

# Superoxide dismutase activity in colostrum, transitional and mature human milk

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**SUMMARY:** Kasapović J, Pejić S, Mladenović M, Radlović N, Pajović SB. Superoxide dismutase activity in colostrum, transitional and mature human milk. *Turk J Pediatr* 2005; 47: 343-347.

Colostrum and mature human milk are rich sources of nutrients and contain biologically active molecules that are essential for specific antioxidant functions. The aim of the present study was to determine the activity of copper, zinc superoxide dismutase (CuZnSOD) and manganese superoxide dismutase (MnSOD) activity in different phases of lactation. Specific enzyme activity was determined in colostrum (3<sup>rd</sup>-5<sup>th</sup> days after delivery), and in mature milk in the third week (15-20 days), and the fourth and seventh months of lactation. In the third week of lactation, the activity of CuZnSOD and MnSOD was significantly higher in comparison to the colostrum phase. In the fourth month of lactation, the activity of both enzymes was suppressed, while in the seventh month of lactation the MnSOD activity was increased, and the CuZnSOD activity was not significantly changed. These findings show that the activities of superoxide dismutases significantly change during different phases of lactation.

*Key words:* superoxide dismutase, colostrum, human milk.

Human colostrum is one of the most potent natural immune and antioxidant booster known to nutrition research. It is a source of immune and antioxidant components and nutrients, and contains more proteins, immunoglobulins, non-protein nitrogen, fat, vitamins, and minerals than milk<sup>1</sup>. Because some components do not cross the placental barrier, colostrum is the primary source of these nutrients for the suckling after birth<sup>2</sup>. An earlier study indicated that colostrum is loaded with polymorphonuclear leukocytes and macrophages, T-lymphocytes, B-lymphocytes, plasma cells, and epithelial cells<sup>3</sup>. It shows marked microbial activity, which is based on the production of free radicals. The surplus of free radicals might represent a great danger for the biomolecules. Therefore, the scavenging activity of antioxidant enzymes such as cytosol copper, zinc superoxide dismutase (CuZnSOD) and mitochondrial manganese superoxide dismutase (MnSOD) significantly contributes to a balance between prooxidants and antioxidants.

Whenever the balance of antioxidants is outweighed by prooxidizing factors, oxidative stress may develop in cells or tissues<sup>4</sup>. There are many factors that increase reactive oxygen species (ROS) concentration in humans. They originate from endogenous sources and are also acquired from the environment. Therefore, we have to focus on the antioxidant properties of nutrition that could be the first condition for a good quality of life.

Concentrations and activity of antioxidants in human milk determine the degree of protection from peroxidation<sup>5</sup>. When explaining the protective effect of any factor, including the antioxidative factor, we must take into consideration that protection in the organism, being the result of evolutive advantage, is provided by synergistic action of different components. This is confirmed by the fact that human milk contains different low- and high-molecular components, which express their antioxidative effect either by connecting to ions

of transition metals and thus preventing their prooxidative action or by removing free radicals by non-enzyme or enzyme activity.

Human milk contains a number of enzymes that are either transported by blood to the mammary gland, found in cells that are present in milk, or synthesized by the mammary gland. Among them are also enzymes with antioxidative activity, superoxide dismutase, as well as glutathione peroxidase<sup>6,7</sup>. Kiyosawa et al.<sup>7</sup> published the results which confirm that cytosol CuZnSOD and mitochondrial MnSOD are present in human milk, both in colostrum and in mature milk. Using the results given by Oyanagui<sup>8</sup> regarding the concentration of SOD in the plasma, they concluded that the concentration of total SOD in human milk is five times lower than in human plasma. Also, comparing their own results with the results obtained by Holbrook and Hicks<sup>9</sup>, they concluded that concentration of SOD in human milk is 2-2.3 times higher than in bovine milk. Their researches additionally indicate higher concentration of MnSOD than CuZnSOD in colostrum and mature milk, unlike in bovine milk in which MnSOD was found in traces. This result is attributed to the large number of cells in human milk, primarily neutrophils and macrophages<sup>6,7</sup>.

