The Effect of Chromium and Selenium Ions on Mitochondrial Transmembrane Transport of Fatty Acids in Healthy and Diabetic Rats

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Abstract

Essential trace elements, chromium (Cr⁵⁺) as well as selenium (Se⁴⁺) and (Se⁻²) exhibits stimulatory effect on β-oxidation of fatty acids, especially after supplementation with carnitine. Presented results allowed to conclude, that the trace elements ions modify the activity of the transport of fatty acids across the mitochondrial membranes. It seems that the limiting step of this process is the activity of carnitine-palmitoyl transferase-1 (CPT-1), which is responsible for transport of substrate across the external membrane. In this process selenium ions (Se⁴⁺) and (Se⁻²) are more active than chromium (Cr⁵⁺). In all studied combinations of ions the activity of carnitine-palmitoyl transferase -2 (CPT-2) remains almost unchanged. Only small stimulatory effect was observed in combination of Cr and Se ions, but in these variants inhibitory effect on CPT-1 occur.

Keywords: CPT-1 and CPT-2; Cr and Se ions; Healthy and diabetic rats

Introduction

Mitochondrial degradation of fatty acid (FA) in β-oxidation pathway requires delivery of substrates. In blood fatty acid are transorted by albumins. In the cell fatty acids must be transferred across the mitochondrial membrane. In this process two enzymes, as well as carnitine are engaged: carnitine-palmitoyl transferase 1 (CPT-1) and carnitine-palmitoyl transferase 2 (CPT-2). First of these enzymes takes a part in transport of palmitic acid across external mitochondrial membrane, while second – across internal mitochondrial membrane to mitochondrial matrix. Carnitine, [(-)-β-hydroxyl-γ-[trimethylamino-]butyrate] is an essential component of the fatty acids transporting system. Activity of CPT-1 is responsible not only for the activity of fatty acids utilization but also for the hepatic level of triglycerides [1,2]. On the other hand CPT-2 deficiency should lead to myopathies accompanied with hypoglycemia [3] or neuromuscular disorders [4]. Disorders at this step of fatty acids metabolism and disturbances in production of FADH₂ and NADH + H⁺ (substrates for oxidative phosphorylation chain) as well as acyl-CoA (the substrate for Krebs' cycle) should lead to energy production disorders.

Supplementation with carnitine increases activity of fatty acids degradation in rat lymphocytes. Decomposition of palmitate increases from 27.46 ± 0.66 pmoles.min⁻¹ mg⁻¹ protein to 31.60 ± 0.77 pmoles.min⁻¹ mg⁻¹ protein in healthy rats and from 24.80 ± 2.02 pmoles.min⁻¹ mg⁻¹ protein to 45.47 ± 4.75 pmoles.min⁻¹ mg⁻¹ protein in diabetic animals. The rate of stimulation after supplementation with 50 µM carnitine was about 20% in healthy and 83% for diabetic animals [5,6].

Chromium in active form of chromodulin [7,8] optimizes insulin action [9], via membrane phosphotyrosine phosphatase [10] and tyrosine kinase [9]. Selenium ions, Se⁴⁺ and Se⁻², are components of several enzymes as glutathione peroxidase, triiodothyrosine deiodase and selenoprotein P. These ions affect glucose uptake in RBC and beta-oxidation activity in WBC obtained from healthy rats [11]. It seems interesting to check if microelements such chromium (Cr⁵⁺), used in therapy of type 2 Diabetes mellitus and selenium (Se⁴⁺ and Se⁻²) should modify fatty acids transport into the mitochondria [12].

Materials and Methods

Agreement issued by III Local Ethic Commission at Life Sciences University in Warsaw, Ciszewskiego 8, 02-786 Warsaw was obtained before starting the experiments.

