa in the ZD rats (1361.7 (SD 109.0) μm) was 24% shorter ($P < 0.001$) than the average of all other groups (1791.7 (SD 33.8) μm) (Fig. 2 (B)). Pictures of spermatozoa are shown in Fig. 2 (A).

Triacylglycerol and cholesterol

The effects of Zn deficiency on testis TAG and cholesterol were also measured. TAG (mg/g testis) showed no significant differences between Zn treatment groups: ZC (0.55 (SD 0.06)); ZD (0.55 (SD 0.20)); PF (0.48 (SD 0.04)). Cholesterol (mg/g testis) was significantly ($P < 0.0375$) higher in the ZD group than in the ZC group while levels in the PF group were comparable with both the ZD and ZC animals: ZC, 0.46

(SD 0.03); ZD, 0.62 (SD 0.07); PF, 0.52 (SD 0.04). Though no significant difference, there was a trend of lower TAG:cholesterol ratio in the testes of ZD and PF animals: ZC, 1.16 (SD 0.05); ZD, 0.90 (SD 0.29); PF, 0.92 (SD 0.01).

Fatty acid composition in phospholipids

Fatty acid composition was determined in phosphatidylcholine (PC) and phosphatidylethanolamine (PE), since these are the most abundant phospholipids in testes (Tables 2 and 3). Dietary Zn significantly altered fatty acid profile in testis phospholipids. In PC, the major long-chain PUFA found in the testes of ZC animals were 18:2n-6, 20:4n-6 and 22:5n-6 at 2.4, 14.3 and 15.0%, respectively. Animals fed a ZD diet had the highest level of 18:2n-6 and the lowest elongated and desaturated product of 22:5n-6, while all other groups had similar levels (Table 2). Similarly, the major long-chain PUFA in ZC PE were 18:2n-6, 20:4n-6 and 22:5n-6, at 2.4, 20.6 and 23.9%, respectively

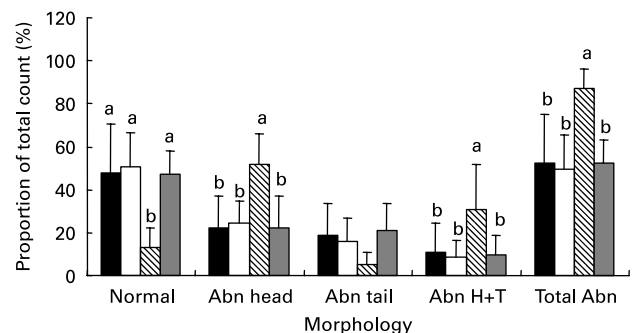


Fig. 1. Sperm morphology (% total count) in testes in response to dietary Zn treatment: (■), Zn control; (□), Zn marginally deficient; (▨), Zn deficient; (▩), pair fed. Two hundred cauda epididymal spermatozoa were analysed per animal using light microscopy at a magnification of 400X. Morphology is defined as follows: normal, hooked head and smooth tail; abnormal (Abn) head, missing head, flattened hook or pinhead; Abn tail, bent or coiled tail; Abn H + T, possessing both a head and a tail abnormality; Total Abn, sum of the three abnormality groups. Values are means ($n = 5$), with standard deviations represented by vertical bars. ^{a,b} Within each morphology category, mean values with unlike letters were significantly different ($P < 0.009$).

