# Influence of Diet on Lipid Oxidation and Membrane Structure in Porcine Muscle Microsomes

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The effect of oxidized dietary lipid (corn oil) and dietary vitamin E ($\alpha$-tocopherol) on the fluidity of porcine muscle microsomal membranes was investigated. Using the membrane probe diphenylhexatriene, fluorescence anisotropy measurements showed that microsomal membrane fluidity decreased during $FeCl_2$-induced lipid peroxidation. Microsomes from pigs fed an $\alpha$-tocopherol-supplemented diet (200 mg of $\alpha$-tocopheryl acetate/kg of diet) were significantly less susceptible ($P < 0.05$) to $FeCl_2$-induced lipid peroxidation and to changes in membrane fluidity compared to muscle microsomes from pigs fed a basal diet (10 mg/kg of diet). Compared to unoxidized dietary corn oil, consumption of oxidized oil (4.5 mequiv of peroxide/kg of diet) did not significantly affect the rate of peroxidation of the microsomal lipids but led to a significant decrease in membrane fluidity. Results suggested that the reduced exudation in pork steaks from pigs fed supplemental $\alpha$-tocopherol, observed in this study and in an earlier study, did not appear to be directly related to oxidation-induced changes in membrane fluidity. However, further studies are needed to more adequately evaluate this observation.

## INTRODUCTION

Biological membranes function as important barriers to deteriorative changes that can affect the quality of foods of both plant and animal origin (Stanley, 1991). The oxidation of unsaturated fatty acids in biomembranes leads to a decrease in the fluidity of the biomembrane (Dobretsov et al., 1977) and disruption of normal membrane structure and function (Machlin and Bendich, 1987; Slater et al., 1987). In muscle foods, previous studies have implicated the oxidation of polyunsaturated membrane lipids in the development of off-flavors (Gray and Pearson, 1987), loss of fresh meat color (Faustman et al., 1989), and the formation of potentially harmful lipid oxidation products (Monahan et al., 1992b). Loss of muscle cell membrane integrity can also affect the ability of the biomembrane to act as a semipermeable barrier and may contribute to exudative loss from meat (Asghar et al., 1991; Stanley, 1991).

Membranal phospholipids are particularly susceptible to oxidation not only because of their high polyunsaturated fatty acid content but also because of their association in the cell membrane with enzymic and nonenzymic systems capable of generating prooxidative free-radical species (Svingen et al., 1979; Sevanian and Hochstein, 1985; Hsieh and Kinsella, 1989; Halliwell and Gutteridge, 1990). To effectively control lipid oxidation in muscle foods at the initiation stage, procedures that stabilize membranal lipids are of interest. Vitamin E ($\alpha$-tocopherol) is a membrane-associated antioxidant and as such can effectively quench highly reactive free-radical species capable of initiating and propagating lipid oxidation at the cell membrane level. Muscle tissue $\alpha$-tocopherol levels in animal and avian species have been shown to respond to dietary vitamin E (Brekke et al., 1975; Marusich et al., 1975; Jensen et al., 1988; Buckley et al., 1989; Faustman et al., 1989). Thus, dietary vitamin E supplementation has been shown to reduce the susceptibility of muscle membranal lipids to oxidation.

The objective of this study was to determine the effect of dietary vitamin E supplementation of pig diets on the integrity of the muscle microsomal membrane under peroxidizing conditions using the fluorescence probe 1,6-diphenyl-1,3,5-hexatriene (DPH).

## MATERIALS AND METHODS

**Reagents.** DPH was obtained from Molecular Probes (Eugene, OR) and 2-thiobarbituric acid from Sigma Chemical Co. Ltd. (St. Louis, MO). All other chemicals were obtained from Sigma, Fisher Scientific (Fair Lawn, NJ), or E. M. Science (Gibbstown, NJ).

**Animals and Diets.** Eighteen 4-month-old Yorkshire × Landrace pigs, averaging 30 kg, were divided into three groups of six. One group was fed a control diet containing 3% fresh corn oil (peroxide value, 2 mequiv of peroxide/kg of oil) as the principal dietary lipid source and vitamin E ($\alpha$-tocopheryl acetate) at a level of 10 mg/kg of diet (Monahan et al., 1993). The other groups were fed diets that differed from the control either in the degree of oxidation of the dietary corn oil (oxidized oil diet) or in the concentration of $\alpha$-tocopheryl acetate (supplemented diet). The oxidized oil diet contained 3% oxidized corn oil (peroxide value, 150 mequiv of peroxide/kg of oil) and the supplemented diet 200 mg of $\alpha$-tocopheryl acetate/kg of diet. The pigs were slaughtered when an average weight of 98 kg was reached, and, following overnight chilling of the carcasses, the Longissimus dorsi muscle was isolated from each carcass. Muscle samples were stored at −20 °C (3 months) prior to preparation of the microsomes.

