INFLUENCE OF ALPHA-LIPOIC AND DIHYDROLIPOIC ACIDS ON LIPID OXIDATION IN A CHICKEN MUSCLE SYSTEM

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BACKGROUND

Concern about the use of artificial antioxidants in foods has led to increased interest in the applications of naturally occurring antioxidants. These include antioxidant substances produced naturally both by plants (e.g. flavonoids) and animals (e.g. carnosine, anserine). α-Lipoic acid (α–LA) has recently gained considerable attention as an antioxidant (Packer *et al.*, 1995). α–LA is an essential coenzyme in animal cells, and plays a vital role in mitochondrial electron transport reactions. α–LA, and its reduced form dihydrolipoic acid (DHLA) scavenge reactive oxygen species, such as superoxide anion, hydroxyl radicals and peroxyl radicals. It also protects membranes by interacting with vitamin C and glutathione, which may in turn recycle vitamin E.

OBJECTIVES

The purpose of this study was to determine the influence of α-lipoic acid (α–LA) and dihydrolipoic acid (DHLA) on iron or iron/ascorbate induced lipid oxidation in a chicken muscle system.

METHODS

Chicken muscle (1g) was homogenised with 9ml KCl (1.15%). The homogenate (100µl) was placed in a test tube with either 200µl of $FeSO_4.7H_2O$ (5mM) or 200µl of $FeSO_4.7H_2O$ plus 200µl of ascorbate (2mM). α-LA or DHLA (50 µl) was added to the incubation medium in ethanolic solutions (Kagan *et al.*, 1992) to a final concentration of 0.5, 1, 5 or 10mM. The mixture was made up to a final volume of 1 ml with tris maleate buffer (80mM, pH 7.4). Control groups contained iron or iron/ascorbate with no α-LA or DHLA. The 1 ml aliquots were incubated at 37°C in a shaking water bath. The extent of lipid oxidation was determined at various time intervals during the incubation by monitoring malonaldehyde (MDA) formation by means of the TBA assay (Beuge & Aust, 1978), except that BHT was added to inhibit further lipid oxidation during the TBA test (Scott *et al.*, 1994). The MDA-TBA complex was determined by first derivative spectral analysis as described previously (O'Neill *et al.*, 1998). Results were reported as MDA-TBA values and expressed as µg MDA/g chicken muscle. All data were subjected to analysis of variance (ANOVA). Where significant differences were detected, means were compared by the method of least significant difference (Snedecor & Cochran, 1967).

RESULTS AND DISCUSSION

The effects of α–LA and DHLA on iron induced lipid oxidation in the chicken muscle system are shown in Figure 1. The addition of α-LA at a concentration of 0.5 or 1mM did not reduce MDA-TBA values compared to control values (Figure 1a). At the 5 mM concentration lipid oxidation was significantly ($P<0.05$) reduced after 90 min incubation, but returned to control values at 300 minutes incubation. MDA-TBA values were significantly ($P<0.05$) reduced by 10mM α-LA from 90 min until the end of the incubation period. The addition of 0.5mM DHLA had no effect on lipid oxidation (Figure 1b). At the 1mM level, lipid oxidation was significantly ($P<0.05$) reduced compared to the control after 90 min, but MDA-TBA values returned to control levels after 180 min. Both the 5 and 10mM levels significantly ($P<0.05$) reduced MDA-TBA values compared to the control throughout the incubation period.

Figure 2 shows the effects of α-LA and DHLA on iron/ascorbate induced lipid oxidation. α-LA had no effect on lipid oxidation at the 0.5, 1 and 5 mM levels (Figure 2a). The addition of 10mM α-LA significantly ($P<0.05$) reduced MDA-TBA values compared to the control from 120min until the end of the incubation period. DHLA significantly ($P<0.05$) reduced MDA-TBA values compared to control values, at all concentrations used (Figure 2b). All concentrations showed similar effects up to 180 minutes. However, after this time, MDA-TBA values increased significantly ($P<0.05$) at the 0.5mM level.

CONCLUSIONS

Overall, these results show that α-LA and DHLA can protect chicken muscle against iron and iron/ascorbate induced lipid oxidation. DHLA exhibited a significant antioxidant effect at lower concentrations than α-LA, suggesting that it may be a more potent antioxidant in muscle systems. The extent of the protection was dependent on the concentration of α-LA or DHLA and upon the type of oxidative stress used to induce lipid oxidation. Further work is required to evaluate the antioxidant effect of α-LA and DHLA in relation to other muscle cell prooxidants and antioxidants.

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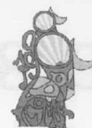
REFERENCES

Beuge, J.A. & Aust, S.D. (1978). *Meth. Enzymol.* **52,** 302-310.
Kagan, V.E., Shvedova A., Serbinova E., Khan S., Swanson C., Powell R. & Packer L. (1992). *Biochem. Pharm.* **44**, 1637-1649.
O'Neill, L.M., Galvin, K., Morrissey, P.A. & Buckley, D.J. (1998). *Meat Sci.* Submitted for publication.
Packer L., Witt E.H. & Tritschler H.J. (1995). *Free Rad. Biol. & Med.* **19,** 227-250.
Scott B.C., Aruoma O.I., Evans P.J., O'Neill C., Van Der Vliet A., Cross C.E., Tritschler H. & Halliwell B. (1994). *Free Rad. Res.* **20**, 119-133.
Snedecor, G.W. & Cochran, W.G. (1967). *Statistical Methods*, 6th edn., Iowa State University Press, Ames, Iowa.