The aim of this work was to determine the activities of cytosol CuZnSOD and mitochondrial MnSOD activity in different phases of lactation. Activity was determined in colostrum milk (3<sup>rd</sup>-5<sup>th</sup> days) and in mature milk in the third week, and the fourth and seventh months of lactation.

## Material and Methods

### Subjects

The research comprised 36 women, aged  $26.2 \pm 4.7$  years. Written informed consent was obtained from each subject prior to participation. All women were from the town of Valjevo and its immediate surroundings. In order to avoid the psycho-physiological effects, the study included lactating women with similar socioeconomic status, hygienic-dietary habits and other characteristics. They gave birth in due time and had healthy babies. Pregnancy and delivery were normal and without any complications in all respondents. There were 24 primiparas (66.7%) and 12 with the second child (33.3%). Out of 36 children, four had an Apgar score lower than eight (11.1%).

Human colostrum and whole milk samples were collected from healthy lactating women in the morning. Milk samples were obtained manually. As soon as milk samples were taken, they were frozen at  $-70^{\circ}\text{C}$  and processed within 15-30 days. Centrifugation was performed at 1600 g for 20 minutes at  $4^{\circ}\text{C}$  (Beckman centrifuge J2-21, Beckman Instruments Inc., Palo Alto, CA). Lipid layer from the surface was removed and the resulting skimmed milk fraction was subjected immediately to enzyme assays. Protein concentrations were determined by the method of Lowry et al.<sup>10</sup>, using BSA as standard.

### Enzyme Assay

Superoxide dismutase activity was measured by the method of Misra and Fridovich<sup>11</sup>. The reaction of auto-oxidation of adrenaline to adrenochrome was run in 3 ml of an incubation mixture containing 0.05 M  $\text{Na}_2\text{CO}_3$ , 0.1 M EDTA, pH=10.2, followed by the addition of 100  $\mu\text{l}$  of milk sample and 100  $\mu\text{l}$  of  $3 \times 10^{-4}$  M adrenaline (obtained from Sigma Chemicals Co.). Inhibition of auto-oxidation was monitored spectrophotometrically (Cecil CE 2040 spectrophotometer, Cecil Instruments Ltd., Cambridge, UK) at 480 nm and at  $26^{\circ}\text{C}$ . After assaying the total SOD activity, the samples were treated with 4 mM KCN in order to inhibit cytosol SOD<sup>12</sup>, and subjected again to the enzyme assay as described above. The values thus obtained and the differences between the two measurements were considered as MnSOD and CuZnSOD activities, respectively. The results were expressed as specific activity of the enzyme in units per mg protein (U/mg prot.). One unit of SOD was defined as the amount of protein that causes 50% inhibition of the conversion rate of adrenaline to adrenochrome between the 3<sup>rd</sup> and 4<sup>th</sup> minute under specified conditions.

### Statistics

The results were analyzed by one way analysis of variance with repeated measures and by paired samples t-test. Differences between means were considered significant at  $p < 0.05$ .

### Results

Variation in MnSOD and CuZnSOD activities was followed in the samples of the same lactating women in the colostrum phase (3<sup>rd</sup>-5<sup>th</sup> days after delivery) as well as in the third week

(15–20 days) and in the fourth and seventh months of mature lactation. The experimental group, which consisted of 36 women in the colostrals phase, was reduced to 34 women in the third week, 26 women in the fourth month, and to 16 women in the seventh month of lactation. Milk samples of the 16 women who participated in all phases of lactation were used for statistical analyses.

