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Effect of Converting Enzyme Inhibitor on Copper and Iron Concentrations of Blood Plasma in Calves During the Neonatal Period

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The converting enzyme catalyzes the conversion of angiotensin I to angiotensin II. Ang II is the key component of the renin-angiotensin-aldosterone (RAA) system, regulating water-electrolyte balance in newborn calves. Captopril is an inhibitor of the angiotensin-converting enzyme. The aim of this study was to examine the effect of captopril-induced reduction of convertase activity on copper and iron concentrations of blood plasma in calves. The experiment was carried out on 10 Holstein-Friesian female calves, during the first week of life. Copper and iron concentrations in blood plasma were examined before and after captopril administration (0.5, 1, 2, 4, and 6 hours after giving the inhibitor) on subsequent days of the experimental period. The results demonstrated that the copper concentration of blood plasma increased with age. On the seventh day, the copper concentration stabilised at the level observed in adult cattle. Measured before captopril administration, the iron concentration in blood plasma changed: the highest iron concentration was observed on the first day of life, which was followed by a decrease on the third day, and thereafter an increase on the seventh day. These changes may significantly influence the neonatal adaptation of newborn calves, particularly hemopoiesis efficiency. Captopril did not cause statistically significant changes in plasma copper concentration in calves. However, the reduction of angiotensin convertase activity induced by captopril administration resulted in a drop of plasma iron concentration, observed already within 1-2 hours after administration of the inhibitor, and especially within two days post partum. The results indicate that an efficient mechanism maintaining a constant concentration of selected minerals may involve changes in the reabsorption of these minerals from the system fluids to tissues.

Key words: Calves, neonatal period, blood plasma, captopril, copper, iron.

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Copper and iron are important trace elements regulating the cellular homeostasis of the organism (BIAŁOŃSKA *et al.* 2002). Copper is a coenzyme of, for example, caeruloplasmin, cytochrome oxidase, lysine and ascorbate oxidase, superoxide dismutase, tyrosinase, and dopamine beta-monooxygenase. Copper deficiency reduces the activity of these enzymes which can lead to metabolic disturbances and to cell, tissue and organ damage. The element is crucial for the normal functioning of the ceardiovascular, nervous and immunological systems (CERONE *et al.* 1998; CERONE *et al.* 1995; KLEVAY 2000; MONDAL *et al.* 2007; PROHASKA 2000; SHARMA *et al.* 2005).

Iron is also indispensable for the normal activity of the nervous, vascular and immunological system (AGARWAL *et al.* 2001; BEARD *et al.* 2003; EKIZ *et al.* 2005; HALLQUIST *et al.* 1992; ORTIZ *et al.* 2004). This element is a component of many proteins, such as haemoproteins (haemoglobin, myoglobin) or enzymes (e.g. catalase, peroxidase, respiratory chain cytochromes, cytochrome P450). Iron deficiencies first induce a reduction of iron tissue reserves, followed by a drop in the blood plasma concentrations of the element. Heavy deficiencies of iron often lead to anemia (JOHNSON 1990).

Captopril is an inhibitor of the angiotensin-converting enzyme. The convertase catalyzes the conversion of angiotensin I to angiotensin II. Ang II is the key component of the renin-angiotensin-aldosterone (RAA) system. Angiotensin II causes contraction of smooth muscles in blood vessels. Moreover, through aldosterone, it influences the regulation of renal electrolyte excretion (FUCHS *et al*. 2004). OŻGO (2001) has demonstrated that the efficiency of RAA in terms of water-electrolyte balance regulation is low in calves during their first week of life. This can be concluded from the decreasing molality of blood plasma despite a high plasma renin activity and an increasing aldosterone concentration.

In another study, the same author demonstrated that the converting enzyme played an important role in the regulation of haemodynamics and renal function of newborn calves by controlling blood plasma aldosterone and angiotensin II concentrations (OŻGO 2009). The convertase can also participate in the regulation of secretion of atrial natriuretic peptides during the first days of postnatal life. In hypertension-affected rats, captopril reduces both the blood plasma angiotensin II concentration and the diastolic and systolic pressure of blood (BOLTERMAN *et al*. 2005; SCHLENKER *et al*. 2004).