**Fluorescence Anisotropy Studies.** Muscle microsomes were prepared as previously described (Kalyanaraman et al., 1979). Protein determinations were made following the Lowry procedure (Lowry et al., 1951).

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Muscle microsomal suspensions (1.45 ± 0.08 mg of protein/mL) were prepared in 2.0 mL of 0.05 M potassium phosphate buffer, pH 7.2, containing 1 mM $FeCl_2$. At 30-min intervals following the addition of $FeCl_2$, aliquots containing 0.5 mg of microsomal protein were removed from the incubation medium for measurement of lipid oxidation using the 2-thiobarbituric acid-reactive substances (TBARS) procedure (Buege and Aust, 1978). TBARS were reported as nanomoles of malonaldehyde (MDA) per milligram of protein. Aliquots equivalent to 0.1 mg of microsomal protein were removed simultaneously for fluorescence anisotropy measurements using the fluorescence probe DPH. DPH was incorporated, from a 0.2 mM solution in dimethylformamide, into microsomes (0.1 mg of microsomal protein/mL of potassium phosphate buffer, pH 7.2) at a concentration of 0.3 μM by hand homogenization with a Potter Elvehjem homogenizer. Fluorescence anisotropy measurements were made in 4 × 10 mm cuvettes at room temperature (22 °C) 15 min after the addition of the probe. Anisotropy measurements were made on an SLM Instruments, Model 4800, spectrofluorometer (Urbana, IL) interfaced to a 386 computer with data acquisition hardware from On-Line Instrument Systems (Bogart, GA). The samples were excited by vertically polarized light at 360 nm, and vertically and horizontally polarized components of the sample fluorescence, $I_\parallel$ and $I_\perp$, emitted through optical filters (Corning 3060) were detected in T-format. The steady-state anisotropy, $r_s$, was calculated as

$$rs = (I_\parallel - I_\perp)/(I_\parallel + 2I_\perp)$$

where $\parallel$ and $\perp$ are vertical and horizontal components of the polarized light, respectively. Corrections were made for intrinsic fluorescence and light scattering on the basis of

$$r = f_1 r_s + f_2 r_b$$

where $r_s$ is the anisotropy of the sample, $r_b$ is the anisotropy resulting from the blank, $r$ is the observed anisotropy, and $f_1$ and $f_2$ are the respective fractional intensities of the sample and blank (Lakowicz, 1983). The corrections never exceeded 7%.

**Lipid Oxidation and Drip Loss in Pork Steaks.** Pork steaks were prepared from fresh muscle and stored in refrigerated display as described previously (Monahan et al., 1992b). Lipid oxidation was assessed by the 2-thiobarbituric acid method of Ke et al. (1977). TBARS were expressed as milligrams of MDA per kilogram of muscle. Drip loss from pork steaks in refrigerated storage was calculated as the percentage reduction in weight from the day zero weight.

**Statistical Analysis.** Statistical significance of the difference between mean values was determined using Fisher's LSD test.

## RESULTS AND DISCUSSION

**Effect of Dietary Treatment on Microsomal Lipid Oxidation.** Figure 1 shows the effect of dietary oxidized lipid and α-tocopherol on TBARS of peroxidizing muscle microsomes. TBARS of microsomes from pigs fed the oxidized oil diet did not differ significantly from those of pigs fed the control diet. In contrast, when dietary peroxides were fed to pigs at twice the level used in the present study, Buckley et al. (1989) showed that oxidized dietary lipid had a destabilizing effect on muscle microsomal lipids. The effect of the oxidized dietary lipid on the peroxidizability of porcine muscle membranal lipids may be dose-related. In general, the pathological effects of oxidized dietary lipids appear to be related to level consumed (Kubow, 1990). Furthermore, dietary peroxides *per se* may not have a direct destabilizing effect on tissue lipids because of the unlikelihood of their being absorbed (Andrews et al., 1960; Bunyan et al., 1968). Rather, diets containing high levels of oxidized lipids may have lower α-tocopherol contents as a result of the destruction of native α-tocopherol during the oxidation process. Membranal α-tocopherol is lower in animals consuming oxidized lipids as reported earlier (Monahan et al., 1993). In the present study, the basal level of dietary α-tocopherol fed

