ARTICLES

Influence of Dietary Treatment on Lipid and Cholesterol Oxidation in Pork

Frank J. Monahan,† J. Ian Gray,*,‡ Alden M. Booren,‡ Elwyn R. Miller,§ D. Joseph Buckley,† Patrick A. Morrissey,∥ and Enayat A. Gomaa‡

Departments of Food Technology and Nutrition, University College, Cork, Republic of Ireland, and Department of Food Science and Human Nutrition and Department of Animal Science, Michigan State University, East Lansing, Michigan 48824

The effect of oxidized corn oil and α-tocopherol acetate in pig diets on oxidation of lipids in raw and cooked pork was studied. Oxidation was significantly influenced by dietary α-tocopherol supplementation but not by oxidized corn oil. The rate of formation of lipid and cholesterol oxidation products was low in raw samples compared to that in cooked samples. After 2 days of refrigerated storage, total cholesterol oxides represented 2.7% of total cholesterol content of cooked pork from pigs fed the basal level of α-tocopherol acetate (10 mg/kg of diet) and 1.6% of total cholesterol in pork from pigs fed the supplemented diet (200 mg/kg). Lipid and cholesterol oxide product formation were positively correlated in cooked pork ($r = 0.88$, $P < 0.01$). The results of the present study indicate that increasing the α-tocopherol content of muscle by dietary means can reduce cholesterol oxide formation in muscle foods.

INTRODUCTION

Unsaturated fatty acids and cholesterol undergo autoxidation by a free-radical mechanism involving the abstraction of a labile hydrogen from the lipid molecule followed by the addition of molecular oxygen to the resultant lipid radical (Lundberg, 1962; Smith, 1981). The lipid peroxy radicals formed undergo a series of reactions leading to the formation of a number of oxidation products (Mottram, 1987; Morrissey and Apte, 1988; Smith, 1981). In muscle foods, lipid oxidation has been associated with quality deterioration caused by the development of off-flavors and off-odors during storage (Gray and Pearson, 1987). A variety of aldehydes, ketones, and organic acids arising from the breakdown of lipid hydroperoxides contribute to the sensory properties of meats particularly after cooking (Mottram, 1987).

More recently, interest in the possible toxicological effects of lipid oxidation products, particularly cholesterol oxidation products (COPS), has increased (Addis and Park, 1989; Kubow, 1990). In vivo free-radical-mediated lipid peroxidation has been implicated in a variety of pathological processes including inflammation and rheumatoid arthritis (Halliwell and Gutteridge, 1985), atherosclerosis (Quinn et al., 1987), mutagenesis and carcinogenesis (Ames et al., 1982; O'Brien, 1982). However, the influence of dietary lipid oxidation products on these processes is largely speculative. Malonaldehyde has been shown to be carcinogenic in mice (Shamberger et al., 1974) and to be mutagenic in the Ames *Salmonella* assay (Mukai and Goldstein, 1976), but the validity of these results has been challenged by Ames et al. (1982).

Animal studies provide more compelling evidence implicating dietary cholesterol oxides as the initiators of atherosclerotic lesions in blood vessels (Taylor et al., 1979; Imai, 1980; Peng et al., 1985, 1987). COPS have been detected in a variety of processed food products, including powdered milk (Nourooz-Zadeh and Appelqvist, 1988), grated cheeses (Finocchiaro et al., 1984), butter (Csiky, 1982), and spray-dried egg products (Morgan and Armstrong, 1987). COPS have also been detected in raw, cooked, and processed meats (Higley et al., 1986; Park and Addis, 1987; Pie et al., 1991). Pie et al. (1991) found that COPS increased significantly on cooking of meats and during subsequent refrigerated storage. Plasma COPS levels in humans have been shown to increase with increased dietary intake of cholesterol oxides (Emanuel et al., 1991).

In a series of studies, we have investigated the effect of α-tocopherol supplementation of pig diets on lipid oxidation in pork (Monahan et al., 1990a,b). Previous studies also examined the effect of degree of unsaturation (Monahan et al., 1992) and peroxide value (Buckley et al., 1989) of dietary lipid on lipid oxidation in pork. The present study was conducted to determine the effect of oxidized dietary lipid on cholesterol oxidation in pork and to investigate the effectiveness of dietary α-tocopherol in controlling cholesterol oxidation. The relationship between lipid and cholesterol oxidation was also examined.