KÖHLER-SAMOUILIDIS *et al*. (1997) demonstrated that administration of captopril to animals dislocates microelements to various tissues and/or boosts their removal from the organism. In 7-month-old rabbit males, a 9-week application of 6.5 mg captopril per 1 kg b.w. resulted in a significant decrease of concentrations of zinc in blood, copper, calcium, and magnesium in the epididymides, as well as a significant increase in concentrations of copper and magnesium in the adrenal gland and copper in the sperm. KOTSAKI-KOVATSI *et al*. (1997) demonstrated that intraperitoneally administered captopril (2 mg per 1 kg b.w. for 9 weeks) to adult Guinea pigs resulted in a considerable decrease in the concentrations of zinc in the liver, copper in the liver, adrenal gland, jejunum, urine, and hair, magnesium in the blood and urine. In the same report a significant increase was found in the concentrations of zinc in testes and epididymides, copper in the heart, epididymides, and faeces, magnesium in the lungs, kidneys, suprarenal gland, jejunum, epididymides, and hair, as well as calcium in the brain, heart, lungs, kidneys, spleen, and stomach. OŻGO *et al*. (2004) observed that captopril applied to calves led to increased potassium and magnesium concentrations in blood plasma and a reduced level of these elements in erythrocytes. In humans affected by hypertension, a two-week captopril treatment reduces the concentration of transferrin, hemoglobin, hematocrit index, and iron in the blood plasma (AVERBUKH *et al*. 2004).

The importance of convertase for water-electrolyte balance in newborn calves instigated this study on the effect of captopril-induced reduction of convertase activity copper and iron concentrations of blood plasma in calves on subsequent days during the first week of life.

Material and Methods

The experiment was carried out on 10 Holstein-Friesian female calves during the first seven postnatal days. The calves lacked any symptoms of disease. For the duration of the experiment, the animals remained under laboratory conditions (in individual pens), in the vivarium of the Department of Animal Physiology and Cytobiology, under unified environmental conditions. They were fed on colostrum and milk of their dams, three times per day. Blood for the analyses was drawn from the external jugular vein previously catheterised. Blood samples were preserved on heparin ("Heparin" – Biochemie, Austria) to prevent clotting.

Blood was collected every day starting from two hours after the morning feeding (a "zero" test before captopril administration). After this, the calves were *per os* administered 0.3 mg per 1 kg b.w. of captopril ("Captopril", Jelfa). Subsequent blood samples were collected 0.5, 1, 2, 4, and 6 hours after the application of the inhibitor. The samples were centrifuged immediately on collection (at 3000 rpm for 15 minutes, temperature 4°C).

Blood plasma was assayed for copper and iron concentrations (following standard procedures of sample preparation) using an atomic absorption spectrometer AAnalyst 400 (Perkin Elmer) with the flame atomic absorption method at wavelengths: 324.75 nm for copper and 248.33 nm for iron. We used lamps with hollow cathodes separately for copper and iron, as well as a deuterium lamp for background correction.

The results were processed statistically by means of ANOVA with repeated measurements (Statistica 6.0 package). Significance of differences between blood plasma iron and copper concentrations before and after captopril administration were tested at $P \leq 0.05$ and $P \leq 0.01$.

Results

The experiment demonstrated that blood plasma copper concentrations increase with the age of calves. On the first day of life, before captopril administration, the concentration of this micromineral was 6.75 μmol/l and significantly ($P \leq 0.01$) increased until the sixth day (15.65 μmol/l). On the seventh

Table 1

Blood plasma concentration of copper and iron in the first seven days of life and significant differences between values in the following days of life [μmol/l]

Element		Day of life							Significance of differences	
		1	2	3	4	5	6	7	0≤0.01	0≤0.05
Cu	$\overline{x}$	6.75	8.62	10.58	12.44	13.90	15.65	16.10	1→3,4,5,6,7	1→2
	SD	0.63	1.23	2.01	2.54	2.94	3.73	3.91	2→4,5,6,7	2→3
Fe	$\overline{x}$	23.32	19.29	8.02	9.93	7.93	10.50	13.24	3→5,6,7	3→4
	SD	8.43	12.07	4.22	8.97	5.12	8.51	8.27	4→6,7	5→6,7

day, copper concentration stabilised at the level observed in adult cattle, i.e. 16.10 μmol/l (Table 1).