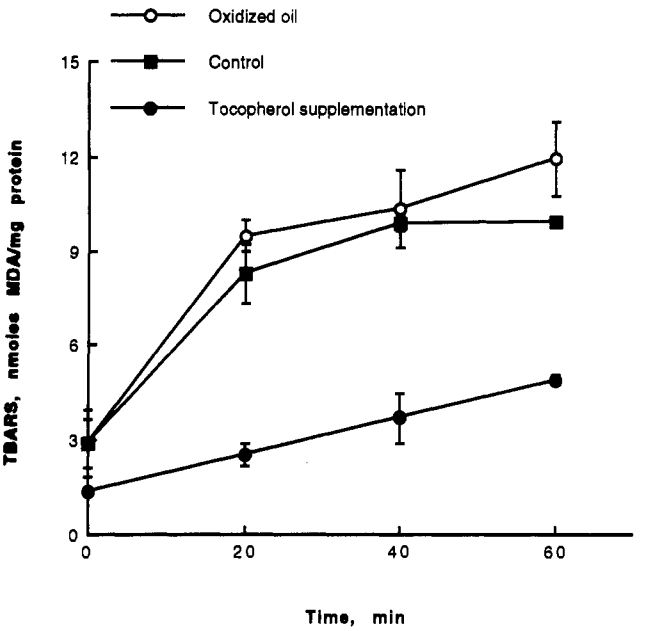


**Figure 1.** Effect of dietary oxidized oil and α-tocopherol supplementation on $Fe^{2+}$-induced lipid oxidation (mean TBARS ± SEM) in porcine muscle microsomes.

was probably sufficient to overcome any destabilizing effect of the peroxidizing lipids and maintain a favorable antioxidant/prooxidant balance in the muscle membranes (Sies, 1986).

In microsomes from pigs fed the α-tocopherol-supplemented diet, TBARS of peroxidizing lipids were significantly lower after 20, 40, and 60 min of incubation with $FeCl_2$ (Figure 1). The inhibitory effect of dietary α-tocopherol on microsomal lipid oxidation is well documented (Asghar et al., 1989; Buckley et al., 1989). The free-radical quenching ability of α-tocopherol of dietary origin has recently been demonstrated in peroxidizing porcine muscle microsomes (Monahan et al., 1993).

**Effect of Dietary Treatment on Membrane Structure.** Figure 2 shows the effect of the dietary treatments on the fluorescence anisotropy of DPH incorporated into muscle microsomes. In the presence of $Fe^{2+}$, fluorescence anisotropy, $r_s$, increased with increasing incubation time in all groups. This increase in $r_s$, indicative of a reduction in the mobility of DPH in the lipid bilayer and a decrease in membrane fluidity, is believed to result from an increase in the molecular order of the fatty acyl chains in the bilayer. Since DPH partitions equally well into the various lipid domains of biological membranes, the $r_s$ values obtained represent the weighted average over all domains (Shinitzky and Barenholtz, 1978). Previous studies using DPH as a fluorescence probe demonstrated an increased ordering of microsomal phospholipid (Eichenberger et al., 1982), erythrocyte membrane (Rice-Evans and Hochstein, 1981), and lecithin liposome (Fukuzawa et al., 1981) acyl chains during lipid oxidation induced by NADPH, phenylhydrazine, and $Fe^{2+}$/ascorbate, respectively. Similarly, using doxyl stearate spin probes with electron spin resonance spectroscopy, Bruch and Thayer (1983) and Ohki et al. (1984) showed that fluidity was markedly decreased in oxidizing soybean phospholipid liposomes and in rat liver microsomes, respectively. A number of reasons have been put forward to explain the decrease in membrane fluidity associated with membranal lipid oxidation. In general, a decrease in the relative proportion of unsaturated fatty acids in biological membranes leads to an increase in the molecular order of the membranal lipids (Nozawa et al.,