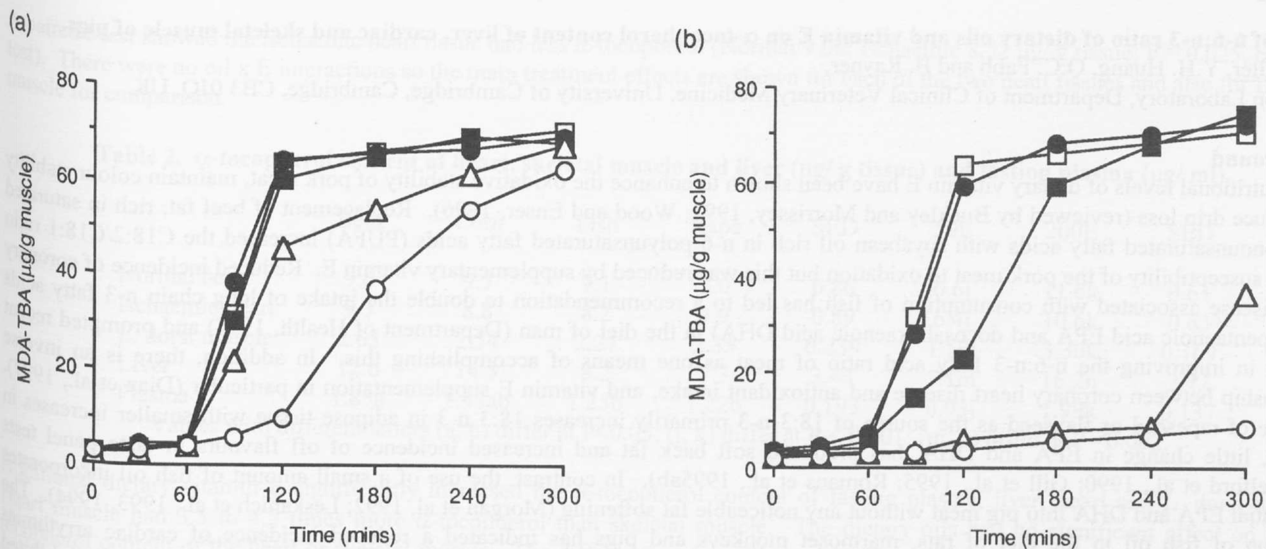


Figure 1. Effect of (a) α-lipoic acid (b) dihydrolipoic acid on iron induced lipid oxidation in chicken muscle homogenate. Values are means ± SEM of 6 analyses.
Control (□), 0.5 mM (●), 1 mM (■), 5 mM (△), 10 mM(○) α-lipoic acid or dihydrolipoic acid.

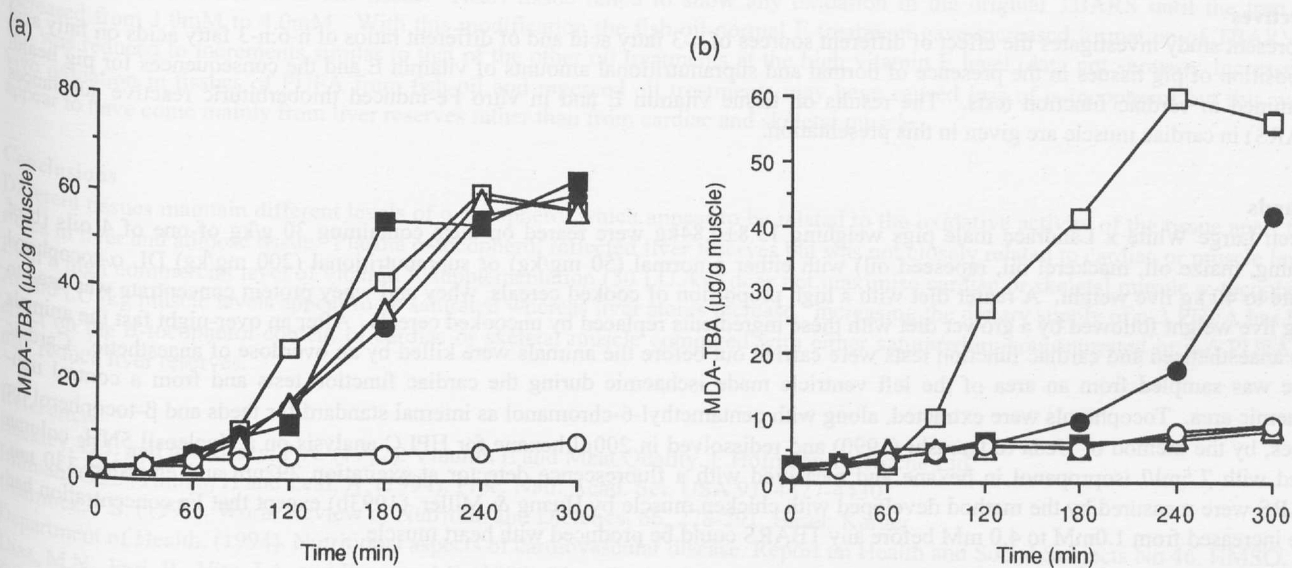


Figure 2. Effect of (a) α-lipoic acid (b) dihydrolipoic acid on iron/ascorbate induced lipid oxidation in chicken muscle homogenate. Values are means ± SEM of 6 analyses.
Control (□), 0.5 mM (●), 1 mM (■), 5 mM (△), 10 mM(○) α-lipoic acid or dihydrolipoic acid.