CHANGES OF SOME BIOCHEMICAL PARAMETERS IN RATS SUPPLEMENTED WITH HIGH DOSES OF ZINC LACTATE*

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The experiment was conducted on 18 Wistar rats during a six-week period; 12 animals were given zinc lactate (120 mg/rat and week) in feed mixture and 6 control animals were fed a standard mixture for rats (ST-1). Sixteen biochemical parameters were measured from blood (serum) samples: total protein (TP), albumin (ALB), urea (UREA), glucose (GLU), triacylglycerols (TAG), non-esterified fatty acids (NEFA), cholesterol (CHOL), creatinine (CREAT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), uric acid (UA), magnesium (Mg), calcium (Ca), phosphorus (P), and trace elements such as Fe and Zn. When compared to the control group, we found that rats fed zinc lactate had higher concentrations of GLU, UA, UREA, Fe, Mg, Ca, TAG, TP, ALB, and ALP in the blood serum. Contrarily, the concentrations of AST, NEFA, CHOL, CREAT, P, and Zn were higher in the blood serum of control rats. Statistically significant differences between rats fed Zn and the control were found only in the concentrations of GLU, AST, ALP, UA, and P.

rat, blood, serum, toxicity, glucose

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INTRODUCTION

Zinc (Zn) is an essential element of exceptional biological and public health importance (Hambidge, Krebs, 2007). Zinc plays an important role in a wide variety of metabolic processes in animal systems. However, it is toxic when taken up in excess. Supplementary Zn in the diet increases the probability of zinc toxicity, especially the chronic type (Mizari et al., 2012). There is also strong evidence that depression is associated with low Zn levels in the blood serum (Tao et al., 2013). To prevent the occurrence of Zn deficiency, Zn fortification and supplementation is widely implemented. However, the potential risks of Zn overdosing are largely underestimated (Yang et al., 2013).

Zinc oxide, one of the most common supplements in the United States, and zinc carbonate are nearly insoluble and poorly absorbed in the body. Allen (1998) described low plasma Zn concentrations after consumption of zinc oxide and zinc carbonate if compared to those reached after zinc acetate and sulphate salts supplementation (Allen, 1998).

However, harmful excess supplementation is a problem among the relatively affluent, and dosage should probably not exceed 20 mg/day in healthy people, although the U.S. National Research Council set a tolerable upper intake of 40 mg/day (Maret, Sandstead, 2006).

Approximately 225–450 mg Zn is known to produce immediate vomiting in adults (Fosmire, 1990). Chronic overdosage of Zn, in the range of 100–300 mg Zn/day for adults, may induce copper deficiency (Prasad et al., 1978) and alterations in the immune response and serum lipoprotein levels. Some of these disturbances may also occur at lower doses (50 mg Zn/day) (Plum et al., 2010). 60 mg of supplementary Zn per day resulted in adverse interactions with other nutrients (WHO, 1996). Individuals may be exposed to high Zn intakes, either through supplementation or by contact with environmental zinc. Overt toxicity symptoms, such as nausea, vomiting, epigastric pain,

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diarrhea, lethargy, and fatigue, may occur with acute, high zinc intakes (F o s m i r e , 1990).

Compared to several other metal ions with similar chemical properties, zinc is relatively harmless. Just the exposure to high doses has toxic effects, making acute zinc intoxication a rare event. In addition to acute intoxication, long-term, high-dose Zn supplementation interferes with the uptake of copper.

Zinc homeostasis seems to be an important factor allowing efficient handling of the excess orally ingested zinc (P l u m et al., 2010).

The objective of the present research was to determine possible homeostasis changes of certain biochemical parameters that occur after administering high doses of zinc lactate, which is commonly used by humans as a dietary supplement, using an experimental animal model (laboratory rats *Rattus norvegicus* var. *alba*), and to point to the possible impact of Zn nutritional supplement overdose on the recipient (mammal).

Our hypothesis was that zinc lactate overdosing causes a glucose disbalance in the blood serum, as well as a disbalance in certain biochemical parameters.

