

THE MEAT IN THE DIET OF AGED SUBJECTS AND THE ANTIOXIDANT EFFECTS OF CARNOSINE

F.M. ANTONINI^a, E. PETRUZZI^{a*}, P. PINZANI^b, C. ORLANDO^b,
M. POGGESI^b, M. SERIO^c, M. PAZZAGLI^b and G. MASOTTI^a

^aGerontology and Geriatrics Unit, Department of Critical Care Medicine and Surgery; ^bClinical Biochemistry, and ^cEndocrinology Unit, Department of Clinical Pathophysiology; University of Florence, Viale Pieraccini 6, I-50139 Florence, Italy

*Corresponding author:

Tel.: +(39-055) 4271-471; Fax: +(39-055) 4271-413; E-mail: epet@unifi.it

SUMMARY

Five volunteer male subjects (49-82 years of age) consumed five different types of meals: (1) typical mediterranean diet + red wine (300 ml, Chianti); (2) typical mediterranean diet without wine; (3) red wine (Chianti) only; (4) meat only (300 g low-cooked Florentine beef-steak); (5) L-carnosine reagent grade (450 mg; Sigma, Milan). The serum total antioxidant capacity (TAC) (expressed as $\mu\text{mol/l}$ Trolox equivalents, by chemiluminescent assay), and serum urate levels were measured in every study, at start (0-time), as well as +1 h and +2 h time intervals. Results of serum TAC values (mean \pm SD) were the following at 0 time, and 1 and 2 hrs after the meals, respectively. (1) 439 ± 127 , +11.6 %, and +11.9 % (for both $p < 0.05$). (2) 360 ± 96 , +7.5 % and 23.6 % (NS, and $p < 0.01$). (3) 378 ± 89 , +10.8 %, and +14.5 % (NS, and $p < 0.05$). (4) 512 ± 133 , +7.9 % and +21.9 % (NS and $p < 0.01$). (5) 530 ± 145 , +13.6 % and +6.3 % ($p < 0.05$ and NS). These results show that different meals significantly influence serum TAC at various time intervals, to different extents. The increased serum TAC induced by red wine ingestion appears to be mediated by urate. If we consider in particular the role of carnosine, the results show a significant increase of TAC (+11.6 %, $p < 0.05$) after 450 mg L-carnosine ingestion, after 1 h but not (+6.25 %, NS) after 2 hrs. Our results are the first evidence of the action of oral administration of carnosine in humans on serum antioxidant capacity. These preliminary results support the potential antioxidant role of carnosine and its potential therapeutic use in humans.

Keywords: antioxidants, free radicals, meat, carnosine, mediterranean diet

INTRODUCTION

The biochemistry of reactive oxygen species is an important field with vast implications. Although oxygen is an essential component of living organisms, the generation of reactive oxygen intermediates is inevitable in aerobically metabolizing cells. Cells expend substantial resources to protect themselves against the potential damaging effects of reactive oxygen species. The first line of defense is composed of enzymes, such as superoxide dismutase, glutathione peroxidases, and catalases, and several vitamins and micronutrients that are active in quenching these free-radical species or required cofactors

for antioxidant enzymes (Cadenas and Packer, 1996). Both preventive and chain breaking antioxidants have a role in the limitation of free radical damage. Some of these may be regarded as classical like vitamins E and C but others are more recently discovered, such as the flavonoids, widespread in plant tissue, and the muscle constituents anserine and carnosine. The potent antioxidant activity of phenolic substances of red wine, in particular, have been proposed as an explanation for the "French paradox" (the apparent incompatibility of a high fat diet with a low incidence of coronary heart diseases) (Renaud and De Longelil, 1992). Recently it has been demonstrated that the cardioprotective effect of red wine is mediated by urate (Day and Stansbie, 1995), while it is also known that urate is a strong antioxidant in vivo. The cellular antioxidant status and its role against the development of certain disease processes, known to be associated with oxidative stress, has gained potential therapeutic significance in view of the beneficial effects of free-radical scavenging drugs or antioxidants. Likewise, epidemiological studies emphasize the relevance of antioxidant vitamins and nutrients in health issues and/or prevention of disease (e.g., cardiovascular disease). There is much controversy over antioxidant supplementation policies, some authorities recommending a massive program of supplementation for all ages and classes, others stressing the value of the traditional mixed diet, as it is the mediterranean diet (Alberti, 2001).