The concentration of iron in the blood plasma of calves during the first week of life, before captopril administration, ranged between 7.93 and 23.32 μmol/l (Table 1). The highest iron concentration was observed on the first day of life, which was followed by a decrease to 8.02 μmol/l on the third day. Thereafter, plasma iron concentration increased to 13.24 μmol/l on day seven. The changes, however, were statistically non-significant ($P \leq 0.05$).

It should be emphasized that on the first two days of life, the copper to iron ratio was 1:3.5 (the first day) and 1:2.3 (the second day). From the third day, the ratio reversed and averaged 1:0.7.

Application of captopril did not result in statistically significant changes in plasma copper concentrations in the calves (Figs 1 to 7). Nevertheless, over the first five days of life, we observed a drop in copper levels within one hour following captopril administration. On each of the seven studied days, within six hours the copper concentration af-

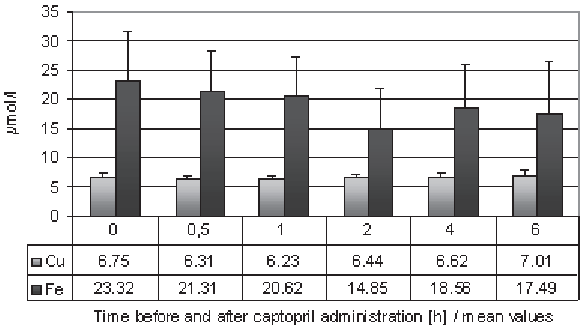


Fig. 1. Blood plasma concentration of copper and iron on the first day of life before and after captopril administration [µmol/l]. Significant differences at the level of $P \leq 0.01$ are marked (**) between values, before and after captopril administration.

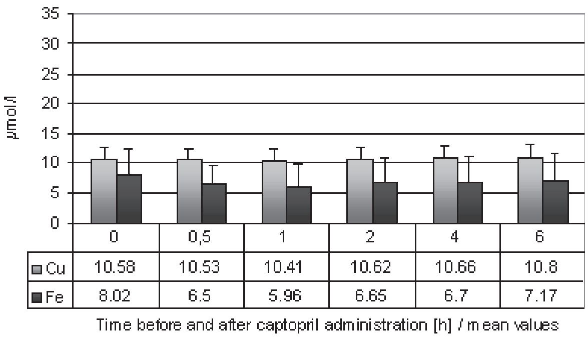


Fig. 3. Blood plasma concentration of copper and iron on the third day of life before and after captopril administration [μmol/l].

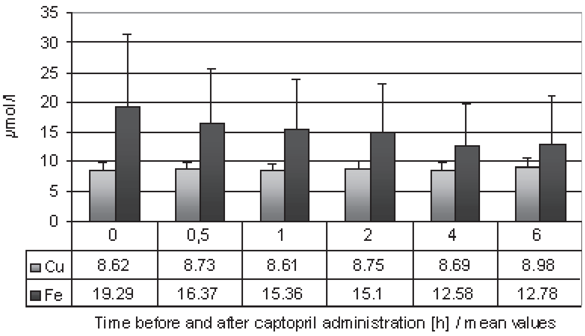


Fig. 2. Blood plasma concentration of copper and iron on the second day of life before and after captopril administration [μmol/l]. Significant differences at levels $P \leq 0.01$ (**) and $P \leq 0.05$ (*) are marked between values, before and after captopril administration.

