ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN - POLONIA
VOL.LX, SUPPL. XVI, 304 SECTIO D 2005

Zakład Biochemii, Akademia Wychowania Fizycznego, Warszawa
Department of Biochemistry, Academy of Physical Education, Warsaw

MARZENA MALARA, MARLENA ŻOŁNOWSKA, GRAŻYNA LUTOSŁAWSKA

The association between zinc nutritional density and circulating free fatty acids, glycerol and triacylglycerol leves in young males and females

Zależność między gęstością pokarmową cynku, a stężeniem wolnych kwasów tłuszczowych, glicerolu oraz triacylogliceroli w osoczu u młodych kobiet i mężczyzn

INTRODUCTION

Zinc (Zn) is an essential catalytic component of over 300 enzymes (e.g. lactate dehydrogenase, carbonic anhydrase, and Cu-Zn superoxide dismutase) (8). Zn also plays a critical structural role in many proteins such as zinc fingers, that are commonly found in transcriptional regulatory proteins (4). It is well recognized that zinc status affects muscle work capacity and performance. Early data have indicated that low zinc concentrations in the medium decrease frog sartorius muscle switch tension and shorten twitch time (3). There is a wealth of studies indicating a close link between whole-body zinc status and insulin action. In vitro ionic zinc has been found to exert insulin-like effects inhibiting ritodrine (β-receptor agonist) stimulated lipolysis and increasing 3-methyl-glucose uptake in the adipose tissue (6). According to our best knowledge there are no in vivo studies concerning the associations between zinc nutritional status and circulating glycerol, a measure of adipose tissue lipolysis, free fatty acid (FFA) and triacylglycerol (TG) in young healthy male and female subjects.

The aim of the present study was to examine the relationship between plasma glycerol, free fatty acid and triacylglycerol levels and daily zinc intakes in young, non-obese males and females.

MATERIALS

A total of 107 subjects volunteered to participate in the study (54 females and 53 males). They were informed about experimental procedures and gave their written consent prior to the study. The experimental protocol was approved by the Ethics Commission at the Academy of Physical Education. All the women were regularly menstruating and none was taking oral contraceptives. None of the subjects had any medical history and they were not taking any medication on a regular basis.

METHODS

Blood was drawn under aseptic conditions from the antecubital vein after overnight fasting into tubes containing either lithium heparin (for zinc determination) or EDTA (for other biochemical assays) as an anticoagulants. Plasma was separated by centrifugation (15 min, 4000 rpm, 4o C) and stored at – 70o until analyses. Plasma glycerol, free fatty acid (FFA) and triacylglycerol were assayed colorimetrically at 520 nm, 550 nm and 500 nm, respectively using Randox commercial kits (Randox Laboratories, United Kingdom). Plasma zinc concentrations were determined using atomic absorption spectrometry. Inta- and interassay coefficients of variation (CV) of all methods did not exceed 5%.

All the subjects were asked not to change their dietary habits throughout the study. Daily intake of energy, macronutrients and zinc was briefly assessed from 24 h food records collected over 4 days

preceding blood collection and analyzed using a computer program. A set of pictures of meals and foods were shown to the participants by an experienced interviewer. The household measures of food intake were converted into gram weights. An interviewer assigned codes to the foods reported by the subjects and performed computer analysis using the Food 2 program purchased from the Institute of Food and Nutrition in Warsaw.

RESULTS

The physical characteristics of the subjects are presented in Table 1. No sex-related differences were found in biochemical variable levels in plasma, except for TG concentrations which were significantly lower in female than in male participants ($p< 0.02$) (Table 2).There were no differences between males and females in the percent of energy derived from protein, fat and carbohydrates (Table 3). In females the mean daily zinc intake (8.2 mg) covered 82% of the safe intake level. In males the mean daily zinc intake of 14,3 mg covered 102,1% of the safe intake level. However, the mean zinc nutritional density did not differ between male and female participants. None of the biochemical variables were correlated with plasma zinc levels. On the contrary, significant and inverse correlation was demonstrated between zinc nutritional density and plasma FFA levels ($p< 0.003$) (Table 4). In addition, the correlation between plasma glycerol and zinc nutritional density ($p<0.06$) was close to the accepted limit of significance.