MATERIAL AND METHODS

Experimental animals

The experiment was conducted during a six-week period on 18 Wistar rats (initial body weight of animals 150 g). During the experiment each animal was placed in a metabolic cage (1 animal per cage) in an air-conditioned room with constant temperature (22–24°C), humidity level (approximately 70%), and day/night cycle (08:00–20:00 h). The animals had free access to water.

Experimental design

Rats in the control group (Group C, 6 animals) were fed a commercially manufactured feed ST-1 (Table 1), while rats in the experimental group (Group P, 12 animals) were fed a commercial feed mixture ST-1 with zinc lactate (25 g of feed contained 20 mg Zn; each individual was given 150 g feed (6 × 25 g) per week, i.e. 120 mg Zn per week, i.e. 720 mg Zn per 6 weeks).

Biochemical analysis

The blood was collected from *cavum abdominis*. The blood samples were coagulated at laboratory temperature and then centrifuged at 1000 *g* for 15 min. Separated serum was deep-frozen (–80°C) until the analysis. Sixteen biochemical parameters were measured from blood (serum) samples: total protein (TP), albumin (ALB), urea (UREA), glucose (GLU), triacylglycerols (TAG), non-esterified fatty acids (NEFA), cholesterol (CHOL), creatinine (CREAT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), uric acid (UA), minerals such as magnesium (Mg), calcium (Ca), phosphorus (P), along with the concentrations of trace elements (Fe, Zn). Zinc was determined by a manual method using a spectrophotometer (Libra S6; Biochrom, Cambourne, UK). The commercial kit Randox Zinc (Randox Laboratories Ltd., Crumlin, UK) was used for colorimetric method performance. Other blood parameters were determined spectrophotometrically using an automatic analyzer ERBA XL 200 (Erba Diagnostics Mannheim GmbH, Mannheim, Germany) in the laboratory of the Department of Veterinary Sciences, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague. The analysis was performed with commercial kits (namely from Randox NEFA (Randox Laboratories Ltd.) and others kits from Erba Diagnostics Mannheim GmbH.

Table 1. Composition of rat diet (ST-1)

Ingredients	Value
Crude protein	25.5 (%)
Ash	6.26 (%)
Dry matter	86.5 (%)
Crude fibre	4.3 (%)
Nitrogen free extract	47.7 (%)
Fats	3.76 (g/100 g)
Ca	12 700 (mg/kg)
K	10 500 (mg/kg)
Mg	2 210 (mg/kg)
Na	1 780 (mg/kg)
P	8 110 (mg/kg)
Zn	70 (mg/kg)

Statistical analysis

Normality of data was tested separately using a Shapiro-Wilk test, and to evaluate the proposed hypothesis we used non-parametric Mann-Whitney U tests. A *P*-value of 0.05 was set for our hypothesis about the significant statistical influence of zinc in rat food. STATISTICA 10 (Statsoft, USA) was used for all computations and statistical analysis.

RESULTS

There were no abnormal signs of toxicity or death recorded in Group P after the six weeks of treatment at the dose of 20 mg zinc lactate per rat and day. Gradual body weight increase was comparable with that of control rats, the mean weight gain in Group C was

Table 2. Mean values ± SD of biochemical parameters in blood serum of rats

	Mg (mmol/l)	Ca (mmol/l)	P (mmol/l)*	CHOL (mmol/l)	TAG (mmol/l)*	UREA (mmol/l)	GLU (mmol/l)*	NEFA (mmol/l)
Group P	0.8 ± ٠,١٩	2.5 ± 0.14	2.8 ± 0.48	1.0 ± 0.14	0.8 ± 0.28	6.3 ± 0.66	14.7 ± 2.17	0.6 ± 0.09
Group C	0.7 ± 0.17	2.2 ± 0.33	5.7 ± 2.28	1.5 ± 0.43	0.3 ± 0.12	6.8 ± 0.81	8.3 ± 1.66	0.7 ± 0.32
	ALP (μkat/l)*	AST (μkat/l)*	TP (g/l)	ALB (g/l)	UA (μmol/l)	CREAT (μmol/l)	Fe (μmol/l)	Zn (μmol/l)
Group P	2.7 ± 0.79	1.7 ± 0.24	51.4 ± 2.7	29.3 ± 0.97	144.0 ± 57.15	41.9 ± 4.16	61.1 ± 7.18	23.4 ± 3.45
Group C	1.6 ± 0.42	3.6 ± 1.78	50.3 ± 6.25	28.1 ± 3.44	113.6 ± 85.13	43.5 ± 11.89	54.8 ± 6.79	26.9 ± 6.48