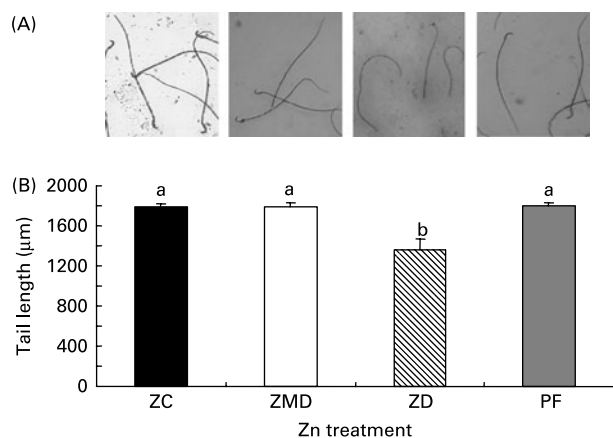


Fig. 2. Spermatozoa pictures (A) and tail length (B) in response to dietary Zn treatment. ZC, Zn control; ZMD, Zn marginally deficient; ZD, Zn deficient; PF, pair fed. Twenty cauda epididymal spermatozoa were analysed using Image Pro Plus 5.0 computer software (Media Cybernetics Inc., Silver Spring, MD, USA). Values are means (*n* 4), with standard deviations represented by vertical bars. ^{a,b} Mean values with unlike letters were significantly different (*P* < 0.001).

(Table 3). The level of 18:2*n*-6 in PE was highest in the rats fed a ZD diet in comparison with the other diet groups. The level of 22:5*n*-6 was lowest in the ZD animals resulting in a higher ratio of 18:2*n*-6 to 22:5*n*-6 (Table 3, Fig. 3). Although it was not statistically significant, 22:6*n*-3 was also lowest in the ZD animals in both PC and PE. The ratio of total *n*-6:*n*-3 fatty acids was in the range of 25–39:1 in PC and 21–28:1 in PE, indicating a highly *n*-6 fatty acid-enriched environment in the testes of rats.

Discussion

The present study examined the impact of a short period of dietary Zn deficiency on long-chain PUFA composition in testis phospholipids and sperm integrity of growing rats.

The major fatty acid changes in PC and PE of Zn-deficient testes were elevated 18:1 (oleic acid) and 18:2*n*-6, and reduced 22:5*n*-6 compared with the other groups. These changes were only identified in animals fed a severely Zn-deficient diet (ZD; ≤ 1 mg/kg) for 3 weeks. The ZMD diet (9 mg Zn/kg diet) did not show the same effect. Oteiza *et al.* (22) found that weanling rats on a Zn-deficient diet (0.5 mg/kg diet) for 2 weeks had 32% lower Zn (247 nmol/g wet tissue) in the testes in comparison with control animals fed a 25 mg Zn/kg diet (169 nmol/g wet tissue). Since our feeding regimen was 3 weeks with a similar diet regimen, its level in testes could be lower. It was also evident that rat testes are highly enriched in *n*-6 long-chain PUFA, especially 22:5*n*-6, which is normally a very minor component in other organs and tissues. Thus, the reduction of this fatty acid may overall influence testis function, spermatogenesis and androgenesis. The finding of the present study implies that the decrease of 22:6*n*-3, enriched in human testes and sperm, could lead to abnormal sperm function. In fact, decreased 22:6*n*-3 in the sperm phospholipid has been identified in infertile men and asthenozoospermic males (23,24).

Testis apoptosis, before detecting any morphological changes, can be achieved at 3 weeks after initiating a diet deficient in Zn (< 1 mg Zn/kg diet) in rats aged 7 weeks (3). Furthermore, testicular atrophy with the loss of sperm cells and spermatocytes with lower relative testis weight to body weight was identified after 10 weeks. These changes depend on the severity of Zn deficiency (2,3). In the present study, animals fed a ZD and a PF diet had higher relative testis weights. This implies that the testis may be the last organ to be affected by Zn deficiency. However, considering the sperm abnormalities found, the present study indicates that the 3-week feeding period of our diet regimen (≤ 1 mg Zn/kg) to growing animals may also have induced apoptosis in testes. Further study is necessary to confirm the threshold of Zn deficiency-induced testis apoptosis regarding dietary Zn level, feeding period and age.