Experiments were performed on 54 healthy and 54 diabetic Wistar rats, 4-weeks old, weighting 135 - 150 g. Animals were fed ad libitum with a standard laboratory diet, having free access to drinking water. Rats were killed by cervical dislocation and blood was collected on standard EDTA and livers were excised and immediately frozen in liquid nitrogen for further studies. Diabetes was provoked by intraperitoneal injection of streptozotocin dissolved in physiological saline. After increasing of blood glucose concentration from 5.35 ± 0.54 mmole/L to 28.74 ± 4.05 mmole/L and falling down the concentration of insulin from 3.16 ± 0.99 ng/ml to 0.26 ± 0.10 ng/ml the animals were kept 1 week at this state before killing.

The assays of the activity of CPT-1 and CPT-2 were performed according to Ventura et al. [13] method. Livers were homogenized in buffer: 250 mmol/L mannitol, 5 mmol/L TRIS-HCl, pH 7.4 and 0.5 mmol/L EGTA. Homogenates were centrifuged 10 min. at 600 x g. Supernatant was recentrifuged second time for 10 min. at 3600 x g. The pellet was resuspended in homogenizing buffer and centrifuged third time for 10 min. at 2700×g. Final pellet was resuspended in buffer: 50 mmol/L HEPES - 150 mmol/L KCl, pH 7.4 and sonicated 3-times for 15 seconds in 45 seconds intervals at the temperature of +4°C. Activities of palmitoyl transferases were measured in incubation mixtures contained: 50 mmol/L HEPES, 150 mmol/L KCl, 1 mmol/L EDTA, 1 mmol/L DTT, 20 µmol/L BSA, 0.5 mmol/L carnitine and 1.0µCi of radioactive L-methyl-³H-carnitine, 100 µmol/L palmitoyl-CoA and 50 µ of mitochondrial preparation in a final volume of 500 µL. Samples were supplemented with chromium acetate to a final concentration of chromium ions of 96.15 µmol/L, selenomethionine or sodium selenite to a final concentration of selenium ions of 6.33 µmol/L or combinations of Cr⁵⁺ with Se⁴⁺ or Se⁻². Ions were added in a volume of 1 µL what had no effect on final concentrations of components of

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incubation mixtures. Activity of CPT-2 was measured after blocking of the CPT-1 with 200 µmol/L. M malonyl-CoA. All other components of the reaction mixture were the same. Incubation was carried out at 37°C for 10 minutes and terminated by addition of 0.5 ml of 1.2 mol/L HCl and 1 mL of n-butanol saturated with distilled water. Samples were vigorously shaken and organic phases was collected. Water fraction was extracted again with water-saturated butanol and organic phases were combined and counted in scintillation β-spectrometer. Subtraction of the results from unblocked and blocked samples allows estimating the activity of CPT-1. The results were expressed as nmoles of transferred fatty acid per 1 min per 1 mg of mitochondrial protein. The results were statistically evaluated using ANOVA test.

**Results**

Activities of carnitine-palmitoyl transferase 1 and carnitine-palmitoyl transferase 2 in mitochondrial preparations from rat’s livers are presented in Table 1 (for healthy animals) and in Table 2 for diabetic rats.

Chromium ions as well as selenium Se⁺⁴ ions exhibit slight inhibitory effect on transmembrane transport of fatty acids. This effect should be reversed by supplementation of selenium Se⁻² ions. The data indicate, that selenium Se⁻² ions had no effect alone and in combination with chromium Cr⁺³ ions on total activity of transmembrane transport of fatty acids. (44.8 ± 0.94 nmoles min⁻¹ mg⁻¹ vs 45.56 ± 1.93 nmoles min⁻¹ mg⁻¹ as compare to control 46.04 ± 1.43 nmoles min⁻¹ mg⁻¹, respectively).