Group P = group treated with zinc lactate, Group C = control group, CHOL = cholesterol, TAG = triacylglycerols, GLU = glucose, NEFA = nonesterified fatty acids, ALP = alkaline phosphatase, AST = aspartate aminotransferase, TP = total protein, ALB = albumin, UA = uric acid, CREAT = creatinine

*statistically significant difference

82 g (mean initial weight 244.5 g and mean final weight 326.5 g); Group P had mean weight gain 61.2 g (mean initial weight 281.4 g and mean final weight 342.6 g). When compared to the control group, we found that rats in Group P showed a higher concentration of GLU, UA, UREA, Fe, Mg, Ca, TAG, TP, ALB, and ALP in blood serum. Contrarily, AST, NEFA, CHOL, CREAT, P, and Zn concentrations were lower in the blood serum of treated rats (Figs. 1–4). Statistically significant differences between groups were in GLU, AST, ALP, UA, and P concentrations in blood serum (Table 2).

DISCUSSION

As shown in Fig. 1, the serum concentrations of Mg, Ca, CHOL, UREA, and NEFA were not significantly affected by treatments with zinc lactate. However, the serum concentrations of GLU, P, and TAG were significantly ($P \leq 0.05$) affected by the addition of zinc lactate to the feed mixture. GLU and TAG concentrations were significantly higher in the blood serum of Group P with Zn supplementation (interestingly, I c i e k et al. (2009) and D v o ř á k o v á et al. (2015) mentioned low levels of CHOL and TAG after garlic consumption). P concentrations were significantly lower in the blood serum of Group P. Rats in Group C had P concentrations exceeding the upper reference limit of 1.0–3.6 mmol/l (J o h n s o n - D e l a n e y , 1996). Since the rats were only 2 months old and still growing, higher levels of P can be attributed to increased intestinal absorption and decreased renal excretion to facilitate bone mineralization (G r u e n b e r g , 2011).

In our study, glucose concentrations were surprisingly significantly higher in Group P with zinc lactate supplementation, although in some studies (e.g.U y a n i k et al., 2001; B a l t a c i et al. 2003; D e h s h a l et al., 2007; B o n a k d a r a n et al., 2009) an increased glucose concentration in serum resulted from Zn deficiency, as Zn is associated with insulin synthesis, storage, and secretion. Zinc lactate is commonly used as an easily digestible form of Zn supplement. But lactate as a gluconeogenetic source of glucose may increase serum glucose level (Fig. 1). The glucose level may be also affected by stress (stress hormones adrenaline and cortisol increase gluconeogenesis). D v o ř á k o v á et al. (2015) described a decrease in plasma glucose levels after garlic consumption, preventing the development of diabetes mellitus.

The high level of glucose in the serum of rats supplemented with zinc lactate (20 mg/day) may be associated with the lactate form of Zn. Lactate is a part for anaerobic glycolysis. It is the starting compound of

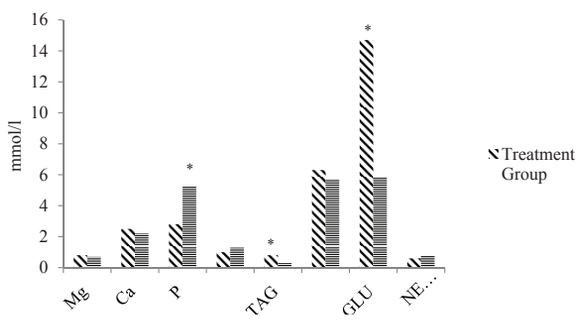

Fig. 1. Effect of oral treatment/intake of zinc lactate (20 mg/rat/day) on biochemical parameters (mmol/l) in rats