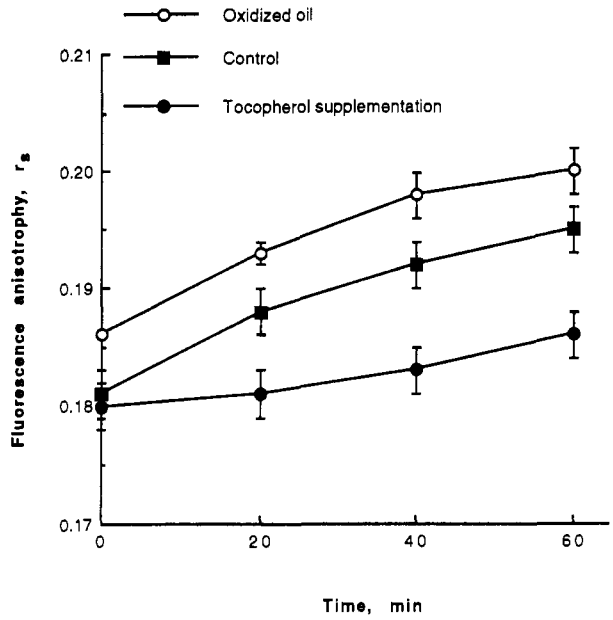


**Figure 2.** Effect of dietary oxidized oil and $\alpha$-tocopherol supplementation on steady-state fluorescence anisotropy (mean $r_s \pm$ SEM) of DPH at 22 °C in porcine muscle microsomes following $Fe^{2+}$-induced lipid oxidation.

1980). It has been proposed that oxidized fatty acyl chains are transported from the hydrophobic core of the membrane to the outside, resulting in a decrease in the unsaturated fatty acid content of the membrane (Witting, 1965; Dobretsov et al., 1977). Cross-linking either between acyl radicals (Eichenberger et al., 1982) or by the bifunctional malonaldehyde molecule, a product of polyunsaturated fatty acid oxidation (Tappel, 1980), may also be responsible for the effect.

Muscle microsomes from pigs fed the oxidized oil diet had significantly higher $r_s$ values than microsomes from pigs fed the control diet at each sampling time (Figure 2). Alterations in the degree of unsaturation of membranal lipids on feeding oxidized lipid may explain this effect. The fatty acid profiles of the polar (phospholipid) fractions from the muscle of pigs fed the various diets are shown in Table 1. The data show that polar lipids from pigs fed the oxidized corn oil had a smaller linoleic/oleic acid (C18:2/ C18:1) ratio than polar lipids from pigs fed the control or supplemental diets. Gould et al. (1987) showed that the fatty acid composition of rabbit muscle phospholipids was significantly altered by dietary lipid. In pigs, we also found that the fatty acid profile of the polar lipid fraction of porcine muscle was influenced by the degree of unsaturation of the dietary lipid (Monahan et al., 1992a). In the preparation of the oxidized oil a decrease in the degree of unsaturation of the oil due to the oxidation of unsaturated fatty acids occurs. Consumption of oxidized oil, containing proportionately more saturated fatty acids than unoxidized oil, may therefore bring about an increase in the saturated fatty acid content of the membranal phospholipids (Table 1). The higher $r_s$ values of microsomes from pigs fed the oxidized oil may reflect an increase in the degree of saturation of the membranal lipids and a concomitant decrease in membrane fluidity due to consumption of the oxidized oil.

In the presence of $Fe^{2+}$, microsomes from pigs fed the $\alpha$-tocopherol-supplemented diet were significantly less susceptible to change in $r_s$ compared to control microsomes (Figure 2). The repressive effect of $\alpha$-tocopherol appears to result from its antioxidative action and not from a physical effect of the molecule itself on membrane

**Table 1. Percentages of Some Fatty Acids in the Polar Lipid Fraction of Muscle from Pigs Fed Fresh and Oxidized Oil-Containing Diets**

| | dietary treatment | | |
|---|---|---|---|
| fatty acid | control (%)[a] | oxidized (%) | supplemented (%) |
| C14:0 | 1.2 ± 0.5[b] | 1.2 ± 0.5 | 0.7 ± 0.1 |
| C16:0 | 23.3 ± 0.8 | 23.3 ± 0.7 | 23.6 ± 0.6 |
| C16:1 | 0.9 ± 0.1 | 1.4 ± 0.2 | 1.3 ± 0.2 |
| C18:0 | 12.3 ± 0.7 | 12.2 ± 0.5 | 11.6 ± 0.3 |
| C18:1 | 20.4 ± 1.3 | 25.8 ± 2.1 | 21.1 ± 1.3 |
| C18:2 | 31.9 ± 2.4 | 27.0 ± 1.7 | 31.8 ± 1.3 |
| C18:3 | 2.0 ± 0.4 | 3.2 ± 0.5 | 1.5 ± 0.2 |
| C20:4 | 7.2 ± 0.9 | 6.0 ± 0.9 | 8.3 ± 0.6 |
| ratio C18:2/C18:1 | 1.6 ± 0.2 | 1.1 ± 0.2 | 1.6 ± 0.2 |

[a] Percent of total peak area of the fatty acids listed. [b] Mean value ± SEM of six analyses.