In healthy rats all examined ions, alone and/or in combinations suppress the activity of CPT-1. Transmembrane transport of fatty acids across external mitochondrial membrane was inhibited, especially by chromium ions in all experimental variants, at least in combination with selenium (5.55 ± 0.32, 5.29 ± 0.42 and 9.5 ± 0.32 nmoles min⁻¹ mg⁻¹ as compare to control 19.62 ± 1.12 nmoles min⁻¹ mg⁻¹, respectively). Inhibitory effect should be partially reversed by selenium Se⁻² ions, but not by Se⁺⁴. Selenium ions, when used alone were also inhibitors of this enzyme, but the effect was much lower than those observed for chromium ions (8.55 ± 0.58 for Se⁺⁴ and 11.09 ± 0.98 nmoles min⁻¹ mg⁻¹ for Se⁻² ions as compare to control - 19.62 ± 1.12 nmoles min⁻¹ mg⁻¹).

On the other hand, chromium Cr⁺³ as well selenium Se⁺⁴ and Se⁻² stimulated the activity of transmembrane transport of fatty acids across internal mitochondrial membrane. The activity of CPT-2 increased from 26.42 ± 0.75 for control to 33.45 ± 1.79 for Cr⁺³, 33.00 ± 1.55 for Se⁻² and 33.39 ± 0.64 nmoles min⁻¹ mg⁻¹ for Se⁺⁴ ions.

In diabetic rats all examined ions, alone and/or in combinations also suppress the activity of both enzymes. Only Se⁻² and its combination with chromium did not exhibit inhibitory effect (41.72 ± 3.96 and 44.24 ± 3.10 nmoles min⁻¹ mg⁻¹ as compare to 45.51 ± 3.71 nmoles min⁻¹ mg⁻¹ for control). Transmembrane transport of fatty acids across external mitochondrial membrane was stimulated by all combinations of supplements, especially by chromium and selenium Se⁻² ions (24.26 ± 4.64 for chromium and 21.75 ± 2.13 for selenium Se⁻² ions min⁻¹ mg⁻¹ as compare to control - 15.94 ± 2.83 nmoles min⁻¹ mg⁻¹). Inhibition of the activity of CPT-2 should be partially reversed by combination of chromium with selenium Se⁻² ions (24.44 ± 3.81) or Se⁺⁴ (22.08 ± 1.69 nmoles min⁻¹ mg⁻¹).

**Discussion**

Chromium is known as a stimulator of glucose transport into the cells and modulator of insulin receptor signaling [14]. It is possible, that observed earlier increased oxidation of fatty acids in the presence of chromium Cr⁺³ ions should be, in part, the effect of increased permeability of cellular membranes for fatty acids [10]. As indicated in presented data chromium activates fatty acids degradation in the absence, as well as in the presence of exogenous carnitine (63.95% and 72.19%, respectively). Stimulation of carnitine acyltransferases was reported earlier by Karlic et al. [15]. Observed activation of β-oxidation by trace elements should result in increased concentration of acetyl-CoA, which may serve as a substrate for Krebs’ cycle, as well as for resynthesis of fatty acids. In this process first reaction is carboxylation of acetyl-CoA to malonyl-CoA, which was recognized as a strong inhibitor of CPT-1. Acetyl-CoA carboxylase should be activated by a number of factors, such as substrate (Acetyl-CoA), leptin or glucose (in central nervous system) [16-18]. Methodological pitfalls, causing the observed differences can be mostly excluded for the following reason: (a) dietary effects should be excluded because all animals had the same diet and free access to them; (b) CPT-1 activity should be affected by inhibitors due to changes in membranes fluidity. This may be omitted when experiments performed in *in vitro*, using isolated, sonicated mitochondria. That why differences observed in such experiments seems to be caused by added trace elements, as well as exogenous carnitine.

