# INFLUENCE OF VITAMIN C SUPPLEMENTATION ON THE OXIDATIVE STATUS OF RAT LIVER

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*Abstract* — Rats have the ability to synthesize vitamin C in the liver. In the present work, we tested whether its additional intake improves antioxidative protection of the tissue. Thus, we studied the liver's oxidative status in rats given two supplements of ascorbic acid over a four-week period of time. Our results confirmed that the additional intake of ascorbate improves the liver's antioxidative defense in a dose-dependent manner. The explanation for the disproportion between the ratio of employed doses of vitamin C and their effects on the studied parameters probably lies in the mechanism of tissue accumulation of ascorbate and balance of its alimentary and endogenous availability.

*Key words*: Vitamin C, liver, antioxidative protection, rats

## INTRODUCTION

Vitamin C (L-ascorbic acid, ascorbate) is a simple glucose-related carbohydrate with rather unique properties. The presence of an enediol group in the molecule confers electron lability, which makes it a member of an oxidation-reduction system with electron-donating or accepting properties. A loss of the first electron results in formation of the ascorbate free radical (M e h l h o r n et al., 1989), which can be further oxidized by another electron loss to give dehydroascorbic acid. Both ascorbic acid and ascorbate free radical have a reducing potential low enough to react with most of the physiologically important radicals and oxidants (B u e t t n e r, 1993), enabling vitamin C to act as a powerful hydrosoluble antioxidant in body fluids (F r e i et al., 1989), scavenging reactive oxygen and nitrogen species (H a l l i w e l l, 1996). Vitamin C also acts as a cosubstrate for some hydroxylase and oxygenase enzymes, maintaining their active center metal ions in a reduced state for optimal enzyme activity (C a r r and F r e i, 1999).

The ascorbic acid biosynthetic pathway in rats involves the liver as the site of its synthesis (S t o n e, 1972), and it is to be expected that this is the way they satisfy normal daily needs for this nutrient.

However, it is possible that additional vitamin C supplies may improve the antioxidative defense of these animals. In order to elucidate this effect, we studied the oxidative status of rats given two different supplements of ascorbic acid over a four-week period of time. Thus, we determined the activities of copper zinc superoxide dismutase (CuZnSOD), manganese superoxide dismutase (MnSOD), and catalase, hydrogen peroxide concentration, the level of lipid peroxidation, and vitamin C content in livers of the experimental animals, as well as their serum vitamin C level.

## MATERIALS AND METHODS

### Design of experiment

Male rats of the Wistar strain (*Rattus norvegicus*) weighing 200±45 g were used for the experiments. The animals were acclimated to 22±1°C and maintained under conditions of 12-h periods of light and dark, with free access to tap water and commercial rat food.

The rats were divided into three groups, each consisting of six animals. The first group consisted of control rats, which drank tap water. The second and third groups were formed by animals whose

diets were supplemented with a low or high dose of vitamin C dissolved in drinking water.

### Vitamin C supplementation

The vitamin C doses chosen were 0.75 and 25 mg of ascorbic acid/kg of rat body weight per day (referred to as low and high doses).

According to our previous experiment performed on a group of six animals over a period of four weeks, the average consumption of water in rats was 240±5 ml of tap water/kg of body weight per day. Both doses of vitamin C were therefore administered every day dissolved in an appropriate volume of tap water and administered to the corresponding group of animals. This supplementation was constant throughout four weeks.

### Sample preparation

At the end of the vitamin C supplementation period, animals were killed by decapitation with a Harvard guillotine without anesthesia according to the rules adopted by the Local Ethical Committee. After decapitation, livers were removed and blood was collected.

The tissue was excised and divided in two equal portions. One portion was homogenized in 25 mM phosphate-buffered saline (PBS), pH 7.0, and centrifuged at 9000 g in a semi-preparative Sorvall Super T21 centrifuge for 20 min at 4°C. The supernatants were used for determination of catalase, CuZnSOD, and MnSOD activities, as well as for measurement of the concentration of hydrogen peroxide and total lipid hydroperoxides (the level of lipid peroxidation). The other portion of livers and blood serums were used for determination of vitamin C concentration by a similar procedure, except that 6% trichloroacetic acid (TCA) was used instead of PBS.

### Methods

Total content of vitamin C was determined by the method of Roe and Kuether (1943) and calculated against its standard curve absorption values.

Total superoxide dismutase activity was deter-mined in the tissue PBS samples by the adrenaline method of Misra and Fridovich (1972) using potassium cyanide as a CuZnSOD inhibitor for differential calculation of MnSOD activity (Weisiger and Fridovich, 1973).