DISCUSSION

The most important finding of our study concerns the inverse relationship between circulating FFA and zinc nutritional density suggesting that low zinc intake possibly increases FFA levels in plasma. Concomitantly, a weak and inverse correlation was noted between zinc density and circulating glycerol. Assuming that glycerol plasma levels reflect adipose tissue lipolysis, it seems feasible that low zinc density decreases its availability to adipose tissue and depresses insulin-like (inhibitory) effect of zinc on lipolysis (6).

It is worth noting that plasma FFA concentrations reflect on the one hand adipose tissue and circulating TG lipolysis, and on the other hand FFA uptake by the peripheral tissues, mainly the liver and the striated muscle (7). In this way, circulating FFA contributes to the synthesis of originating from the liver TG in the blood and to the synthesis of intramuscular TG stores. However, in our study there was no relationship between plasma TG and zinc density in the diet. Thus, it could be tentatively postulated that zinc content in the diet affects mostly FFA uptake by the muscle under fasting conditions when glucose contribution to muscle metabolism is negligible (1).

Taking into account that insulin stimulates FFA uptake by the muscle it seems feasible that zinc may be of importance in insulin action on fat metabolism in this tissue (2). It should be pointed out that recently it has been found that zinc phosphorylates insulin receptor substrate-1 (IRS-1) and potentiates insulin-mediated activation of IRS-1 in the muscle (5). On the other hand, Wilmsen et al (9) have indicated that improvement in insulin sensitivity by insulin-sensitizing drugs (tiazolidienediones, TZD) upregulate FFA uptake in skeletal muscle affecting FFA translocase (FAT/CD36) expression. Thus, it could not be excluded that a close link between insulin signaling and zinc affects not exclusively glucose but also FFA disposal in the body. In conclusion, our study indicated that zinc density slightly, but significantly affects circulating FFA and low zinc density has an adverse effect increasing FFA concentration in plasma. It is postulated that insulin-like effects of zinc is not limited to its action on glucose disposal but possibly include plasma FFA uptake by the muscle at least under fasting conditions.

REFERENCES

1. Anders R. i wsp. The quantitatively minor role of carbohydrate in oxidative metabolism by skeletal muscle in infant man in the basal state. Measurements of oxygen and glucose uptake oral carboy dioxide and lactate production in the forearm. J Clin Invest 1956; 3: 671-78.
2. Dyck D.J. i wsp. Insulin increases FA uptake and esterification but reduces lipid utilization in isolated contracting muscle. Am J Physiol Endocrinol Metab 2001; 281: E600-E607.

3. Isaacson A., Sandow A. Effects of zinc on response of skeletal muscle. J Gen Physiol 1963; 46:655-677.
4. Krishna S.S. i wsp. Structural classification of zinc fingers: survey and summary. Nucleic Acids Res 2003; 31: 532-550.
5. Miranda E.R., Dey C.S. Effect of chromium and zinc on insulin signaling in skeletal muscle cells. Biol Trace Elem Res 2004; 101: 19-36.
6. Shisheva A. i wsp.. Insulinlike effects of zinc ion in vitro and in vivo. Preferential effects on desensitized adipocytes and induction of normoglycemia in streptozocin-induced rats. Diabetes 1992; 41: 982-988.
7. Teusink B. i wsp. Contribution of fatty acids released from lipolysis of plasma triglycerides to total plasma fatty acid flux and tissue-specific fatty acid uptake. Diabetes 2003; 52: 614-20.
8. Vale B.L., Auld B.S. Zinc coordination, function, and structure of zinc enzymes and other proteins. Biochemistry 1990; 29: 5647-5659.
9. Wilmsen H.M. i wsp. Thiazolidinediones upregulate impaired fatty acid uptake in skeletal muscle of type 2 diabetic subjects. Am J Physiol Endocrinol Metab 2003; 285: E354-E362.

This study was supported by grant I.3 from the Academy of Physical Education

Table 1. Physical characteristics of the subjects (means ± SD)

	Females (n=54)	Males(n=54)
Age (years)	21.0 ± 1.2	22.4 ± 1.4
Body mass (kg)	169.4 ± 7.1	181.3 ± 5.8
Body height (cm)	61.7 ± 7.6	81.1 ± 13.3
BMI (kg/m2)	21.2 ± 1.9	24.6 ± 3.1

Table 2. Plasma triacylglycerol, glycerol, free fatty acid and zinc levels in males and females (means ± SD)

	Females (n=54)	Males (n=53)
TG (mmol/L)*	0.74 ± 0.30a	0.95 ± 0.46
FFA (mmol/L)	0.302 ± 0.190	0.362 ±0.295
Glycerol (μmol/L)	67.3 ± 25.5	58.9 ± 27.3
Zinc (μmol/L)	14.4 ± 2.0	14.2 ± 1.9