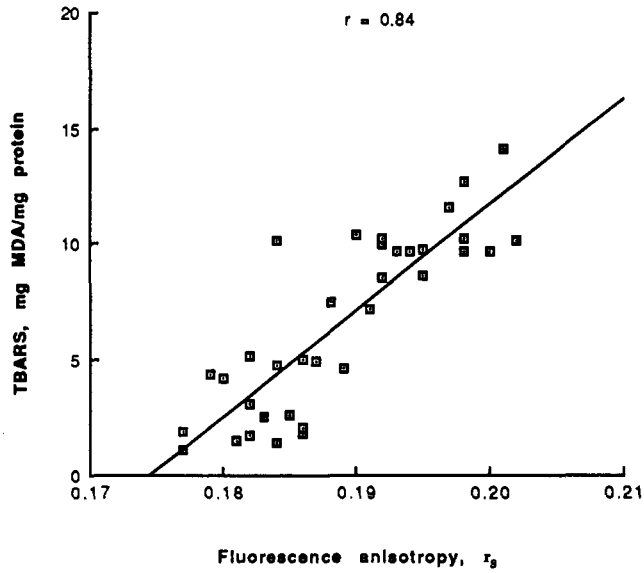


**Figure 3.** Relationship between lipid oxidation (TBARS) and steady-state fluorescence anisotropy ($r_s$) of DPH in muscle microsomes following $Fe^{2+}$-induced lipid oxidation.

structure since time zero anisotropy values (Figure 2) of control and supplemented microsomes did not differ. However, the temperature dependence of $r_s$ was not determined in this study. Changes in the $\alpha$-tocopherol content of the microsomal membrane could alter the membrane phase transition temperature so that, while there was no detectable difference in $r_s$ at room temperature (22 °C) immediately following the addition of $FeCl_2$ (time zero) (Figure 2), the $r_s$ values of microsomes from control and supplemented animals at 37 °C could be significantly different. Therefore, the possibility that the change in membrane fluidity could reflect both structural and antioxidant effects of $\alpha$-tocopherol cannot be ruled out. Ohki et al. (1984) showed that at concentrations in excess of physiological levels $\alpha$-tocopherol may alter membranal phospholipid mobility. However, at physiological concentrations our results agree with those of Ohki et al. (1984); i.e., $\alpha$-tocopherol exerts its effect on acyl chain mobility by inhibiting oxidation of unsaturated fatty acids. Similarly, Fukuzawa et al. (1981) showed that incorporation of 0.4 mol % of $\alpha$-tocopherol into lecithin liposomes did not affect the fluorescence anisotropy of DPH but $r_s$ increased when a 10-fold higher concentration of $\alpha$-tocopherol was incorporated. At both concentrations $\alpha$-tocopherol effectively inhibited $Fe^{2+}$/ascorbate-induced lipid peroxidation.

Figure 3 shows the relationship between fluorescence anisotropy ($r_s$) and lipid oxidation product formation (TBARS). The data indicate a strong correlation ($r$ =