Catalase activity was measured in the tissue PBS samples by the method of Beutler (1982), which is based on the rate of $H_2O_2$ degradation by the action of catalase contained in the examined samples.

Measurement of total lipid hydroperoxide and $H_2O_2$ content was in both cases based on the ferrous ion oxidation by xylenol orange (FOX) assay (Gupta, 1973; Wolf, 1994). Two versions of FOX assays are described in the literature, FOX-1 and FOX-2 (Banerjee et al., 2003). The concentration of $H_2O_2$ was measured by the FOX-1 assay (Gay and Gebicki, 2000) and calculated against hydrogen peroxide standard curve absorption values.

The concentration of lipid hydroperoxides was measured by the FOX-2 assay (Jiang *et al.*, 1991), with the level of lipid peroxidation in samples expressed as percent of the lipid peroxidation level of the control group.

### Statistical analysis

The data are expressed as means ± SEM. The values obtained from rats without vitamin C supplementation were used as a control. One-way ANOVA was undertaken for multiple range comparison, while significant differences among groups were determined by the Tukey test. The probability of significance of differences was set at $P < 0.05$.

## RESULTS

Vitamin C supplementation elevates the serum's total ascorbate content in a dose-dependent manner. This is not the case with the liver, in which only the low dose increases ascorbate tissue content. However, in this tissue both vitamin C doses reduce the level of lipid peroxidation (Fig. 1).

As indicated by Figs. 2 and 3, the activity of CuZnSOD is elevated in the liver of rats fed vitamin C, MnSOD activity and hydrogen peroxide concen-
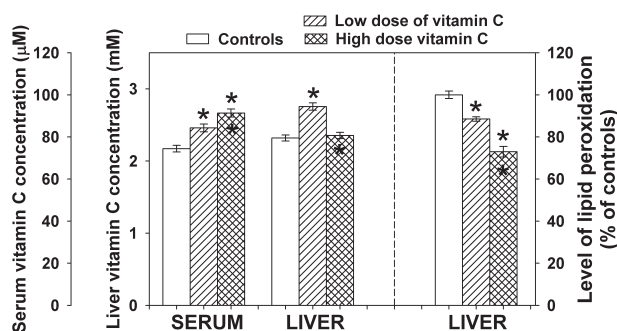
**Fig. 1.** Influence of low and high doses of vitamin C supplementation on endogenous concentration of vitamin C in the rat serum and liver (left), as well as on the level of liver lipid peroxidation (right). Statistically significant differences (P < 0.05) in relation to the control are marked with an asterisk above the columns, while statistically significant differences between the two doses of the vitamin C are marked with an asterisk inside the columns.
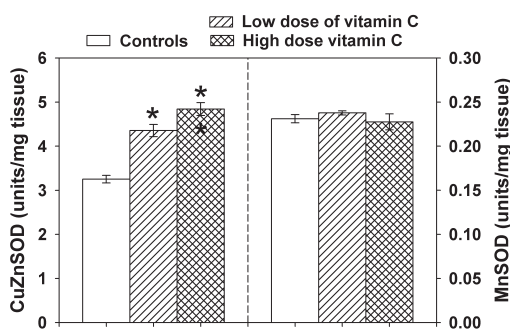


**Fig. 2.** Influence of low and high doses of vitamin C supplementation on activity of CuZnSOD (left) and MnSOD (right) in the rat liver. Statistically significant differences (P < 0.05) in relation to the control are marked with an asterisk above the columns, while statistically significant differences between the two doses of the vitamin C are marked with an asterisk inside the columns.
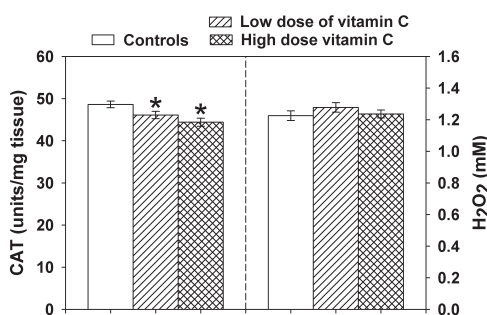


**Fig. 3.** Influence of low and high doses of vitamin C supplementation on activity of catalase (left) and concentration of $H_2O_2$ (right) in the rat liver. Statistically significant differences (P < 0.05) in relation to the control are marked with an asterisk above the columns.

tration are unchanged, while catalase activity is even depressed in relation to the control level.

