

THE EFFECT OF ZINC DIET ON DISTRIBUTION OF FATTY ACID IN BLOOD PLASMA CHYLOMICRONS

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Summary: The effect of dietary zinc on the distribution of fatty acids in blood plasma chylomicrons was studied. The experiment was performed on male Mongolian Gerbils, fed *ad libitum* for 3 weeks with standard diet, containing 8 or 38 mg of Zn per kg of food (low zinc diet group, LZ, and saturated zinc diet group, SZ, respectively). At the 21th day gerbils were given sunflower oil by gavage. After 2.5 hours, blood was collected from abdominal vein, and samples were pooled (five animals per one sample). Chylomicron fractions were isolated by ultracentrifugation and mass of dried chylomicrons was measured. Fatty acid composition was analyzed by gas-liquid chromatography. Significantly higher amount of chylomicrons in blood plasma of SZ than in LZ group was found. In chylomicrons, following fatty acids were found: 16:0, 16:1, 17:0, 17:1, 18:0, 18:1, 18:2, 18:3, 20:0, 21:0 and 20:4. The amount of individual fatty acids in chylomicrons in both groups was similar, except 20:4 where lower amount in SZ group was found. Zinc diet did not affect fatty acid distribution in chylomicrons of both groups. Animals fed with zinc saturated diet had higher amount of fatty acids in blood plasma. Observed results suggest that dietary zinc influences the quantity of fatty acids absorption but not its distribution in chylomicrons.

Key words: low zinc diet, saturated zinc diet, fatty acids, chylomicrons

Introduction

Zinc, an essential trace element in eukaryotes (1), plays a structural role in a wide range of important proteins, as the component of zinc finger motif (2). Due to unadequate intakes of zinc, appeared clinical features in rats include growth retardation, dermal lesions, immunodeficiency, alopecia, male and female infertility and increased capillary permeability (3).

The results of animal studies consistently showed association between Zn-deficient diet with lipid absorption (4–6). Earlier studies investigated zinc effect on chylomicrons formation (7), composition of erythrocytes membranes, essential fatty acids metabolism (8), activity of fatty acid synthase (9) and lipoprotein lipase (10), but not fatty acids composition in chylomicrons.

Dietary fats (triacylglycerols, sterols, phospholipids) enter the gastrointestinal tract, and digestion begins. Digestive enzymes involved in the breakdown of dietary lipids are esterases that cleave the ester bonds within triacylglycerols (lipase), phospholipids (phospholipases), and cholesteryl esters (cholesterol esterase). Most dietary triacylglycerol digestion is completed in the lumen of the small intestine, resulting in a complex mixture of diacylglycerols, monoacylglycerols and free fatty acids. Esterified cholesterol, meanwhile, undergoes hydrolysis to free cholesterol and a fatty acid, catalyzed by the enzyme cholesterol esterase. The C-2 fatty acid of the phospholipid is hydrolytically removed by a specific esterase called phospholipase A₂, producing lysophospholipid and still another free fatty acid (11).

The products of the partial digestion of lipids, primarily monoacylglycerols, lysophospholipids, cholesterol and fatty acids, combine with bile salts, forming negatively charged polymolecular aggregates called micelles.

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These lipid products then pass by monomolecular diffusion into the mucosal cells (enterocyte) of the small intestine. In the mucosa cell, monoacylglycerols and fatty acids are recombined into triacylglycerols. The triacylglycerols are incorporated into lipoproteins called chylomicrons (12). Chylomicrons consist of 2.1% free cholesterol, 3.9% esterified cholesterol, 4.3% phospholipids (PL), 87% triacylglycerol and 2% proteins (13). Chylomicron PL is mostly phosphatidilcholin (PC) and is derived largely from biliary PL secreted into the intestinal lumen (14). Intestinal absorption of lipids is impaired in zinc deficiency. The impaired absorption appeared not to be due to defects in the luminal digestion of lipids, or in the mucosal uptake of hydrolytic products, but primarily to the defective intestinal formation of chylomicrons (7). Enterocyte of Zn-deficient animals accumulates lipid droplets in the enterocyte and fails to transport the lipids via chylomicrons (15). These lipid droplets exhibited a strong tendency to coalesce within the cytoplasm of the enterocyte, which suggests of altered composition of chylomicron coats caused by lack of surface components such as phospholipid (PL) and apoB (16, 17).

It is well known that the structure and distribution of incorporated fatty acids in chylomicrons are related to the kind of lipids ingested (18). In addition, diets with high concentrations of polyunsaturated fatty acids lead to bigger chylomicrons than do diets with low levels of polyunsaturated fatty acids, which can then be degraded more rapidly (19, 20).