*- TG – triacylglycerol; FFA – free fatty acid; a - $p < 0.02$ in comparison with males

Table 3. Energy, macronutrients and zinc daily intakes and zinc density in males and females (means ± SD)

	Females (n=54)	Males (n=53)
Energy (kcal)	1734 ± 581a	3056 ± 876
Protein (g)	57.9 ±16.3a (14 ± 3)	107.4 ± 33.2 (14 ± 3)
Fat (g)	64.9 ±31.2a (33 ± 8)	121.4 ± 45.6 (35 ± 6)
Carbohydrates (g)	243.2 ±80.4a (53 ± 8)	406.6 ± 119.9 (51 ± 6)
Zinc (mg)	8.2 ±2.3a	14.3 ± 4.5
Zinc (mg/1000 kcal)	4.9 ± 1.1	4.7 ± 0.9

* In brackets-percent of energy derived from respective macronutrients
a – $p < 0.001$ in comparison with males

Table 4. Spearman rank correlation coefficients between zinc density and biochemical variable levels in plasma in taken together male and female subjects

Variable	r
TG	- 0.121
FFA	- 0.285a
Glycerol	- 0.186b

a – $p < 0.003$; b – $p < 0.06$

ABSTRACT

This study aimed at evaluation of the relationship between zinc nutritional density and circulating free fatty acids (FFA), glycerol and triacylglycerol (TG) in young male and female subjects. A total of 107 subjects volunteered to participate in the study. Plasma zinc levels were assayed using absorption atomic spectrometry, FFA, glycerol and TG concentrations was determined colorimetrically using commercial kits (Randox Laboratories, United Kingdom). Subjects' dietary intake was assessed from 24 h dietary records collected 4 times from each participant. Females were characterized by significantly lower zinc intake compared to males due to much lower energy and macronutrient intakes. However, zinc nutritional density did not differ with respect to sex. A significant and inverse relationship has been found between circulating FFA and zinc density ($p<0.003$) and a weak and inverse correlation between zinc density and circulating glycerol ($p<0.06$). There were no associations between circulating TG and zinc density. Our data indicated that zinc nutritional density slightly affected lipolysis of either adipose tissue or circulating lipoproteins and inadequate zinc intake promotes lipolysis. A much more pronounced effect of zinc density was observed for circulating FFA, suggesting that low zinc intake may contribute to depressed FFA uptake by peripheral tissues.

STRESZCZENIE

Celem niniejszych badań była ocena zależności między gęstością pokarmową cynku, a stężeniem wolnych kwasów tłuszczowych (WKT), glicerolu oraz triacylogliceroli (TG) w osoczu u młodych kobiet i mężczyzn. W badaniach wzięło udział 107 uczestników osób. Krew do badań biochemicznych pobierano rano, na czczo z żyły łokciowej. Stężenie cynku w osoczu oznaczano metodą absorpcyjnej spektrometrii atomowej, natomiast stężenia WKT, glicerolu oraz TG oznaczane były metodą kolorymetryczną z zastosowaniem gotowych zestawów firmy Randox (Randox Laboratories, Wielka Brytania). Dzienne spożycie energii, białka, tłuszczów i węglowodanów oceniano na podstawie czterech 24 godzinnych wywiadów żywieniowych . Kobiety w porównaniu do mężczyzn charakteryzowały się znacznie niższym spożyciem cynku w dziennych racjach pokarmowych. Nie stwierdzono jednak różnic między kobietami, a mężczyznami w gęstości pokarmowej (mg/1000 kcal). Wykazano istotną i ujemną korelację między stężeniem WKT w osoczu , a gęstością pokarmową cynku ($p<0.003$). Jednocześnie stwierdzono, że ujemna korelacja między gęstością cynku a stężeniem glicerolu znajduje się na granicy istotności statystycznej ($p<0.06$). Nie stwierdzono zależności między gęstością pokarmową cynku, a stężeniem TG w osoczu. Uzyskane wyniki wskazują, że gęstość pokarmowa cynku w znacznie większym stopniu może wpływać na stężenie WKT, niż na stężenie glicerolu w osoczu i sugerują, że zawartość cynku w diecie w większym stopniu wpływa na pobieranie WKT przez tkanki obwodowe niż na lipolizę w tkance tłuszczowej.