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Effect of dietary CLA isomers on selenium, zinc, copper, chromium, magnesium and calcium levels in rat liver*

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ABSTRACT

The effects of diets enriched with 1-2% conjugated linoleic acid isomers (CLA) and/or 2 ppm selenium (as Na_2SeO_4) on the level of Se, Zn, Cu, Cr, Mg and Ca in the rat liver were studied. Feeding selenium (Se) and/or CLA isomers resulted in significant changes or trends changing the level of Zn, Cu, Cr, Mg and Ca in the liver. The dosage of Se, regardless of the presence of CLA isomers, significantly increased the Se level in the liver. *Trans10cis12*CLA in the diet most efficiently elevated Zn, Cu, Mg and Ca levels, and, numerically, the Cr content in the liver.

KEY WORDS: Se, Zn, Cu, Cr, Mg, Ca, CLA, interactions, liver, rat

INTRODUCTION

Numerous beneficial effects on health have been attributed to conjugated linoleic acid (CLA) isomer in view of the anticarcinogenic, antiatherosclerotic, antidiabetogenic and antiadipogenic properties ascribed to them (Belury, 2002; Lavillonniere et al., 2003). The predominant isomer in food is the *cis9trans11*CLA (*c9t11* CLA), followed by *t7c9* CLA, *11,13*CLA (*ct/tc*), *8,10*CLA (*ct/tc*) and *t10c12* CLA isomer. On the other hand, selenium (Se), an essential trace element for mammals, has been found to be an integral part of the active site of cytosolic

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and mitochondrial glutathione peroxidases (cGPx), as well as phospholipid hydroperoxide glutathione peroxidase (PHGPx) (Czauderna et al., 2004). These enzymes reduce hydrogen peroxides, organic hydroperoxides, protecting tissues and polyunsaturated fatty acids (PUFA) in particular, from peroxidation damage. Moreover, it is well established that dietary selenate leads to increasing the CLA content in the rat liver. Therefore, the principal objective of this study was to examine the effect of feeding CLA on the level of Se, Zn, Cu, Cr, Mg and Ca in rat liver. The mechanism of the interaction between CLA and the assayed elements was considered. Rats were used as a model of monogastric animals.

MATERIAL AND METHODS

Ten groups of 7 female rats each (Wistar, Ifz: BOA), 8 weeks of age and an initial body mass ~200 g were housed individually as described previously (Czauderna et al., 2004). Rats were fed Labofeed *ad libitum* or diets enriched in CLA isomers and/or 2 ppm Se (as Na_2SeO_4) (Table 1). After 28 days the rats were killed by CO_2 and their livers removed, weighed and freeze-dried. The Se level in the livers was analysed by the fluorimetric method of Rodriguez et al. (1994), while Zn, Cu, Cr, Mg and Ca contents by CPI-MS (Czauderna et al., 2004). The effects of CLA isomers or Se treatments were subjected to statistical analysis by the nonparametric Mann-Whitney U test, while those of the combined Se and CLA treatments, by two-factorial analysis. The Statistica (version 6) and Excel 2000 programs were used.

RESULTS AND DISCUSSION

Feeding diets enriched in CLA isomers and/or Se resulted in changes of the assayed elements in the liver (Table 1). No lesion or symptoms of Se or CLA isomer intoxication were found. It was found that diets with only CLA isomers (Groups 3-6) resulted in significant ($P<0.01$ or <0.05) decrease in liver Se concentrations. In contrast, experimental diets enriched in Se, regardless of the presence of CLA isomers (Groups 2_{+se}, 7_{+Se}-10_{+Se}), significantly ($P<0.01$) stimulated accumulation of Se in the liver compared with control rats (Group 1) and the Cr level in the liver tended to increase. The presence of CLA isomers in the diets enriched in Se (Groups 7_{+Se}-10_{+Se}) significantly decreased or tended to reduce the content of Se in the liver compared with the liver of rats fed the diet with only Se (Group 2_{+Se}). Moreover, administration of *trans10cis12*CLA significantly ($P<0.01$ or <0.05) elevated the content of Zn, Cu, Mg and Ca in the liver, with an increasing trend in the Cr level. The results clearly demonstrate that feeding selenate, regardless of the presence of CLA isomers, only slightly promotes the accumulation of Se in the liver compared with dietary selenite.