Studies on zinc-deficient rats showed different activity of lipoprotein lipase in animals fed diets with oil which contained predominately saturated fatty acids in comparison to the oil rich with unsaturated fatty acids, but the activity of lipoprotein lipase did not differ between zinc-adequate rats fed on the same way (10).

The aim of this study was to explore influence of dietary zinc on the distribution of fatty acids in chylomicrons.

Materials and Methods

Animals

Thirty male mongolian gerbils, weight 55–60 g, 9–10 weeks old, were used in this study. They were housed in cages, 5 animals per cage, and held under controlled conditions of illumination (light on 7 am–7 pm) and temperature (25 ± 3 °C).

Meal feeding and dietary treatment

Gerbils were assigned randomly to the following two groups: group fed with basal diet low in zinc (LZ) and group (SZ) fed with the same basal diet, but supplemented with zinc sulfate.

Recommended requirement for *mongolian gerbile* for Zn is 25 mg/kg (21). Both groups were fed for 3 weeks. LZ group was given deionized water, while SZ group was given a tap water. The access to diet and water was *ad libitum*.

The composition of the basal diet is given in Table I. It was prepared as described by Noh and Koo (15), with the following modification: amounts of vitamins and minerals were justified, according to the recommendations for *mongolian gerbile* (Table I) (21). This basal diet contained 8 mg of Zn/kg (LZ-diet) and was supplemented with zinc sulfate to prepare the SZ diet, 38 mg of Zn/kg.

After 20 days, diet was taken away from all gerbils, at 1 pm while water was available for consumption all the time. On following day gerbils were given 0.5 mL sunflower oil by gavage, always between 08.30–09.00, to avoid any possible rhythmic variations in metabolic status. 2.5 hours after per-os taking sunflower oil, the gerbils were anesthetized by inhalation diethylether and blood was collected from abdominal vein.

Chylomicron preparation

Blood samples were pooled (five animals per one samples). Whole pooled blood was collected into

Table I Composition of basal diet

Ingredient	Amount (g/kg)	Ingredient	Amount (mg/kg)	Ingredient	Amount (mg/kg)
Egg white	197.8	Vitamin A	0.72	Thiamin	5
Corn starch	535.3	Vitamin E	22	B ₁₂	0.010
Dextrinized corn starch	91.2	Vitamin D	0.025	Ca	5000
Sunflower oil	79.7	Vitamin K	1	NaCl	3600
Cellulose	56.9	Niacin	15	P	3000
Vitamin, mineral mix	39.1	Folic acid	0.5	Mg	1500
		Ca pantothenat	16	Fe	35
		Riboflavin	3	Mn	10
		Biotin	0.2	Cu	5
		Pyridoxine	8	Se	0.3

EDTA tubes and plasma was harvested by centrifugation at $1500\times g$ for 15 min immediately after collection. Chylomicrons were isolated by sequential flotation ultracentrifugation, following the method of Borel (22). Chylomicrons were stored at -80°C until analyzed.

Chylomicron analysis procedure

One mL of chylomicron phase was introduced into the appropriate flask, 1.2 mL of absolute ethanol containing 0.05% BHT (w/v) was added and warmed up for 2 minutes. After addition of 0.4 mL of 60% KOH (w/v) to the mixture, samples were vortexed for 1 minute. Reflux condenser was fit the to the flask and boiled for 1 hour. One mL of water and 3 mL of hexane were added to the samples, and vortexed. The upper layer was removed (hexane), and procedure was repeated with addition of 2 mL of hexane. Five ml of 20% HCl (w/w) was added into water residue layer and extracted three times with 8, 3 and 2 mL of diethylether. Three diethylether extracts were combined and washed three times with 6 mL of water, until free for acid, using the methyl red solution as indicator. Diethylether extracts were transferred into conical flask and evaporated to dryness by vacuum evaporator (23). Due to chylomicron composition, we used the amount of dried total fatty acids to representing the mass of chylomicrons.

Determination of chylomicron fatty acids compositions by GL-chromatography

Dried samples were methylated by the addition of 1 mL of the methanolic boron trifluoride solution into obtained dry residue. Reflux condenser was fit to the conical flask and warmed up exactly 3 minutes, and cooled to room temperature. Then, the condenser was removed. Liquids in samples were transferred into tube, 1 mL of water was added and extracted three times with 2, 1 and 1 mL hexane. Three hexane extracts were combined and transferred into second conical flask and evaporated by vacuum evaporator to approximately 0.5 mL. This solution containing methyl esters of fatty acid may be injected directly into the column for gas-liquid chromatography (24). Fatty acids composition was analyzed using Varian 1400 fitted with a hydrogen flame ionization detector. Metallic column (3 m \times 3.2 mm) packed with 20%LAC-3R-728 on Chromosorb W/AW, 80 mesh was used. The column detector and injector port were maintained at 180, 235 and 235 $^{\circ}\text{C}$, respectively. Nitrogen was used as carrier gas (24 mL/min).