Table 2. Phosphatidylcholine fatty acid profile (% total fatty acids) in testes in response to dietary zinc treatment (Mean values and standard deviations)

Zn treatment...	ZC†		ZMD†		ZD‡		PF‡		<i>P</i>
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
14:0	0.23	0.07	0.21	0.06	0.22	0.10	0.23	0.03	NS
16:0	39.06	3.11	38.85	1.27	39.07	1.67	37.10	5.34	NS
16:1	0.85	0.13	0.79	0.05	0.98	0.26	1.10	0.27	NS
18:0	4.68 ^a	0.51	4.99 ^a	0.32	4.49 ^a	0.58	3.79 ^b	0.18	0.0157
18:1(9+11)	15.11 ^b	1.00	14.73 ^b	0.53	17.56 ^a	1.15	15.85 ^{a,b}	1.54	0.0142
18:2 <i>n</i> -6	4.21 ^{b,c}	0.97	3.60 ^c	0.52	5.96 ^a	1.14	5.08 ^{a,b}	0.76	0.0127
20:3 <i>n</i> -6	1.30	0.20	1.25	0.05	1.67	0.51	1.48	0.10	NS
20:4 <i>n</i> -6	14.25	1.32	13.25	0.91	15.32	2.62	16.00	1.39	NS
22:4 <i>n</i> -6	1.44	0.19	1.37	0.04	1.22	0.09	1.18	0.19	NS
22:5 <i>n</i> -6	15.24 ^a	1.13	15.52 ^a	1.03	11.62 ^b	2.73	16.05 ^a	1.67	0.0158
22:6 <i>n</i> -3	1.26	0.32	1.22	0.09	0.87	0.20	1.32	0.21	NS
Σ SFA	44.30	2.59	44.43	1.43	44.16	1.75	41.43	5.18	NS
Σ MUFA	16.04 ^b	1.15	15.51 ^b	0.52	18.67 ^a	0.87	17.07 ^b	1.59	0.0074
Σ <i>n</i> -6	36.56	2.35	35.06	1.08	35.92	1.10	39.93	3.75	NS
Σ <i>n</i> -3	1.38	0.37	1.35	0.13	0.92	0.18	1.35	0.21	NS

ZC, Zn control; ZMD, Zn marginal deficient; ZD, Zn deficient; PF, pair fed.

^{a,b,c} Mean values within a row with unlike superscript letters were significantly different (*P* < 0.05).

† *n* 5 per group.

‡ *n* 3 per group.

Table 3. Phosphatidylethanolamine fatty acid profile (% total fatty acids) in testes in response to dietary zinc treatment
(Mean values and standard deviations)

Zn treatment...	ZC†		ZMD†		ZD‡	PF‡	Pooled sd
	Mean	SD	Mean	SD	Mean	Mean	
14:0	0.12	0.22	0.20	0.16	0.08	0.06	0.02
16:0	23.94	4.56	17.97*	0.93	23.87	27.04	1.86
16:1	0.35	0.34	0.31	0.09	0.31	0.27	0.03
18:0	8.47	1.51	7.14	0.36	9.44	8.82	0.44
18:1(9 + 11)	8.34	1.46	6.53	0.32	9.56	7.67	1.15
18:2n-6	2.38	0.39	2.26	0.23	3.82	3.12	1.15
20:3n-6	0.63	0.05	0.63	0.07	0.74	0.71	0.07
20:4n-6	20.60	2.49	17.83	1.88	22.19	18.43	2.75
22:4n-6	3.44	0.55	3.57	0.11	3.88	3.48	0.46
22:5n-6	23.94	2.23	24.66	1.47	22.88	26.65	2.54
22:6n-3	2.01	0.48	2.08	0.14	1.85	2.37	0.52
Σ SFA	33.65	6.06	25.68*	1.09	34.40	36.92	1.52
Σ MUFA	9.45	1.76	9.17	0.98	10.04	8.07	1.19
Σ n-6	51.04	3.05	48.97	1.17	53.55	52.44	0.85
Σ n-3	2.19	0.68	2.33	0.17	1.89	2.39	0.51

ZC, Zn control; ZMD, Zn marginally deficient; ZD, Zn deficient; PF, pair fed.