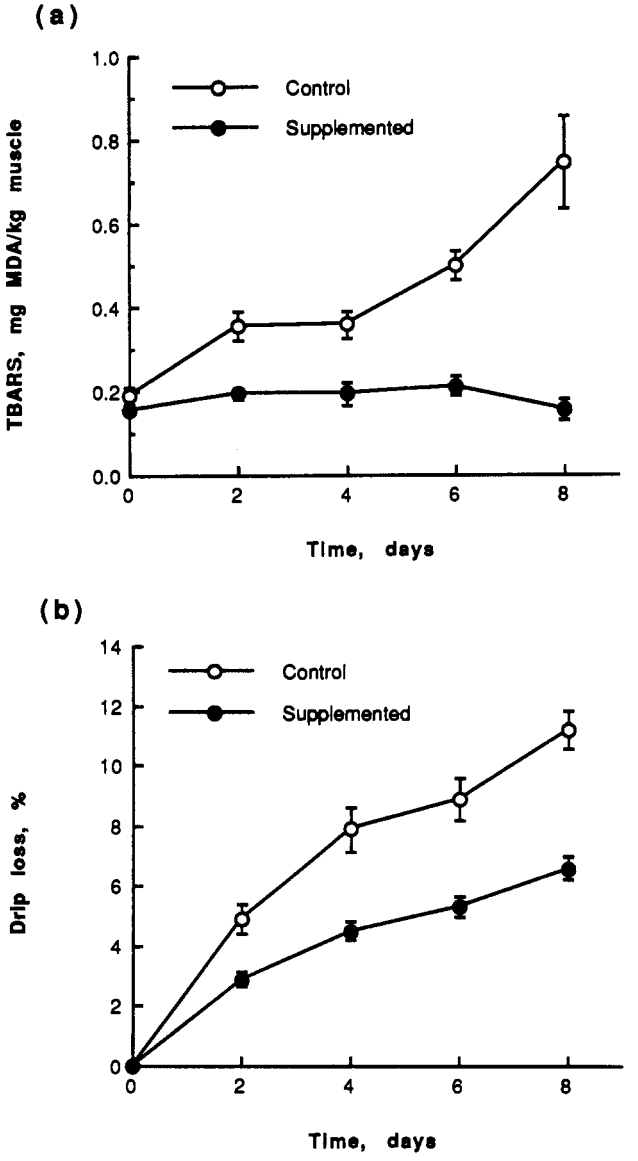


**Figure 4.** Effect of dietary α-tocopherol supplementation on (a) lipid oxidation and (b) drip loss from pork steaks in refrigerated storage.

0.84) between TBARS and $r_s$ of muscle microsomes in the presence of $Fe^{2+}$ and lend further support to the contention that a relationship exists between lipid oxidation and alteration in membrane structure.

**Relationship between Lipid Oxidation and Surface Exudation in Muscle.** Asghar et al. (1991) reported that drip loss from thawing pork steaks was lower in muscle from pigs fed α-tocopherol-supplemented diets (100 and 200 mg of α-tocopherol acetate/kg of diet) compared to control animals. As a possible mechanism, the authors suggested that α-tocopherol could preserve the integrity of muscle cell membranes by preventing the oxidation of membranal phospholipids during refrigerated storage. The results presented so far indicate that there may be a relationship between lipid oxidation in a model muscle microsomal system undergoing lipid oxidation and the fluidity of the lipid bilayer.

Figure 4 shows the time courses of lipid oxidation product formation (TBARS) and drip loss in fresh pork steaks placed in refrigerated storage. While dietary α-tocopherol supplementation led to a reduction in both lipid oxidation and drip loss, the two processes are not directly related: drip loss increased at a more rapid initial rate than TBARS in control pork, and in the supplemented pork considerable drip loss occurred even though lipid oxidation was negligible. It is possible that measurement of primary oxidative changes, such as the formation of lipid peroxides, would give a better estimate of lipid oxidation occurring during the first days of storage and hence better evaluate the relationship (if any) between lipid oxidation and drip loss. As suggested earlier, the effect of α-tocopherol on membrane fluidity may arise from the physical interaction of α-tocopherol with the membrane lipids and not solely from its antioxidative action (Diplock, 1985; Asghar et al., 1991). This appeared not to be the case since, in the present study, the initial (time zero) fluorescence anisotropy measurements in control and α-tocopherol-supplemented microsomes were not different (Figure 2). However, the system under study is a complex one, and the experimental results may be influenced by probe type and membrane location (Bruch and Thayer, 1983; Kitagawa et al., 1991) and by the temperature at which measurements are made. Altering the α-tocopherol content of microsomal membranes by dietary means may have had a structural effect on the microsomal membrane that was not detectable using the methodology employed in this study. In addition, should α-tocopherol exert a structural effect on one particular lipid domain in the membrane, this may not have been detected since acyl chain mobility, determined by DPH fluorescence anisotropy, represents a weighted average over all domains. Alternatively, small changes in the lipid microenvironment as a result of lipid peroxidation or changes in α-tocopherol concentration may affect the activity of phospholipases (Dennis, 1983; Asghar et al., 1991). Since phospholipases cleave fatty acid moieties from phospholipids, changes in phospholipase activity, as opposed to the oxidation of membrane lipids, may explain the differences in drip loss between groups.

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LITERATURE CITED

Andrews, J. S.; Griffith, W. H.; Mead, J. F.; Stein, R. A. Toxicity of air-oxidized soybean oil. *J. Nutr.* **1960**, *70*, 199–210.