Statistical analysis

Data are expressed as the mean \pm SD and statistical significance and other statistical tests were established by protocols described in Hinkle et al (25).

Results

Chylomicron concentration

Chylomicrons consisted mostly of triacylglycerol (87%) and small amounts of free cholesterol (2.1%), esterified cholesterol (3.9%), and phospholipids (PL) (4.3%) (13) and that is the reason why the mass of fatty acids after saponification can be used to represent the mass of chylomicrons. Obtained values of chylomicron concentrations in plasma in LZ- and SZ- groups treated with oral dose of sunflower oil show that SZ-group had significantly ($p < 0.001$) higher amount of chylomicrons in plasma (5.42 ± 0.28) than LZ-group (3.96 ± 0.24).

Fatty acids composition of chylomicrons

In chylomicrons, following fatty acids were found: 16:0, 16:1, 17:0, 17:1, 18:0, 18:1, 18:2, 18:3, 20:0, 21:0 and 20:4. The concentrations of fatty acids in chylomicron, in LZ- and SZ- groups are shown in *Table II*. Dominant fatty acids identified in chylomicrons, in both groups were: 18:2, 18:1, 16:0, and 18:0, which represent 49.6, 26.9, 13.3 and 5.9% of chylomicron mass in LZ-group and 41.4, 30.2, 15.3 and 7.0%, in SZ group, respectively. The percentage of dominant fatty acids in chylomicrons in both groups was similar, to the percentage of the same fatty acids in sunflower oil (26), due to their dietary origin. The concentration of other fatty acids in chylomicrons, which were not dietary origin: 16:1, 17:0, 17:1, 18:3, 20:0 in both groups was similar, except for arachidonic acid (20:4). The results show that LZ-group had significantly ($p < 0.01$) higher concentration of 20:4 in chylomicrons (4.2 ± 1) than SZ-group (0.8 ± 0.4), (*Table II*).

Table II Concentration of fatty acids in chylomicron, in LZ- and SZ- groups treated with oral dose of sunflower oil (n=5) N.S. non significant

Fatty acids	Low Zn group ($\mu\text{g}/\text{mg}$)	Suppl Zn group ($\mu\text{g}/\text{mg}$)	Significance
16:0	133 ± 5	153 ± 12	N.S.
16:1	16.4 ± 4.4	18.4 ± 4.3	N.S.
17:0	5.6 ± 2.4	18.9 ± 9.5	N.S.
17:1	8.2 ± 3.3	14.2 ± 4.9	N.S.
18:0	59 ± 5	70 ± 9	N.S.
18:1	269 ± 6	302 ± 21	N.S.
18:2	496 ± 16	414 ± 58	N.S.
20:0	3.5 ± 0.7	3.7 ± 0.6	N.S.
18:3	3.7 ± 0.7	3.6 ± 0.3	N.S.
21:0	1.7 ± 0.4	2.1 ± 0.4	N.S.
20:4	4.2 ± 1	0.8 ± 0.4	** $p < 0.01$

Discussion

The present study confirmed that absorption of dietary fat is significantly lowered impaired in gerbils fed with a low zinc diet. The data provide clear evidence that SZ-group had significantly higher amount of chylomicrons in plasma than LZ-group. Some earlier studies have shown that enterocyte of Zn-deficient animals accumulates lipid droplets in the enterocyte and fails to transport the lipids via chylomicrons. The tendency to accumulate lipid droplets appears to be due to a lack of surface components such as phospholipid, which are required for chylomicron synthesis (15).

The surface coat of chylomicron contains apolipoproteins apo B-48, apo C-II, apo C-III, apo E, apo A-I and apo A-IV (12). Study on animals showed that zinc deficiency produced an increase in apo A-I (16) and decrease in apo B-48 (17). Changes in chylomicron apoproteins produced by zinc deficiency are due in part to postsynthetic modification of intestinal chylomicrons (16). Defect in luminal PC hydrolysis is the primary cause of the impaired absorption of fat observed in zinc deficiency (15). The most of the PC of biliary or dietary origin is hydrolyzed by pancreatic phospholipase A₂ (PLA₂) to lysophosphatidylcholine (lysoPC) (27). According to Koo (7, 28), the luminal availability of lysoPC, rather than intact PC, could be limited in LZ group, resulting in impaired absorptions of fat and fat soluble vitamins. PC and lysoPC can be taken up directly by the brush-border membrane of the enterocyte (29). LysoPC, once taken up by the enterocytes, is reacylated to PC, which is utilized for formation of the PC coat of chylomicrons (14).