* Mean value was significantly different from that of the ZC group ($P < 0.05$).

† n 3 per group.

‡ n 2, therefore pooled sd is presented. Trend analysis (R^2) was performed on the two independent datasets (R^2 0.9957 and 0.9963, in the ZD and PF groups, respectively).

The present study demonstrated that spermatozoa integrity is adversely affected with a Zn-deficient diet. Stoltenberg *et al.* (25) have demonstrated *in vitro* that Zn distribution in the spermatozoa varies depending on its stage of maturation within the epididymis. Once formed in the testis seminiferous tubules, immature spermatozoa migrate to the caput of the epididymis, when Zn is mostly concentrated in the tail. Spermatozoa then mature and gain mobility as they continue through the epididymal duct to the cauda epididymis, in which Zn is highly and almost uniquely concentrated in the acrosome of the head. Our findings reveal that spermatozoa head and combined head and tail abnormalities are the most frequent abnormalities and tail length is stunted in Zn deficiency. This indicates that Zn is involved in not only promoting the maturation of testis germinal cells into spermatozoa (1,2) but also in epididymal sperm maturation.

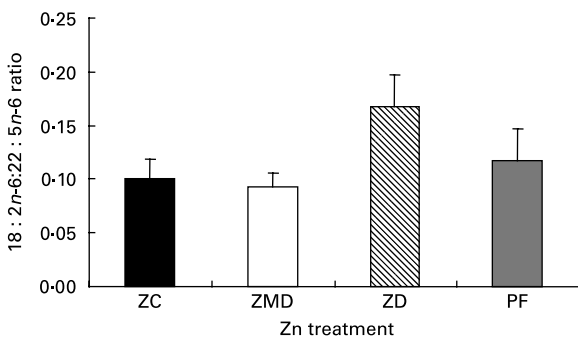


Fig. 3. Effect of Zn treatment on the ratio of 18:2 to 22:5n-6 in testis phosphatidylethanolamine. ZC, Zn control; ZMD, Zn marginally deficient; ZD, Zn deficient; PF, pair fed. For ZC and ZMD, values are means (n 3), with standard deviations represented by vertical bars. For ZD and PF, because the data represent n 2, values are means, with pooled standard deviations.

The present study showed that essential fatty acid metabolism is interrupted by Zn deficiency in testes. Specifically, there is an interruption in the n -6 essential fatty acid desaturation and elongation from 18:2n-6 to 22:5n-6 when Zn is severely but not marginally deficient. Cunnane *et al.* (11) also found high levels of 18:2n-6 with a decrease of 20:4n-6 in testes of Zn-deficient rats. The present study showed a decrease in 22:5n-6, a major fatty acid in testes. In consideration of precursors and metabolites in essential fatty acid metabolism, the ratios of 18:2n-6:20:4n-6, 18:2n-6:22:4n-6 and 18:2n-6:22:5n-6 were all higher in the ZD group than the other diet groups (Fig. 4). These data confirm the effects of inhibited activity of the Δ 6 and Δ 5 desaturases as put forth by Ayala & Brenner (10). However, the existence of two different Δ 6 desaturases has been proposed for the synthesis of long-chain PUFA (26), one in microsomes and the other in peroxisomes. Whether both desaturases are affected by Zn deficiency requires further investigation. Although the specific function of 22:5n-6 is not well known, the present results indicate that the reduction of this fatty acid could be one of the mechanisms for Zn deficiency-induced inhibited spermatogenesis or testis atrophy. In addition, long-chain PUFA are membrane-associated fatty acids rather than used as substrates for oxidation or energy production, thus the low level of 22:5n-6 may affect the membrane integrity in both testes and spermatozoa. In another study conducted in our laboratory, we have found underdeveloped rat testes to have a uniquely low concentration of 22:5n-6 that is not shared with normal-sized testes (M. Suh, unpublished results).