SUBJECTS AND METHODS

On the basis of these remarks, 5 volunteer normal male subjects (age range 49-82 years) consumed five different types of meals. They were considered normal according to the criteria of the Eurage Senieur Protocol (Ligthart et al., 1990). All subjects gave a full medical history and underwent physical and of routine biochemical examinations. Furthermore, they gave oral and written informed consent for taking part in the study. In each case, the measurement of serum urate (considering its possible role in mediating the antioxidant activity of some foodstuff), and serum total antioxidant capacity (TAC) (expressed as $\mu\text{mole/l}$ Trolox equivalents, by chemiluminescent assay) were performed at start (0 time), then +1 h and +2 h after the meals. The chemiluminescent assay (Whitehead et al., 1992) is based on an enhanced chemiluminescence reaction catalyzed by horse radish peroxidase conjugate, which determines oxidation of the chemiluminescent substrate luminol in the presence of hydrogen peroxide. The use of p-iodophenol as enhancer of the chemiluminescent signal determines a more intense, prolonged and stable light emission

(Figure 1). Serum urate was measured by using the method based on the use of uricase, and expressed as $\mu\text{mole/l}$.

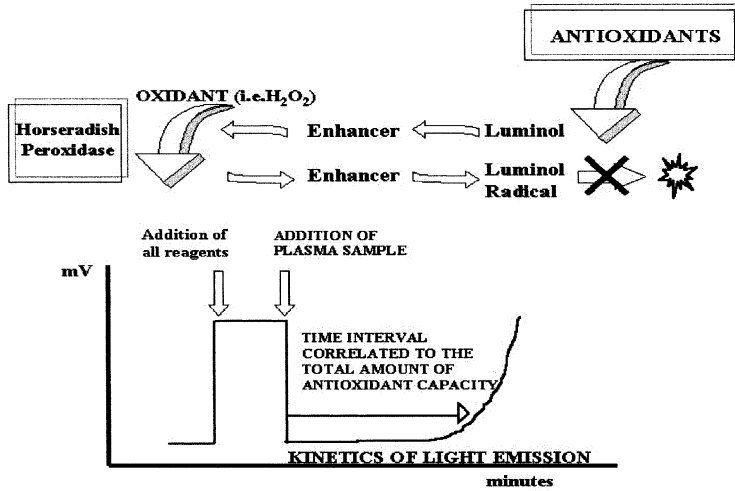


Figure 1. Schematic representation of the enhanced chemiluminescence assay for TAC (Whitehead et al., 1992).

Radical scavenging antioxidants can interfere with the constant production of free radical intermediates and hence stop light emission. The signal is restored after consumption of the antioxidants and the time period of suppression is directly related to the amount of antioxidants (Whitehead et al, 1992). A calibration curve with Trolox standard solutions (a water-soluble vitamin E analogue; Hoffman-La Roche, obtained from Aldrich Chemical Co., Gillingham, Dorset, UK), was processed together with the serum samples for the measurement of the TAC in the samples under investigation, and were expressed as $\mu\text{mole/l}$ Trolox equivalents.

The experimental model was based on the use of five different meals. They were: (1) typical mediterranean diet (100 g of pasta with tomato sauce; 300 g low-cooked Florentine beef-steak; 70 g of fresh salad dressed with 10 g olive oil; 70 g of fresh strawberries dressed with lemon juice) + red wine (300 ml, Chianti); (2) typical mediterranean diet described above, but without wine; (3) red wine (300 ml, Chianti) only; (4) meat (300

g low-cooked Florentine beef-steak) only; (5) L-carnosine, reagent grade (450 mg; Sigma, Milan).