Asghar, A.; Lin, C. F.; Gray, J. I.; Buckley, D. J.; Booren, A. M.; Crackel, R. L.; Flegal, C. J. Influence of oxidized dietary oil and antioxidant supplementation on membrane-bound lipid stability in broiler meat. *Br. Poult. Sci.* **1989**, *30*, 815–823.

Asghar, A.; Gray, J. I.; Booren, A. M.; Gomaa, E. A.; Abouzied, M. M.; Miller, E. R.; Buckley, D. J. Effect of supranutritional vitamin E levels on subcellular deposition of α-tocopherol in the muscle and on pork quality. *J. Sci. Food Agric.* **1991**, *57*, 31–41.

Brekke, C. J.; Al-Hakim, S. H.; Highlands, M. E.; Hogan, J. M. The relation of *in vivo* and *in vitro* tocopherol supplementation to stability of fowl fat. *Poult. Sci.* **1975**, *54*, 2019–2024.

Bruch, R. C.; Thayer, W. S. Differential effect of lipid peroxidation on membrane fluidity as determined by electron spin resonance probes. *Biochim. Biophys. Acta* **1983**, *733*, 212–222.

Buckley, D. J.; Gray, J. I.; Asghar, A.; Booren, A. M.; Crackel, R. L.; Price, J. F.; Miller, E. R. Effects of dietary antioxidants and oxidized oil on membranal lipid stability and pork product quality. *J. Food Sci.* **1989**, *54*, 1193–1197.

Buege, J. A.; Aust, S. D. Microsomal lipid peroxidation. *Methods Enzymol.* **1978**, *52*, 302–310.

Bunyan, J.; Green, J.; Murrell, E. A.; Diplock, A. T.; Cawthorne, M. A. On the postulated peroxidation of unsaturated lipids in the tissues of vitamin E-deficient rats. *Br. J. Nutr.* **1968**, *22*, 97–110.

Dennis, E. A. Phospholipases. In *The Enzymes*; Boyer, P. D., Ed.; Academic Press: New York, 1983; Vol. 16, pp 307–353.

Diplock, A. T. Vitamin E. In *Fat-soluble Vitamins*; Diplock, A. T., Ed.; Heinemann: London, 1985; pp 154–224.

Dobretsov, G. E.; Borschevskaya, T. A.; Petrov, V. A.; Vladimirov, Y. A. The increase of phospholipid bilayer rigidity after lipid peroxidation. *FEBS Lett.* **1977**, *84*, 125–128.

Eichenberger, K.; Bohni, P.; Winterhalter, K. H.; Kawato, S.; Richter, C. Microsomal lipid peroxidation causes an increase in the order of the membrane lipid domain. *FEBS Lett.* **1982**, *142*, 59–62.

Faustman, C.; Cassens, R. G.; Schaefer, D. M.; Buege, D. R.; Williams, S. N.; Scheller, K. K. Improvement of pigment and lipid stability in Holstein steer beef by dietary supplementation with vitamin E. *J. Food Sci.* **1989**, *54*, 858–862.

Fukuzawa, K.; Chida, H.; Tokumura, A.; Tsukatani, H. Antioxidative effect of α-tocopherol incorporation into lecithin liposomes on ascorbic acid-$Fe^{2+}$-induced lipid peroxidation. *Arch. Biochem. Biophys.* **1981**, *206*, 173–180.

Gould, G. W.; McWhirter, J. M.; East, J. M.; Lee, A. G. Effects of diet on the function of sarcoplasmic reticulum. *Biochem. J.* **1987**, *245*, 751–755.

Gray, J. I.; Pearson, A. M. Rancidity and warmed-over flavor. *Adv. Meat Res.* **1987**, *3*, 221–269.

Halliwell, B.; Gutteridge, J. M. C. Role of free radicals and catalytic metal ions in human disease: an overview. *Methods Enzymol.* **1990**, *186*, 1–85.

Hsieh, R. J.; Kinsella, J. E. Oxidation of polyunsaturated fatty acids: mechanisms, products, and inhibition with emphasis on fish. *Adv. Food Nutr. Res.* **1989**, *33*, 233–241.

Jensen, M.; Hakkarainen, J.; Lindholm, A.; Jonsson, L. Vitamin E requirement of growing swine. *J. Anim. Sci.* **1988**, *66*, 3101–3111.

Kalyanaraman, B.; Mason, R. P.; Perez-Reyes, E.; Chignell, C. F.; Wolf, C. R.; Philpot, R. M. Characterization of the free radical formed in aerobic microsomal incubations containing carbon tetrachloride and NADPH. *Biochem. Biophys. Res. Commun.* **1979**, *89*, 1065–1072.