## DISCUSSION

Vitamin C supplementation dose-dependently increases ascorbate concentration in the blood. In the liver, an increase is present only in the case of a low vitamin C dose, which implies down-regulation of its hepatic synthesis under the influence of a high dose. This is in accordance with findings of T s a o and Y o u n g (1989; 1990), who showed that long-term feeding with vitamin C increases its concentration in circulation and reduces its synthesis in the liver (B a n h e g y i et al., 1997).

Our results indicate that the activity of CuZnSOD is elevated in the liver of rats fed vitamin C. It is known that vitamin C increases hepatic activity of cP4502E1 (an isoform of cytochrome P450) and accompanying superoxide anion radical production, which suggests a possible pro-oxidant effect of ascorbate in the liver (P a o l i n i et al., 1999). This cannot be concluded on the basis of our results, since apart of CuZnSOD activity, not one of the other studied parameters indicates increased oxidative stress. Moreover, the level of liver lipid peroxidation is even reduced, confirming the protective effect of vitamin C supplementation.

The disproportion between the employed vitamin C doses and their effects on the studied parameters needs to be emphasized. For example, the two ascorbate doses, which differ from each other more than 33-fold, cause far less different changes of serum ascorbate concentration (less than 20%). Similar results were obtained in a human study, where application of 200 mg of vitamin C daily caused about 80% of the plasma ascorbate saturation achieved with a dose of 2500 mg (L e v i n e et al., 1996). A possible explanation could be the existence of a specific mechanism of tissue ascorbate accumulation. In one system, which is Na⁺-independent, dehydroascorbic acid is transported by some members of the family of glucose transporters (V e r a et al., 1993). Ascorbic acid, on the other hand, is accumulated by Na⁺-dependent transport realized by sodium-vitamin C co-transporters,

SVCTs (Tsukaguchi et al., 1999). It is clear that CVCTs have major influence on tissue vitamin C accumulation, since in normal conditions ascorbic acid represents more than 95% of the total body vitamin C content (Rose, 1988). Thus, the fact that SVCTs are fully saturated at low extracellular concentrations of ascorbic acid (Washko et al., 1989) probably explains the relative inefficiency of its high doses. Also, it has been demonstrated that the use of high doses of ascorbic acid causes strong decrease in expression of SVCT1 in the apical surface of the intestinal membrane (MacDonald et al., 2002), which supports the old thesis that the main factor limiting the bioavailability of vitamin C is its intestinal absorption (Hodges et al., 1969). In conclusion, our results confirm that additional intake of ascorbate improves the liver's antioxidative defense. The dose-dependent nature of this protection emphasizes importance of the mechanisms governing tissue accumulation of ascorbate, as well as relevance of the balance of its alimentary and endogenous availability.

## REFERENCES

*Banerjee, D., Madhusoodanan, U. K., Sharanabasappa, M., Ghosh, S.,* and *J. Jacob* (2003). Measurement of plasma hydroperoxide concentration by FOX-1 assay in conjunction with triphenylphosphine. *Clin. Chim. Acta* **337**, 147-152.

*Banhegyi, G., Braun, L., Csala, M., Puskas, F.,* and *J. Mandl* (1997). Ascorbate metabolism and its regulation in animals. *Free Radic. Biol. Med.* **23**, 793-803.

*Beutler, E.* (1982). Catalase, In: *Enzymes* (Ed. E. Beutler), 105-106. Grune and Stratton, New York.

*Buettner, G. R.* (1993). The pecking order of free radicals and antioxidants: lipid peroxidation, alpha-tocopherol, and ascorbate. *Arch. Biochem. Biophys.* **300**, 535-543.

*Carr, A.,* and *B. Frei* (1999). Does vitamin C act as a pro-oxidant under physiological conditions? *Faseb. J.* **13**, 1007-1024.

*Frei, B., England, L.,* and *B. N. Ames* (1989). Ascorbate is an outstanding antioxidant in human blood plasma. *Proc. Natl. Acad. Sci. U S A* **86**, 6377-6381.

*Gay, C.,* and *J. M. Gebicki* (2000). A critical evaluation of the effect of sorbitol on the ferric-xylenol orange hydroperoxide assay. *Anal. Biochem.* **284**, 217-220.

*Gupta, B. L.* (1973). Microdetermination techniques for $H_2O_2$ in irradiated solutions. *Microchem. J.* **18**, 363-374.

*Halliwell, B.* (1996). Vitamin C: antioxidant or pro-oxidant in vivo? *Free Radic. Res.* **25**, 439-454.