Whether supplementation of 22:5n-6 reverses Zn deficiency-related defects in sperm development is not known. Chanmugam *et al.* (27) supplemented Zn-deficient rats with 22:5n-6 (0.58%, w/w of total fatty acids) in a 10% fat-containing diet for 6 weeks. This study found that there were no changes in the level of 22:5n-6 in the testis phospholipids of the Zn-deficient rats. The supplementation of 22:5n-6 did not alter the body

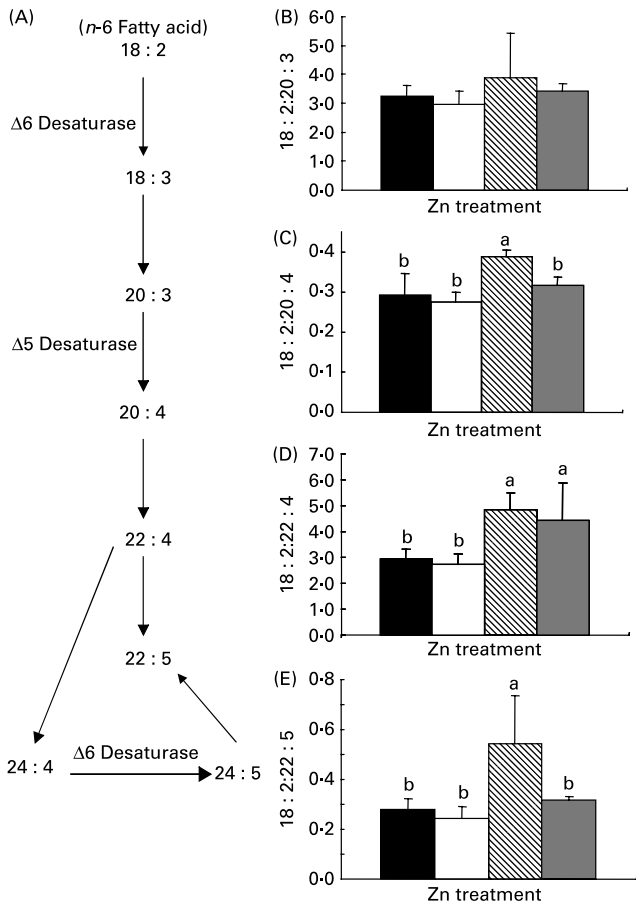


Fig. 4. Effect of Zn treatment on desaturation and elongation of *n*-6 fatty acids in testis phosphatidylcholine. (A) Desaturation and elongation metabolic pathway, showing Sprecher's pathway⁽²⁸⁾. (B) 18:2:20:3 precursor:product ratio; (C) 18:2:20:4 precursor:product ratio; (D) 18:2:22:4 precursor:product ratio; (E) 18:2:22:5 precursor:product ratio. (■), Zn control (*n* 5); (□), Zn marginally deficient (*n* 5); (▨), Zn deficient (*n* 3); (▩), pair fed (*n* 3). Values are means, with standard deviations represented by vertical bars. ^{a,b} Mean values with unlike letters were significantly different (*P* < 0.04).

weight, plasma Zn, liver weight, testis weight and spermatid number. But 22:5*n*-6 increased the level of testis Zn with no clear explanation. This indicates that when Zn deficiency is established, providing 22:5*n*-6 does not reverse the effect induced by Zn deficiency. It will be of interest to see the level of 22:5*n*-6 if the Zn-deficient animals are re-supplemented. Overall, this study confirms our findings that Zn deficiency interrupts essential lipid metabolism or incorporation of fatty acids to testes.

The present study demonstrates that dietary Zn deficiency during sexual maturation alters testis fatty acid composition in rats and affects sperm integrity. These results suggest that Zn deficiency-associated abnormal testicular function is perhaps preceded by alterations in membrane fatty acid composition, especially 22:5*n*-6 in rats. Further studies are necessary to determine which stages of sperm cell spermatogenesis are affected by dietary Zn or Zn status.

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