CHOL = cholesterol, TAG = triacylglycerol, GLU = glucose, NEFA = nonesterified fatty acids

*statistically significant difference

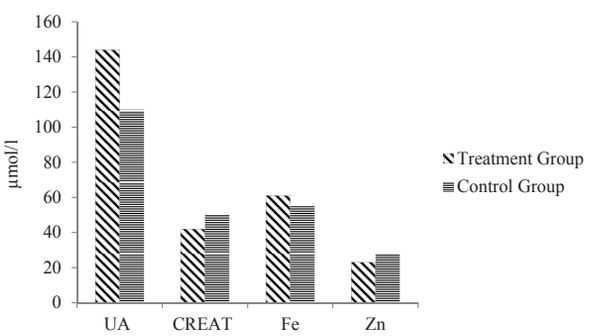

Fig. 2. Effect of oral treatment/intake of zinc lactate (20 mg/rat and day) on biochemical parameters (μmol/l) in rats

UA = uric acid, CREAT = creatinine

gluconeogenesis. Increased amount of zinc lactate in feed mixture promotes the formation of glucose in the liver (Cori cycle). Cori cycle refers to the metabolic pathway in which lactate produced by anaerobic glycolysis in the muscles moves to the liver and is converted to glucose, which then returns to the muscles and is metabolized back to lactate (Nelson, Cox, 2005).

Disorder in mineral status may impact lipid and glucose metabolism, and also mineral dependent enzyme activity, such as that of superoxide dismutase and catalase in the body (Sulibursk a et al., 2014).

Mizari et al. (2012) described significant changes in Na^+, Ca^{2+}, and K^+ concentrations both in saliva and in plasma of rats with oral Zn intoxication. The serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, glucose levels in the plasma, and urine creatinine levels were also altered in experimental groups in comparison with the control group. Furthermore, results showed that zinc toxicity affects the liver and renal function (Mizari et al., 2012).

The primary importance of measuring alkaline phosphatase (ALP) is to check the possibility of bone or liver disease. Since the mucosal cells that line the bile system of the liver are the source of ALP, the free flow of bile through the liver and down into the biliary tract and gallbladder is responsible for maintaining the proper level of this enzyme in the blood. When the liver, bile ducts or gallbladder systems are not functioning properly or are blocked, this enzyme is not excreted through the bile and ALP is released into the blood stream. Higher ALP concentrations in rats from the treatment group can be related to Zn excess. Thus, the serum ALP is a measure of the integrity of the hepatobiliary system and the flow of bile into the small intestine. Increased ALP is typical for bile ducts disease. The causes of decreased serum ALP may be zinc deficiency, hypothyroidism, vitamin C deficiency/ Scurvy, folic acid deficiency, excess vitamin D intake, low P levels (hypophosphatasia), celiac disease, malnutrition with low protein assimilation (including low stomach acid production/ hypochlorhydria), insufficient parathyroid gland function, pernicious anemia, or vitamin B_6 insufficiency (Kaslow, 2014). ALP activity is inhibited by a low Zn concentration. In our study, the concentration of ALP in blood serum was significantly higher in Group P. It can be the result of higher Zn concentration in rat body.

Aspartate aminotransferase (AST) catalyses the reversible transfer of α-amino group between aspartate and glutamate and, as such, it is an important enzyme in the amino acid metabolism. AST is found in the liver, heart, skeletal muscle, kidneys, brain, and red blood cells, and it is commonly measured clinically as a marker for liver health. The blood test for AST is usually used to detect liver damage (Zhang et al., 2004). In our study, the concentration of AST in rat serum significantly differed between the treatment Group P and the control Group C (Fig. 3). However, AST concentration was higher in Group C compared to Group P rats (Fig. 3). Awe, Banjoko (2013) reported that the activity of AST showed insignificant change in rats supplemented with various doses of *Petrosellinium crispum* ethanol extracts (10, 100, and 1000 mg/kg body weight). However, the activity of plasma ALP increased (78 and 88 IU/l) at doses of 100 and 1000 mg/kg body weight.