RESULTS AND DISCUSSION

The effects of the typical mediterranean diet + red wine (model 1) on serum TAC and urate levels are reported in Figure 2.

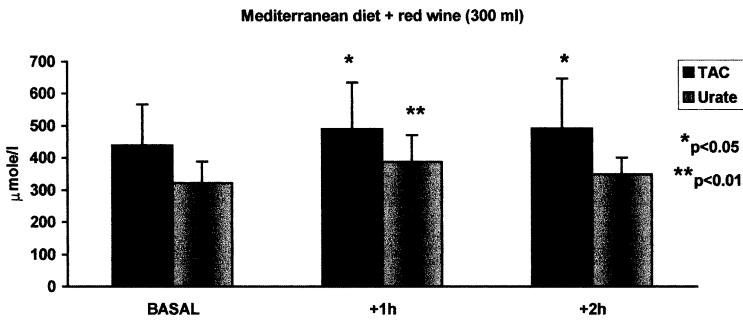


Figure 2. Serum TAC and urate levels (mean ± SD) in the model (1).

The results show a significant increase of serum TAC values throughout the whole period of the study (0-time = 439 ± 127 ; +1 h = +11.6 %, $p<0.05$; +2 h = +11.9 %, $p<0.05$) accompanied by an increase of urate after the first hour (0-time = 322 ± 67 , +1 h = +18.75 % $p<0.01$, +2 h = +6.25 %, NS).

The effects of the typical mediterranean diet without red wine (model 2) on serum TAC and urate levels are reported in Figure 3.

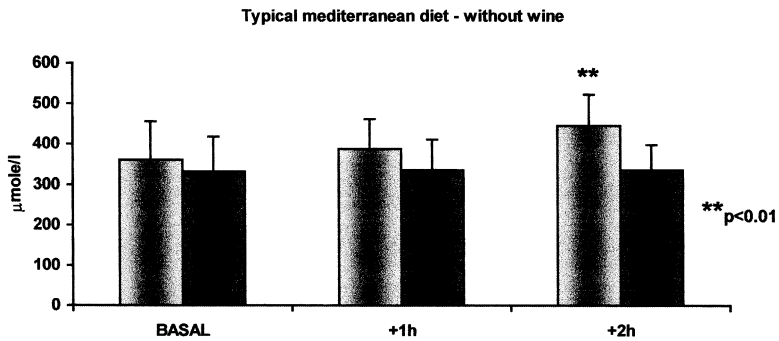


Figure 3. Serum TAC and urate levels (mean ± SD) in the model (2).

The results show a significant increase of serum TAC values at the 2nd hour of the study (0-time = 360 ± 96 , +1 h = +7.5 %, NS; +2 h = +23.6 %, $p < 0.01$), whereas in this case no contribution was due to urate level increase: 0-time = 332 ± 86 , +1 h = +2.5 %, NS; +2 h = +2.7 % NS.

The effects of the administration of red wine only (model 3) on serum TAC and urate levels are reported in Figure 4.

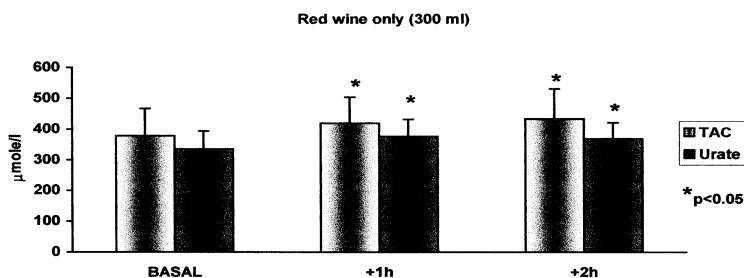


Figure 4. Serum TAC and urate levels (mean \pm SD) in the model (3).