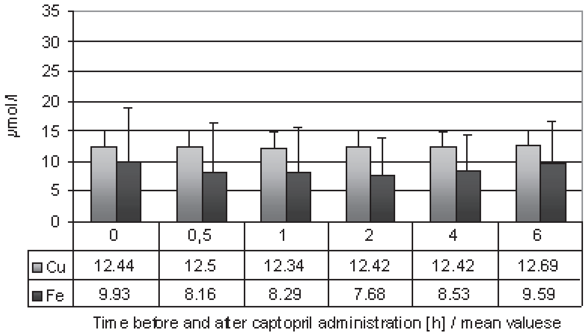
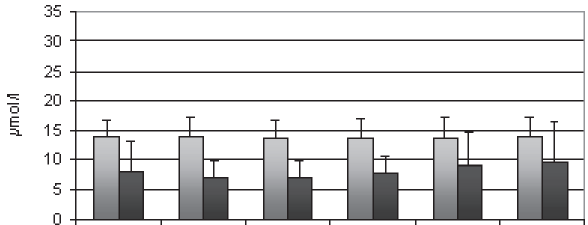


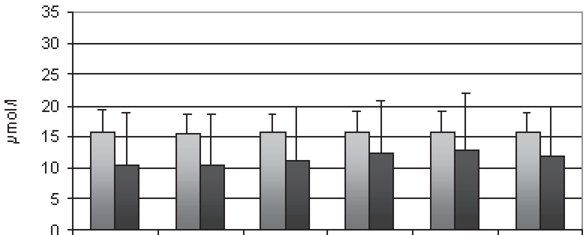
Fig. 4. Blood plasma concentration of copper and iron on the fourth day of life before and after captopril administration [μmol/l].



	0	0,5	1	2	4	6
Cu	13.9	13.93	13.53	13.74	13.81	13.85
Fe	7.93	7.08	7.11	7.78	9.08	9.66

Time before and after captopril administration [h] / mean values

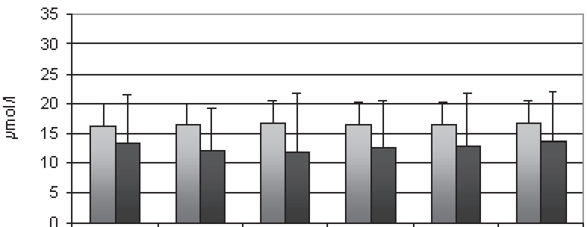
Fig. 5. Blood plasma concentration of copper and iron on the fifth day of life before and after captopril administration [μmol/l].



	0	0,5	1	2	4	6
Cu	15.65	15.44	15.73	15.86	15.76	15.93
Fe	10.5	10.52	11.19	12.32	12.82	11.99

Time before and after captopril administration [h] / mean values

Fig. 6. Blood plasma concentration of copper and iron on the sixth day of life before and after captopril administration [μmol/l].



	0	0,5	1	2	4	6
Cu	16.1	16.49	16.77	16.62	16.64	16.65
Fe	13.24	11.98	11.73	12.61	12.98	13.77

Time before and after captopril administration [h] / mean values

Fig. 7. Blood plasma concentration of copper and iron on the seventh day of life before and after captopril administration [μmol/l].

ter captopril administration was higher than before this treatment.

A reduction of angiotensin convertase activity induced by captopril administration resulted in a drop of plasma iron concentration within 1-2 hours after administration of the inhibitor (Figs 1 to 7). Over the first two days, these changes were significant. On the first day, the iron concentration dropped from 23.32 μmol/l (before captopril) to 14.85 μmol/l (within two hours), whereas on the second day it dropped from 19.29 μmol/l (before captopril) to 12.58 μmol/l (after four hours). It should be emphasized that over the first three days of life we detected a lower concentration of iron in blood plasma than the initial value until six hours after captopril administration. On the other hand, as observed on subsequent days of the experiment, following a slight decrease (after 1-2 hours), iron levels in blood increased within six hours to reach similar values to those measured before treatment.

Discussion

The increase in copper concentration in calf blood plasma with age in this experiment confirms the observations of BOSTEDT *et al.* (1990). These authors reported a low concentration of the electrolyte on the first day post partum (4.8±1.7 μmol/l), which increased over the subsequent days of the first week of life. In the weeks that followed, the authors did not record any changes in blood concentration of this electrolyte.