In our study, a higher uric acid (UA) concentration in Group P was observed (Fig. 2); the increase of serum UA values is a consequence of renal injury after a high intake of metal-containing substances (Garban et al., 2013). There was no statistically significant difference in blood serum Zn concentrations between Groups P and C (Table 2). Zn concentration was surprisingly a little higher (21.7–40.5 µmol/l) in Group C (rats without zinc lactate supplementation) in comparison to Group P supplemented with zinc lactate (17.8–29.0 µmol/l) (Fig. 2). Zn supplementation did not increase the serum Zn concentration, indicating that Zn levels were not reflected in blood Zn concentrations. However, persistent intake of high doses of Zn can lead to copper deficiency (Rink, Gabriel, 2000).

Protein in the plasma is made up of albumin and globulin. Albumin is mainly produced in the liver. It helps keep the blood from leaking out of blood vessels. Albumin also helps carry some medicines and other substances through blood and is important for tissue growth and healing. In blood plasma, zinc is bound to and transported by albumin (60%) and transferrin (10%) (Rink, Gabriel, 2000). Since transferrin also transports iron, excessive Fe reduces Zn absorption, and *vice versa*. A similar reaction occurs with copper. The concentration of Zn in blood plasma stays relatively constant regardless of the Zn intake (Whitney, Rolfes, 2013)). Zinc lactate in the feed mixture did not significantly influence total protein and albumin concentrations in rat blood serum.

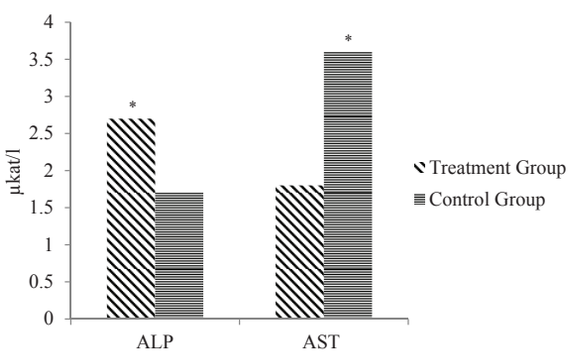

Fig. 3. Effect of oral treatment/intake of zinc lactate (20 mg/rat and day) on biochemical parameters (µkat/l) in rats
ALP = alcalic phosphatase, AST = aspartate aminotransferase
*statistically significant difference

The results of Yang et al. (2015) indicate that Zn plays an important role in hippocampus-dependent learning, memory, and brain-derived neurotrophic factor (BDNF) expression. A high dose supplementation with Zn induces specific Zn deficiency in the hippocampus; this deficiency further impairs learning and memory due to a BDNF deficit and a decreased availability of synaptic Zn. Elevated temperature, infection, stress, or pregnancy decreased the Zn concentration in plasma, while it was increased by fasting and catabolism (decomposition) (King et al., 2000). Interestingly, zinc lactate overdosing did not increase Zn concentrations in blood serum in our study.

Negative effects of some elements, however, arise not only from anthropogenic pollution (Berchová-Bímová et al., 2014; Weingartová et al., 2015), but also from excessive use of nutritional supplements which was confirmed also by the present results.

CONCLUSION

Blood is the main tissue that transports the metabolites and xenobiotics in the organism. The interactions of various metal compounds with biological systems are very important. Zinc lactate is well absorbed by humans and animals, and that is why it is commonly used as a dietary supplement. The present research revealed statistically significant differences between concentrations of GLU, AST, ALP, TAG, and P in the group given a zinc lactate overdose and the control group. Zinc lactate overdosing significantly increased GLU, ALP, and TAG levels, while significantly decreased AST and P. We have come to the conclusion that increased levels of GLU, ALP, TAG and decreased levels of AST and P could indicate a zinc lactate overdose. These findings should be taken into consideration when diagnosing suspected Zn overdose.

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