The role of selenium is not yet well recognized. Low level of this trace element should result in myopathies and cardiomyopathies [19], disturbances in spermatogenesis [20] and in elevated risk of carcinogenesis [21], as well as bacterial [22] and viral infections [23]. Other function of selenium ions is participation in red-ox processes as the structural element of glutathione peroxidases. Glutathione peroxidases are the family of four distinct mammalian selenoproteins. The most popular is classical enzyme CGPx. Other proteins of this family are gastrointestinal isoenzyme (GI-GPx), the most related to CGPx, plasma (pGPx) and glutathione peroxidase of phospholipids peroxidases (PHGPx). Other proteins showing activity modified by the selenium ions are 5’-iodotyronine deiodase, thioredoxin reductase and selenoprotein P [24-26]. Observed differences in the activity of fatty acids decomposition following chromium and/or selenium supplementation.
may suggest that these micronutrients should play a role in some steps ofenergetical processes. Moderatory effect of investigated trace elements on efficiency of fatty acids β-oxidation was observed in the presence of exogenous carnitine. It is possible that these ions are a part ofcarnitine shuttle of fatty acids across the mitochondrial membranes. Increased degradation of fatty acids should be caused by its increasedconcentration in mitochondrial matrix. However, supplementation with ions resulted in modification of the activity of CPT-1. It seems thatchromium ions are inhibitors of this enzyme when used alone (5.55 ± 0.32 nmol min⁻¹ mg⁻¹ as compare to 19.66 ± 1.12 nmol min⁻¹ mg⁻¹ for control), as well as in the presence of selenium (5.29 ± 0.42 nmol min⁻¹ mg⁻¹ and 9.51 ± 0.32 nmol min⁻¹ mg⁻¹ for Se⁴⁺ and Se⁻², respectively). Its inhibitory effect should be partially ameliorated by addition of Se²⁻ ions. Slight difference in the action of Se⁴⁺ and Se⁻² should be observed. Selenium Se⁴⁺ are less effective in stimulation of CPT-1 as compare to control), as well as in the presence of Se⁻² (5.55 ± 0.32 nmol min⁻¹ mg⁻¹ and 11.09 ± 0.98 nmol min⁻¹ mg⁻¹, respectively), as well as in the presence of Cr⁺⁶. These results indicate that the activity of the mitochondrial degradation of fatty acid is rather regulated by the activity of CPT-1 than of CPT-2 and should be, at least in part, controlled by selenium ions. Karlic et al. [15] found that expression of CPT-1 and CPT-2 genes in rats is age-related and is less effective in adult animals because of accumulation of oxidatively damaged products. Selenium is known as anti-oxidant agent, what should reflect in protection of membrane lipids against ROS oxidation and increased activity of β-oxidation as well as carnitine-palmitoyltransferases—CPT-1 and CPT-2.

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References

1. Stefanovic-Racic M, Perdomo G, Mantell BS, Sipula U, Brown NF, et al. (2008) A moderate increase in carnitine palmitoyltransferase 1a activity is sufficient to substantially reduce hepatic triglyceride levels. Am J Physiol Endocrinol Metab 294: 969 - 977.

2. Sierra AV, Gratacós E, Carrasco P, Clotet J, Ureña J, et al. (2008) CPT1c is localized in endoplasmic reticulum of neurons and has carnitine palmitoyltransferase activity. J Biol Chem 283: 6878-6885.

3. Deutsch M, Vassilopoulos D, Sevastos N, Papadimitriou A, Vasilikou K, et al. (2008) Severe rhabdomyolysis with hypoglycemia in an adult patient with carnitinepalmitoyltransferase II deficiency. Eur J Intern Med 19: 289-291.

4. Bruno C, Dimasro S (2008) Lipid storage myopathies. Curr Opin Neurol 21: 601-606.

5. Kury T, Adamowicz M, Debiski B, Bertrand J, Martynek K (2001) Degradation of [9,10] - 3H - myristic acid by lymphocytes. Screening test of inherited disorders of activation, transport and mitochondrial oxidation of fatty acids. Atherosclerosis.

6. Kury T, Debiski B, Milczarek M, Bertrand J, Klos A (2011) Effect of carnitine and microelements (chromium and selenium) on fatty acids metabolism in healthy and type I diabetic rats. Probl Hig Epidemiol 92: 583-586.

7. Wada O, Wu GY, Yamamoto A, Manabe S, Ono T (1983) Purification and chromium-excretory function of low-molecular-weight, chromium-binding substances from dog liver. Environ Res 32: 228-239.