Pancreatic PLA₂ hydrolytically removes the Sn-2 fatty acyl moiety from phospholipids. Although the enzyme is known to require Ca⁺⁺ for its activity (30), evidence also indicates that the enzyme binds zinc avidly *in vitro* and its activity is stimulated markedly by zinc in the presence of Ca⁺⁺ and bile salts (31). Noh and Koo (15) reported that luminal hydrolysis of phosphatidylcholine by pancreatic PLA₂ in rats fed with

lower amount of zinc was impaired and the absorption of fats decreased.

Our data noted no significant differences in distribution of fatty acids in chylomicrons in LZ- and SZ-groups, except for arachidonic acid (20:4), where lower amount in SZ group was measured.

Fatty acids profile of diet is reflected in the fatty acid profile of the chylomicron (32). Sunflower oil given to gerbils do not contain arachidonic acid (26). The origin of this fatty acids determined in chylomicrons was from phospholipids.

Phospholipids contain a large portion of polyunsaturated fatty acids (PUFAs), 18:2, 20:3, 20:4, 22:4, 22:5 (33), derived from dietary linoleic acid and alpha-linoleic acid by elongation and desaturation processes. Some studies on animals showed, higher levels of n-3 PUFA and lower levels of n-6 PUFA, included arachidonic acid, in liver phosphatidylcholine from Zn-deficient rats than from Zn-adequate rats (34, 35). This was confirmed on Zn-deficient rats fed with various types of dietary fat (34–36).

Results from other studies reported increased amount of arachidonic acid in total phospholipids (37, 38) and total lipids (39) in Zn-deficient rats. As it could be seen, the results reported in literature are contradictory. The fatty acid composition of total lipids depends on the ratio between triacylglycerols and phospholipids, thus the pool size of triglycerides influences its fatty acid composition (40).

Increased levels of arachidonic acid found in chylomicron fraction of Zn- deficient gerbils might be due to decreased concentration of chylomicrons dietary triacylglycerol of Zn-deficient gerbils.

In summary, the present study confirms earlier findings that the intestinal fat absorption is regulated by a mechanism sensitive to the zinc diet. Our observations here provide new evidence that distribution of fatty acids in chylomicrons is mainly unaffected by zinc status, except for 20:4.

EFEKAT ISHRANE CINKOM NA DISTRIBUCIJU MASNIH KISELINA U HILOMIKRONIMA PLAZME

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Kratak sadržaj: Ispitivan je efekat unosa dijetarnog cinka na distribuciju masnih kiselina u hilomikronima plazme. Eksperiment je rađen na mužjacima gerbila, hranjenim standardnom dijetom, *ad libitum*, u toku 3 nedelje hrana je sadržala 8 ili 38 mg cinka po kg hrane (grupa sa smanjenim unosom cinka, LZ i grupa sa povećanim unosom cinka, SZ, respektivno). Dvadeset prvog dana, životinjama je dato suncokretovo ulje sondom. Nakon 2,5 časa, krv je uzimana iz abdominalne vene i uzorci su pulovani (5 životinja za jedan uzorak). Hilomikronske frakcije su izolovane ultracentrifugiranjem i merena je masa suvih hilomikrona. Masnokiselinski sastav lipida u hilomikronima je određivan gasno-tečnom hromatografijom. Nađena je značajno veća količina hilomikrona u plazmi SZ grupe nego u LZ grupi. U hilomikronima su nađene sledeće masne kiseline: 16:0, 16:1, 17:0, 17:1, 18:0, 18:1, 18:2, 18:3, 20:0, 21:0 i 20:4. Zastupljenost pojedinačnih masnih kiselina u hilomikronima obe grupe bila je slična, izuzev za 20:4 gde je izmerena niža količina u SZ grupi. Ishrana cinkom ne utiče na distribuciju većine masnih kiselina u hilomikronima. Dobijeni rezultati ukazuju da cink utiče na metabolizam masti i to u delu formiranja hilomikrona, ali je uticaj ograničen na količinu formiranih hilomikrona, ali ne i distribuciju pojedinih masnih kiselina u hilomikronima.

Ključne reči: dijeta sa smanjenim unosom Zn, dijeta sa povećanim unosom Zn, masne kiseline, hilomikroni

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