Table 1. Concentration of Se, Zn, Cu, Cr, Mg and Ca in the liver[1] of rats fed experimental diets[2]

Group	Additive	Content of additive in the diet	Content of Se, Zn, Mg and Ca in rat livers, μg/g of DM[3]					
			Se	Zn	Cu	Cr	Mg	Ca
1	Control group	-	$4.48^{ABCDEFGHa}$	46^{A}	10^{A}	2.21	591^{a}	133^{a}
2_{+Se}	Se	2 ppm	5.77^{AbIJ}	35	9	2.52	515	102
3	CLA mixture	1 %	4.17^{a}	45	11	4.78	641	115
4	*Cis-9trans-11*CLA	1%	3.91^{B}	48	12	5.58	662	122
5	*trans*-10*cis-12* CLA	1%	4.07^{C}	72^{A}	16^{A}	4.60	904^{a}	183^{a}
6	CLA mixture	2%	3.89^{D}	52	12	4.85	697	137
3_{+Se}	Se	2 ppm	5.15^{EI}	48	11	3.08	664	148
	CLA mixture	1 %						
4_{+Se}	Se	2 ppm	5.50^{F}	70	16	2.40	789	221
	Cis-9trans-11 CLA	1 %						
5_{+Se}	Se	2 ppm	5.23^{Gb}	34	8	3.29	464	78
	*trans-10cis-12*CLA	1 %						
6_{+Se}	Se	2 ppm	5.12^{HJ}	35	8	3,81	470	116
	CLA mixture	2 %						

[1] for 4 weeks rats were fed diets enriched in the CLA and/or Se (as Na_2SeO_4)

[2] composition of the mixture of CLA isomers, %: *t*11*t*13 -2.9, *t*10*t*12 -.1, *t*9*t*11 -4.3, *t*8*t*10 -2.9, *c*11*t*13 -13.4, *c*10*t*12 -28.0, *c*9*t*11 -28.6, *c*8*t*10 -9.6, *c*11*c*13 - 1.6, *c*10*c*12 -1.5, *c*9*c*11 -1.4, *c*8*c*10 -0.7. The composition of the *cis*- 9*trans*-11 CLA and *trans*-10*cis*-12 CLA -95 of *c*9*t*11 and *t*10*c*12 isomer, respectively (Czauderna et al., 2004)

[3] means in columns with the same letter are significantly different at ^{A,B} $P<0.01$ or ^{a,b} $P<0.05$

This finding may be explained by the different metabolic pathways of selenate and selenite. In contrast to selenite, which is immediately taken up by and reduced in red blood cells and then transferred to the liver, approximately 20% of selenate given to rats is excreted into the urine without any changes in its chemical form; the major portion of selenate is taken up by the liver, reduced and then utilized for the synthesis of selenoproteins or excreted by urine after being methylated (Shiobara et al., 1999). Recent results reinforce the finding that CLA isomers, especially *trans10cis12*CLA, promote oxidation in the liver. *Trans10 cis12*CLA most efficiently enhanced oxygen consumption and energy expenditure in rats. In addition, *cis9trans10*CLA in cultured cancer cells also led to increased lipid peroxidation metabolites in examined cells, causing their apoptosis (Lavillonniere et al., 2003). So, CLA induced peroxidation by cellular antioxidant defense enzymes (e.g., cGPx, SOD or catalase). Indeed, conjugated trienoic fatty acids derived from the added CLA isomers (i.e. their metabolites) increased the formation hydroperoxides. Considering the above evidence and our presented work, we can suggest that CLA isomers (especially *trans10 cis12* CLA), as an oxidative agent, stimulated biosynthesis of metallothionein (MT) (Czauderna and Rochalska, 1989; Moriarty-Craige and Jones, 2004), so the content of Zn, Cr and Cu markedly increased in the liver. Indeed, the increase of the content of MT, possessing free sulphydryl groups, resulted in an increased accumulation yield of sulphur-philic

metal ions (e.g., Zn, Cu, Cr, Hg or Ag) in the liver. MT, a cysteine-rich protein, played the principal role in reducing oxidative stress or liver toxins (such as heavy metal ions). On the other hand, it could be hypothesized that interactions between added selenate and CLA isomers (Groups 7_{+Se}-10_{+Se}), especially *trans10cis12*CLA (Group 9_{+Se}), reduced the availability of added Se for formation of selenoproteins (like cGPx or PHGPx) due to the pro-oxidative activity of administered CLA isomers. Therefore, the Se concentration in the liver of rats fed diets enriched in Se and CLA isomers was lower (Groups 7_{+Se}-10_{+Se}) compared with rats fed diets enriched only with selenate (Group 2_{+Se}).

CONCLUSIONS

Adding selenate and/or CLA to the diet for 4 weeks resulted in small changes of the concentration of the assayed elements. Unexpectedly, the interaction between CLA isomers and selenate in the liver of monogastric animals may be considered antagonistic.

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STRESZCZENIE

Wpływ izomerów CLA w diecie na poziom selenu, cynku, miedzi, chromu, magnezu i wapnia w wątrobie szczurów

Badano wpływ diety zawierającej selenian sodowy (Se) i/lub izomery sprzężonego kwasu linolowego (CLA) na poziom Se, Zn, Cu, Cr, Mg i Ca w wątrobie szczurów. Dodatek Se i/lub CLA statystycznie stymulował gromadzenie się Se w wątrobie, dodatek *trans10 cis12* CLA zwiększył stężenie Zn, Cu, Mg i Ca w wątrobie, natomiast liczbowo poziom Cr.