Ke, P. J.; Ackman, R. G.; Linke, B. H.; Nash, D. M. Differential lipid oxidation in various parts of frozen mackerel. *J. Food Technol.* **1977**, *12*, 37–47.

Kitagawa, S.; Matsubayashi, M.; Kotani, K.; Usui, K.; Kametani, F. Asymmetry of membrane fluidity in the lipid bilayer of blood platelets: fluorescence study with diphenylhexatriene and analogs. *J. Membr. Biol.* **1991**, *119*, 221–227.

Kubow, S. Toxicity of dietary lipid oxidation products. *Trends Food Sci. Technol.* **1990**, *1* (3), 67–71.

Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Plenum Press: New York, 1983.

Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265–275.

Machlin, L. J.; Bendich, A. Free radical tissue damage: protective role of antioxidant nutrients. *FASEB J.* **1987**, *1*, 441–445.

Marusich, W. L.; De Ritter, E.; Ogrinz, E. F.; Keating, J.; Mitrovic, M.; Bunnell, R. H. Effect of supplemental vitamin E in control of rancidity in poultry meat. *Poult. Sci.* **1975**, *54*, 831–844.

Monahan, F. J.; Buckley, D. J.; Morrissey, P. A.; Lynch, P. B.; Gray, J. I. Influence of dietary fat and α-tocopherol supplementation on lipid oxidation in pork. *Meat Sci.* **1992a**, *31*, 229–241.

Monahan, F. J.; Gray, J. I.; Booren, A. M.; Miller, E. R.; Buckley, D. J.; Morrissey, P. A.; Gomaa, E. A. Influence of dietary treatment on cholesterol oxidation in pork. *J. Agric. Food Chem.* **1992b**, *40*, 1310–1315.

Monahan, F. J.; Gray, J. I.; Asghar, A.; Haug, A.; Shi, B.; Buckley, D. J.; Morrissey, P. A. Effect of dietary lipid and vitamin E supplementation on free radical production and lipid oxidation in porcine muscle microsomal fractions. *Food Chem.* **1993**, *46*, 1–6.

Nozawa, Y.; Kasai, R.; Kameyama, Y.; Ohki, K. Age-dependent modifications in membrane lipids: lipid composition, fluidity and palmitoyl CoA desaturase in *Tetrahymena* membranes. *Biochim. Biophys. Acta* **1980**, *599*, 232–245.

Ohki, K.; Takamura, T.; Nozawa, Y. Effect of α-tocopherol on lipid peroxidation and acyl chain mobility of liver microsomes from vitamin E-deficient rat. *J. Nutr. Sci. Vitaminol.* **1984**, *30*, 221–234.

Rice-Evans, C.; Hochstein, P. Alterations in erythrocyte membrane fluidity by phenylhydrazine-induced peroxidation of lipids. *Biochem. Biophys. Res. Commun.* **1981**, *100*, 1537–1542.

Sevanian, A.; Hochstein, P. Mechanism and consequences of lipid peroxidation in biological systems. *Annu. Rev. Nutr.* **1985**, *5*, 365–390.

Shinitzky, M.; Barenholz, Y. Fluidity parameters of lipid regions determined by fluorescence polarization. *Biochim. Biophys. Acta* **1978**, *515*, 367–394.

Sies, H. Oxidative stress: introductory remarks. In *Oxidative Stress*; Sies, H., Ed.; Academic Press: Orlando, FL, 1986; pp 1–8.

Slater, T. F.; Cheeseman, K. H.; Davies, M. J.; Proudfoot, K.; Xin, W. Free radical mechanisms in relation to tissue injury. *Proc. Nutr. Soc.* **1987**, *46*, 1–12.

Stanley, D. W. Biological membrane deterioration and associated quality losses in food tissues. *Crit. Rev. Food Sci. Nutr.* **1991**, *30*, 487–553.

Svingen, B. A.; Buege, J. A.; O'Neal, F. O.; Aust, S. D. The mechanism of NADPH-dependent lipid peroxidation. *J. Biol. Chem.* **1979**, *254*, 5892–5899.

Tappel, A. L. Measurement of and protection from *in vivo* lipid peroxidation. In *Free Radicals in Biology*; Pryor, W. A., Ed.; Academic Press: New York, 1980; Vol. IV, pp 1–47.

Witting, L. Lipid peroxidation *in vivo*. *J. Am. Oil Chem. Soc.* **1965**, *42*, 908–913.

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