*Hodges, R. E., Baker, E. M., Hood, J., Sauberlich, H. E.,* and *S. C. March* (1969). Experimental scurvy in man. *Am. J. Clin. Nutr.* **22**, 535-548.

*Jiang, Z. Y., Woollard, A. C.,* and *S. P. Wolff* (1991). Lipid hydroperoxide measurement by oxidation of Fe2+ in the presence of xylenol orange. Comparison with the TBA assay and an iodometric method. *Lipids* **26**, 853-856.

*Levine, M., Conry-Cantilena, C., Wang, Y., Welch, R. W., Washko, P. W., Dhariwal, K. R., Park, J. B., Lazarev, A., Graumlich, J. F., King, J.,* and *L. R. Cantilena* (1996). Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. *Proc. Natl. Acad. Sci. USA* **93**, 3704-3709.

*MacDonald, L., Thumser, A. E.,* and *P. Sharp* (2002). Decreased expression of the vitamin C transporter SVCT1 by ascorbic acid in a human intestinal epithelial cell line. *Br. J. Nutr.* **87**, 97-100.

*Mehlhorn, R. J., Sumida, S.,* and *L. Packer* (1989). Tocopheroxyl radical persistence and tocopherol consumption in liposomes and in vitamin E-enriched rat liver mitochondria and microsomes. *J. Biol. Chem.* **264**, 13448-13452.

*Misra, H. P.,* and *I. Fridovich* (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem.* **247**, 3170-3175.

*Paolini, M., Pozzetti, L., Pedulli, G. F., Marchesi, E.,* and *G. Cantelli-Forti* (1999). The nature of prooxidant activity of vitamin C. *Life Sci.* **64**, 273-278.

*Roe, J. H.,* and *C. A. Kuether* (1943). The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid. *J. Biol. Chem.* **147**, 399-407.

*Rose, R. C.* (1988). Transport of ascorbic acid and other water-soluble vitamins. *Biochim. Biophys. Acta* **947**, 335-366.

*Stone, I.* (1972). The natural history of ascorbic acid in the evolution of the mamals and primates and its significance for present day man. *Orthomol. Psychiatry* **1**, 82-89.

*Tsao, C. S.,* and *M. Young* (1989). Effect of exogenous ascorbic acid intake on biosynthesis of ascorbic acid in mice. *Life Sci.* **45**, 1553-1557.

*Tsao, C. S.,* and *M. Young* (1990). Enzymatic formation of ascorbic acid in liver homogenate of mice fed dietary ascorbic acid. *In Vivo* **4**, 167-169.

*Tsukaguchi, H., Tokui, T., Mackenzie, B., Berger, U. V., Chen, X.*

*Z., Wang, Y., Brubaker, R. F.,* and *M. A. Hediger* (1999). A family of mammalian Na+-dependent L-ascorbic acid transporters. *Nature* **399**, 70-75.

*Vera, J. C., Rivas, C. I., Fischbarg, J.,* and *D. W. Golde* (1993). Mammalian facilitative hexose transporters mediate the transport of dehydroascorbic acid. *Nature* **364**, 79-82.

*Washko, P., Rotrosen, D.,* and *M. Levine* (1989). Ascorbic acid transport and accumulation in human neutrophils. *J.*

*Biol. Chem.* **264**, 18996-19002.

*Weisiger, R. A.,* and *I. Fridovich* (1973). Mitochondrial superoxide dismutase. Site of synthesis and intramitochondrial localization. *J. Biol. Chem.* **248**,4793-4796.

*Wolf, S. P.* (1994). Ferrous ion oxidation in presence of ferric ion indicator xylenol orange for measurement of hydroperoxides. *Methods Enzymol.* **233**:182-189

## ПРОМЕНЕ ОКСИДАТИВНОГ СТАТУСА ЈЕТРЕ ПАЦОВА ПРЕХРАЊИВАНИХ ВИТАМИНОМ С

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Испитиван је ефекат прехране пацова са две дозе витамина С на оксидативни статус јетре. Одређиване су активност и бакар цинк супероксид дисмутазе, манган супероксид дисмутазе и каталазе, ниво липидне пероксидације и концентрација водоник пероксида и укупног витамина С у јетри експерименталних животиња, као и концентрација витамина С у серуму. Добијени резултати указују да егзогени витамин С повећава антиоксидативну заштиту у јетри пацова, на начин који указује на специфичан значај механизама његове ткивне акумулације и баланса.