The results demonstrated the role of red wine on serum TAC, showing a significant increase of TAC values throughout the whole period of the study: at 0-time = 378 ± 89 ; at +1 h = +10.8 %, $p < 0.05$ and at +2 h = +14.5 %, $p < 0.05$. The simultaneous determination of urate showed a significant increase: at 0-time = 335 ± 59 , at +1 h = +13.4 %, $p < 0.05$, and at +2 h = +11.9 %, $p < 0.05$, demonstrating that the increment of serum TAC is mainly due in this case to urate level increase.

The effects of the administration of 300 g low-cooked Florentine beefsteak only (see experimental model 4 are reported in Figure 5.

The results show a slight increase of serum TAC values at +1 h, but a significant increase at +2 h (0-time = 512 ± 133 ; +1 h = +7.9 %, NS; +2 h = +21.9 %, $p < 0.01$). In spite of the well known correlation between meat ingestion and urate level increase, in model 4 the ingestion of meat alone was able to induce an increment in serum TAC values without any contribution of serum urate level increase; serum urate level was: at 0-time = 322 ± 83 ; +1 h = +2.9 %, NS; +2 h = +3.4 %, NS.

The effects of L-carnosine oral administration (model 5) on serum TAC and urate levels are reported in Figure 6.

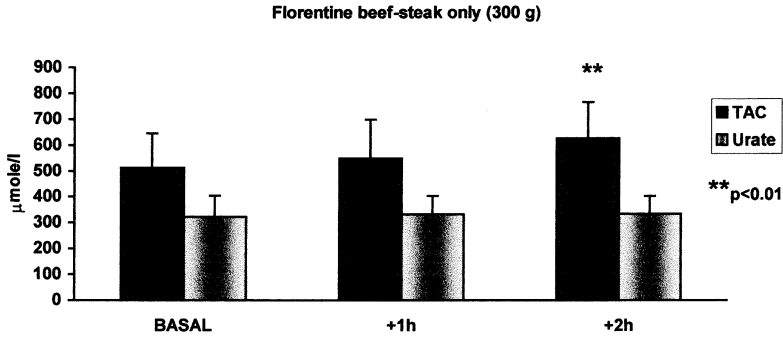


Figure 5. Serum TAC and urate levels (mean ± SD) in the model (4).

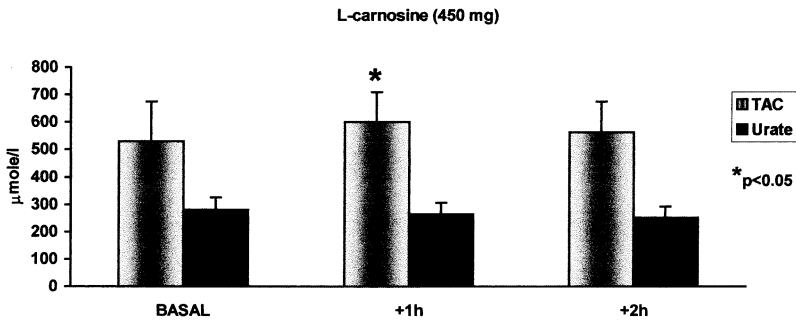


Figure 6. Serum TAC and urate levels (mean ± SD) in the model (5).

The results show that oral administration of L-carnosine, supposed to be the principal antioxidant compound in the meat, also increases serum TAC level even imore rapidly than meat, significantly at +1 h, slightly at +2 h (0-time = 530 ± 145; +1 h = +13.6 %, p<0.05; +2 h = +6.3 %, NS). Serum urate levels remained unchanged (0-time = 280 ± 45; +1 h = -1.8 %, NS; +2 h = -2.4 %, NS).

If we consider, in particular, the role of carnosine, the results show a significant increase of TAC (+11.6 % p<0.05) after 450 mg L-carnosine ingestion, at 1 h but not at 2 h (+6.3 %, NS).

Skeletal muscle is susceptible to oxidative deterioration due to a combination of lipid oxidation catalysts and membrane lipid systems that are high in unsaturated fatty acids. To prevent or delay oxidation reactions, several endogenous antioxidant systems are found in muscle tissue. These include alpha-tocopherol, histidine-containing dipeptides, and anti-oxidant enzymes such as glutathione peroxidase, superoxide dismutase, and catalase.