It should be emphasized that the escalation of copper levels in blood plasma was very dynamic; as soon as on the fourth day of life, the concentration was twice as high as that recorded on the day of birth. At the end of the earliest week of life, the copper concentration in plasma in the studied calves reached the level reported by LAVEN and LIVESEY (2006) in lactating heifers (15.9 μmol/l). According to KUME and TANABE (1993), the increase in copper levels in the plasma of newborn calves is a consequence of the release of copper by the liver, and does not come from colostrum or milk. These authors observed a low concentration of copper in the colostrum of cows, i.e. 0.12 ppm (on the day of birth), 0.09 ppm (12 hours post partum), and 0.08 ppm (24 and 74 hours post partum).

Our experiment demonstrates a decrease in plasma iron concentration in newborn calves. A similar pattern in iron level changes in calves was described by other authors (BOSTEDT *et al.* 1990; KUME & TANABE 1994; MILTENBURG *et al.* 1991). Reduction in the concentrations of iron during the neonatal period have been observed in other animals such as lambs (ANTUNOVIC *et al.* 2005), piglets (ILIC *et al.* 2006; RICKENER *et al.* 2004), and goat kids (SKRZYPCZAK *et al.* 2009). MILTENBURG *et al.* (1991) observed a decrease in iron concentration over the first three weeks of life in calves and concluded that it resulted from an increasing volume of blood plasma and a rapid rate of hemoglobin synthesis. An increasing plasma level in calves over the earliest week of life may also result from a large intake of fluids (KATUNGUKA-RWAKISHAYA *et al.* 1985) and/or changes in water distribution between the intra- and extracellular fluid space (SKRZYPCZAK 1991). KUME and TANABE (1994) state that a high concentration of iron in calf plasma on the day of birth (higher than in their dams) implies dynamic transport of this element through the placenta to the fetus.

The presented experiment did not detect an influence of captopril on changes in copper concentrations in calf plasma. OŻGO (2009) showed that captopril in a dose of 0.3 mg per 1 kg b.w. results in a significant reduction of converting enzyme activity and modifies haemodynamics as well as renal function in newborn calves. Such changes may be expected to be reflected in the concentration of minerals in blood plasma. GARROW *et al*. (1991) demonstrated that elevated blood pressure in rats was accompanied by a higher copper concentration. Studies by KOTSAKI-KOVATSI *et al*. (1997) on Guinea pigs and by KÖHLER-SAMOUILIDISA *et al.* (1997) on rabbits have shown that captopril may contribute to copper retention in various organs and tissues, or may lead to a higher rate of copper removal from the organism.

The results of the presented experiment indicate that changes in reabsorption of minerals from the system fluids to tissues may be an efficient mechanism of maintaining their constant concentrations in blood plasma.

Administration of angiotensin-convertase inhibitor results in a significant reduction of iron concentration in the plasma of calves, especially over the first 2-3 days after birth. AVERBUK *et al.* (2004), who studied humans, noticed that after two weeks of captopril treatment, the plasma iron concentration, transferrin and hemoglobin levels, as well as hematocrit, dropped considerably. The authors also state that captopril, due to the sulfhydryl group in its chemical structure, easily binds iron reducing it from Fe^{3+} to Fe^{2+}. In the blood, complexes of converting-enzyme inhibitor with Fe^{2+} are created, which according to SCHAEFER *et al.* (1998) do not hamper the effective activity of captopril. Binding captopril to iron raises its total concentration in blood and extends its half-life. Moreover, long-term administration of captopril leads to a decrease in hemoglobin level (AVERBUKH *et al*. 2004).

In conclusion, it should be emphasized that copper and iron plasma concentrations exhibit dynamic changes over subsequent days of the earliest week of postnatal life in calves. A significant increase in copper concentration with age, a decrease in iron concentration over the first three-four days post partum, and the variable copper-to-iron ratio should be noted. These changes may significantly influence the neonatal adaptation of newborn calves, particularly hemopoiesis efficiency. Reduced activity of angiotensin convertase due to captopril administration significantly reduces plasma iron concentration in calves, especially within two days post partum, whereas it exerts little influence on copper concentrations in plasma.

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