The results are summarized in Figure 1 and Table I. The activities of both SODs showed significant variations in the examined periods of lactation (Ano1- rep.com: MnSOD  $F_{3,63}=12.24$ ,  $p=0.00004$ ; CuZnSOD  $F_{3,63}=8.13$ ,  $p=0.0004$ ). The activity of MnSOD in the colostrals phase was  $0.32 \pm 0.05$  U/mg protein and of CuZnSOD was  $1.47 \pm 0.25$  U/mg protein. In the third week of lactation, the activity of MnSOD was significantly higher ( $0.94 \pm 0.17$  U/mg protein;  $t = -3.31$ ,  $p = 0.0024$ ) in comparison to the colostrals phase. Similarly, the activity of CuZnSOD was significantly elevated ( $3.07 \pm 0.3$  U/mg protein;  $t = -3.43$ ,  $p=0.0019$ ). When compared to enzyme activities in the third week, the activities of MnSOD

**Table I.** Specific Activity (U/mg protein) of Manganese Superoxide Dismutase (MnSOD) and Copper, Zinc (CuZn)SOD in Different Phases of Lactation

Phases of lactation	MnSOD (U/mg protein)	CuZnSOD (U/mg protein)
3-5 days	$0.32 \pm 0.05$	$1.47 \pm 0.25$
3 <sup>rd</sup> week	$0.94 \pm 0.17^*$	$3.07 \pm 0.30^*$
4 <sup>th</sup> month	$0.13 \pm 0.04‡$	$0.83 \pm 0.25‡$
7 <sup>th</sup> month	$0.32 \pm 0.08†$	$1.44 \pm 0.44$

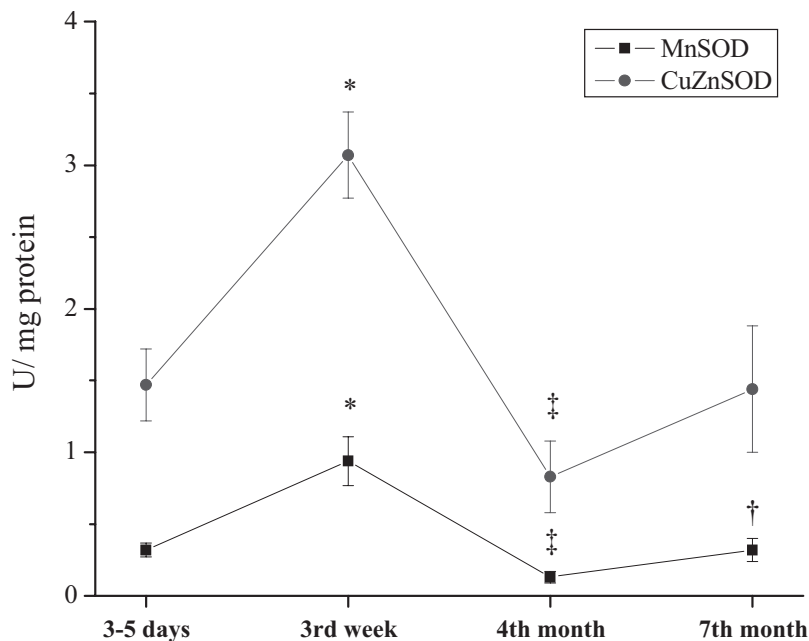
Data are given as means  $\pm$  SEM, n=16.

\* Comparison between 3-5 days and the 3<sup>rd</sup> week was significant,  $p < 0.05$ .

‡ Comparison between the 3<sup>rd</sup> week and the 4<sup>th</sup> month was significant,  $p < 0.05$ .