The oxidative stability of skeletal muscle is also influenced by the histidine-containing dipeptides, carnosine and anserine. Anserine and carnosine are thought to inhibit lipid oxidation by a combination of free radical scavenging and metal chelation (Decker et al., 2001).

In vitro experimental evidences indicate that carnosine and related histidine-containing dipeptides can scavenge superoxide, quench singlet oxygen and hydroxyl radical (Klebanov et al., 1998). In vitro, carnosine not only traps the radicals, but also modifies the activity of the enzyme systems responsible for the production of reactive oxygen species (Hipkiss et al., 1998).

In vivo, several studies have been carried out on animal models demonstrating that carnosine can enhance the resistance of animals towards physical factors (Dunnet and Harris, 1999), lowering mortality of the animals and improving the recovery of them. A few experiments with carnosine have been performed in humans, principally concerning the administration of essential aminoacids or of beta-alanine and L-histidine (Alvestrand et al., 1978).

Our results represent the first evidence of the action of oral administration of carnosine in humans on serum antioxidant capacity. These preliminary results support the antioxidant role of carnosine and its potential therapeutic use in humans.

ACKNOWLEDGEMENTS

This paper was supported by grants from University of Florence and from Ente Cassa di Risparmio di Firenze, Italy.

REFERENCES

- Alberti, A. (2001): Do we need more adequate quality control indices for the "reference Mediterranean diet"? *Diab. Nutr. Metab.*, 14, 179-180.
- Alvestrand, A., Bergstrom, J., Furst, P., Germanis, G. and Widstam, U. (1978): Effect of essential amino acid supplementation on muscle and plasma free amino acids in chronic uremia. *Kidney Int.*, 14, 323-329.
- Cadenas, E. and Packer, L. (1996): Preface. *Handbook of Antioxidants*, Cadenas, E. and Packer, L. (eds), M. Dekker, New York.
- Day, A. and Stansbie, D. (1995): Cardioprotective effect of red wine may be mediated by urate. *Clin. Chem.*, 41, 1319-1320.
- Decker, E.A., Ivanov, V., Zhu, B.Z. and Frei, B. (2001): Inhibition of low-density lipoprotein oxidation by carnosine histidine. *J. Agric. Food Chem.*, 49, 511-551.
- Dunnet, M. and Harris, R.C. (1999): Influence of oral beta-alanine and L-histidine supplementation on the carnosine content of the gluteus medius. *Equine Vet. J. Suppl.* 30, 499-504.
- Hipkiss, A.R., Preston, J.E., Himsworth, D.T., Worthington, V.C., Keown, M., Michaelis, J., Lawrence, J., Mateen, A., Allende, L., Eagles, P.A. and Abbott, N.J. (1998): Pluripotent protective effects of carnosine, a naturally occurring dipeptide. *Ann. N.Y. Acad. Sci.*, 854, 37-53.

- Klebanov, G.I., Teselkin, Y., Babenkova, I.V., Lyubitsky, O.B., Rebrova, O.Y., Boldyrev, A.A. and Vladimirov, Y.A. (1998): Effect of carnosine and its components on free-radical reaction. *Membr. Cell. Biol.*, 12, 89-99.
- Ligthart, G.J., Corberand, J.X., Geertzen, H.G.M., Meinders, E., Knook, D.L., Hijmans, W. (1990): Necessity of the assessment of health status in human immunogerontological studies: evaluation of the senieur protocol. *Mech. Ageing Dev.*, 28, 47-55.
- Reanud, S. and De Longiril, M. (1992): Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet*, 339, 1523-1526.
- Whitehead, T.P., Thorpe, G.H.G. and Maxwell, S.R.J. (1992): Enhanced chemiluminescent assay for antioxidant capacity in biological fluids. *Anal. Chim. Acta*, 266, 265-277.