8. Yamamoto A, Wada O, Ono T (1987) Isolation of a biologically active low-molecular-mass chromium compound from rabbit liver. J Biochim Biophys 165: 627-631.

9. Evans GW, Bowman TD (1992) Chromium picolinate increases membrane fluidity and rate of insulin internalization. J Inorg Biochem 46: 243-250.

10. Davis CM, Sumrah KH, Vincent JB (1996) A biologically active form of chromium may activate a membrane phosphotyrosine phosphatase (FTP). Biochemistry 35: 12963-12969.

11. Davis CM, Royer AG, Vincent JB (1997) Synthetic multinuclear chromium assembly activates insulin receptor kinase: functional model for low-molecular-weight chromium-binding substance. Inorg Chem. 36: 5316-5320.

12. Kury T, Debiski B, Bertrand J, Klos A, Gralak M (2005)Wpływ in vitro jonówchromu Cr⁺³ orazselenu Se⁺⁴ i selenu Se⁻² na procesyenergetyczne u szczurów. Zywieniowictwo i metabolizm, XXIII: supl. Nr 1, cz. 1: 301-6.

13. Ventura VF, Iltist L, Ruitter J, Ofman R, Costa CG, et al. (1998) Carnitinepalmitoyltransferase II specificity towards beta-oxidation intermediates—evidence for a reverse carnitine cycle in mitochondria. Eur J Biochem 253: 614-618.

14. Vincent JB (2000) The biochemistry of chromium. J Nutr 130: 715-718.

15. Karlic H, Lohninger S, Koeck T, Lohninger A (2002) Dietary l-carnitine stimulates carnitineacetyltransferases in the liver of aged rats. J Histochem Cytochem 50: 205-212.

16. Gao S, Kinzig KP, Aja S, Scott KA, Keung W, et al. (2007) Leptin activates hypothalamic acetyl-CoA carboxylase to inhibit food intake. Proc Natl Acad Sci U S A 104: 17358-17363.

17. Wolfgang MJ, Cha SH, Sidhaye A, Chohnan S, Cline G, et al. (2007) Regulation of hypothalamic malonyl-CoA by central glucose and leptin. Proc Natl Acad Sci U S A 104: 19285-19290.

18. Aja S, Landreee LE, Klieman AM, Medghalchi SM, Vadlamudi A, et al. (2008) Pharmacological stimulation of brain carnitine palmitoyltransferase-1 decreases food intake and body weight. Am J Physiol Regul Integr Comp Physiol 294: R352-R361.

19. Kanekura T, Yotsumoto S, Maeno N, Kamenosono A, Sarawutti H, et al. (2005) Selenium deficiency: report of a case. Clin Exp Dermatol 30: 348-348.

20. Pfeifer H, Conrad M, Rotstein D, Kyriakopoulos A, Brielmeier M, et al. (2001) Identification of specific speech nuclei seelenoencezyme necessary for protamine thiol cross-linking during sperm maturation. FASEB J 15: 1236-1238.

21. Kim YY, Mahan DC (2003) Biological aspects of selenium in farm animals. Asian-Austr J AnimSci 16: 435-444.

22. Malbe M, Attila M, Atroschi F (2006) Possible involvement of selenium in Staphylococcus aureus inhibition in cow's whey. J Anim Physiol Anim Nutr (Berl) 90: 159-164.

23. Li W, Beck MA (2007) Selenium deficiency induced an altered immune response and increased survival following influenza A/Puerto Rico/8/34 infection. Exp Biol Med (Maywood) 232: 412-419.

24. Johnson IT (2004) Micronutrients and cancer. Proc Nutr Soc 63: 587-595.

25. Richardson DR (2005) More roles for selenoprotein P: local selenium storage and recycling protein in the brain. Biochem J 384: e5-7.

26. Dhinigra S, Bansal MP (2006) Hypercholesterolemia and tissue-specific differential mRNA expression of type-1 S-iodothyronine deiodinase under different selenium status in rats. Biol Res 39: 307-319.