† Comparison between the 4<sup>th</sup> month and the 7<sup>th</sup> month was significant,  $p < 0.05$ .

and CuZnSOD in the fourth month were significantly suppressed, decreasing to levels even lower than recorded in the colostrals phase (MnSOD:  $0.13 \pm 0.04$  U/mg protein,  $t=4.35$ ,  $p=0.0003$ ; CuZnSOD:  $0.83 \pm 0.25$  U/mg protein,  $t=4.93$ ,  $p=0.00009$ ). In the seventh month of lactation, MnSOD activity was increased ( $0.32 \pm 0.08$  U/mg protein,  $t = -2.17$ ,  $p=0.023$ ),



**Fig. 1.** The specific activity of manganese superoxide dismutase (MnSOD) and copper, zinc (CuZn)SOD in the colostrals phase (3-5 days), the third week, and the fourth and seventh month of lactation.

Data are given as means  $\pm$  SEM, n=16.

\* Comparison between 3-5 days and the 3<sup>rd</sup> week was significant,  $p < 0.05$ .

‡ Comparison between the 3<sup>rd</sup> week and the 4<sup>th</sup> month was significant,  $p < 0.05$ .

† Comparison between the 4<sup>th</sup> month and the 7<sup>th</sup> month was significant,  $p < 0.05$ .

whereas the CuZnSOD activity ( $1.44 \pm 0.44$ ,  $t = -1.29$ ,  $p = 0.109$ ) was not changed significantly in comparison to enzyme activities observed in the fourth month.

Comparison of MnSOD and CuZnSOD activity between the 16 subjects who participated in all phases of the study and the "drop-out" subjects [i.e. 20 subjects (36-16) of the 3rd-5th day colostrum group, 18 subjects (34-16) of the 3rd week lactating group, and 10 subjects (36-26) of the 4th month lactating group] showed similar levels of SOD activity in each examined period of lactation ( $t$ -test,  $p > 0.05$ ).

### Discussion

All aspects of the function of free radicals, the significance of maintaining good antioxidative status as a prerequisite of cell proliferation and differentiation, as well as their role in the maturation of the organic system, primarily endocrine and immunological, clearly show that nutrition at the earliest age should provide not only nutritive, but also other biologically significant components, including antioxidants, that are necessary for meeting these requirements<sup>13</sup>.

Breast milk (premature and mature) is a rich source of nutrients and contains biologically active molecules that are essential for specific antioxidant functions<sup>14,15</sup>. It has the potential to help reconstitute the immune system while enhancing cell growth and tissue repair. Colostrum and mature human milk are outstanding nutritional supplements, a food that protects and promotes development, differentiation and growth<sup>16</sup>.

Our results showed that the activity of both SODs varied significantly during the examined lactation period. When compared with the results of Kiyosawa et al.<sup>7</sup>, it can be noted that the activities of CuZnSOD coincided with its concentrations in colostrum and mature milk in the 3rd week of lactation, whereas the activities of MnSOD did not. This might point to different regulatory mechanisms of these enzyme activities during lactation.

The lowered values of MnSOD and CuZnSOD activity during the fourth month of lactation might be a physiological trend or a transient imbalance between higher antioxidant requirements and intake, which reflects on the newborn's total antioxidant status. Since

the antioxidant enzymes in human milk have specific tertiary structure, which distinguishes them from other antioxidant enzymes of different origin, they are considered more hydrophobic and less sensitive to proteolysis and denaturation. This definitively adds to their physiological role in the gastrointestinal system of a newborn<sup>5,6</sup>. Suppression of SOD activity in the fourth month and increment in MnSOD activity in the seventh month of lactation may also be related to the mother's diet during pregnancy and lactation<sup>16</sup>.

Optimal nutrition should be designed so as to provide for proper growth and development, and give the best results from the aspect of morbidity prevention<sup>17,18</sup>. For newborns and infants, human milk definitively meets these conditions in all aspects<sup>19,21</sup>. Therefore, efforts should be made to improve the mother's dietary habits during pregnancy and lactation, in order to optimize the total antioxidant capacity of breast milk.

In conclusion, our results show significant changes in SOD activity in different phases of lactation, which might reflect the various antioxidant needs during development of newborns as well as differences in the mother's diet in pregnancy and lactation.

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