MATERIALS AND METHODS

Reagents. Cholest-5-en-3β-ol (cholesterol), cholest-5-ene-3β,7α-diol (7α-hydroxycholesterol), cholest-5-ene-3β,7β-diol (7β-hydroxycholesterol), cholest-5-ene-3β,20α-diol (20α-hydroxycholesterol), cholest-5-ene-3β,25-diol (25-hydroxycholesterol), 7-oxocholest-5-en-3β-ol (7-ketocholesterol), cholestan-3β,5α,6β-triol (triol), 5β,6β-epoxycholestan-3β-ol (β-epoxide), 5α,6α-epoxycholestan-3β-ol (α-epoxide), 6-oxo-5α-cholestan-3β-ol (6-ketocholesterol), and 5α-cholestane were obtained from

* Author to whom correspondence should be addressed.
† Department of Food Technology, University College.
‡ Department of Food Science and Human Nutrition, Michigan State University.
§ Department of Animal Science, Michigan State University.
∥ Department of Nutrition, University College.

0021-8561/92/1440-1310$03.00/0 © 1992 American Chemical Society

Steraloids Inc., Wilton, NH. Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was obtained from Pierce Chemical Co., Rockford, IL. All other chemicals were obtained from Sigma Chemical Co. Ltd., St. Louis, MO, Fischer Scientific, Fair Lawn, NJ, or E. M. Science, Gibbstown, NJ.

Feeding Regimen. Seventy-two Yorkshire × Landrace pigs (barrows and gilts), 80–90 days old and averaging 30 kg in weight, were divided into six groups of 12. Each group was balanced with respect to litter mate, body weight, and sex by a restricted randomization technique. Pigs were allocated randomly to receive a grower diet (Loudenslager et al., 1986) containing either 3% fresh corn oil (peroxide value, 2 mequiv/kg of oil) or 3% oxidized corn oil (peroxide value, 150 mequiv/kg of oil) with 10, 100, or 200 mg of α-tocopherol acetate/kg of diet. Diets were prepared at 2-week intervals and stored at ambient temperatures. The pigs were housed in an environmentally controlled, complete confinement, slatted floor swine facility at the Michigan State University Swine Research Farm. The pigs were given feed and water ad libitum. The average weight of the pigs at the time of slaughter was 98 kg.

Sampling Procedure. Feed samples, for α-tocopherol analysis, were taken from each batch on the day of manufacture and at the end of each 2-week storage period. Blood samples, for plasma α-tocopherol analysis, were taken at 4-week intervals during the feeding trial and at slaughter. Six pigs were randomly selected from each group for slaughtering at a local commercial slaughtering facility. Following evisceration, the carcasses were chilled overnight. One loin was removed from each chilled carcass and held at 0–4 °C prior to processing. Boneless pork chops (approximately 1.5 cm in thickness) were obtained from each loin within 12 h of removal from the carcasses. The remainder of each loin was vacuum packaged and stored at −20 °C until required for further analysis. At various time intervals over a 6-month period, additional pork chops were removed from each frozen loin for α-tocopherol analysis and lipid and cholesterol oxidation studies.

Sample Preparation. Pork chops were placed on polystyrene trays (six per tray), wrapped with a retail commercial oxygen-permeable PVC stretch overwrap, and stored at 4 °C under fluorescent light for up to 8 days. The extent of lipid and cholesterol oxidation was assessed at 48-h intervals. To simulate the manufacture of a processed meat product, chops were trimmed of extramusclar fat and ground while still partially frozen through a grinder plate with 8-mm holes (Hobart Manufacturing Co., Troy, OH). Samples (120 g) of the ground muscle were placed in open retortable bags (22 × 17.5 cm) and cooked by immersion in a water bath at 70 °C for 30 min. Following cooking, the samples were cooled and assessed for lipid and cholesterol oxidation, immediately and at 48-h intervals over a 4-day refrigerated storage period.

Measurement of Lipid Oxidation. Lipid oxidation was assessed by the 2-thiobarbituric method of Ke et al. (1977). Thiobarbituric acid reactive substances (TBARS) values were expressed as milligrams of malonaldehyde per kilogram of muscle.

Cholesterol and Cholesterol Oxide Determination. Cholesterol was extracted from muscle samples following the direct saponification procedure of Adams et al. (1986). The cholesterol content of underivatized cholesterol extracts in dimethylformamide was quantified by GC analysis on a HP 5840A gas chromatograph equipped with a flame ionization detector and a glass column (2 m × 2 mm i.d.) containing 1% SE-30 on 100/120 Gas Chrom Q. The carrier gas was nitrogen, and the GC was operated isothermally at 230 °C. Injector port and detector temperatures were 220 and 275 °C, respectively.

Total lipid extracts, for cholesterol oxide analysis, were prepared from 5-g muscle samples (raw and cooked) according to the method of Marmer and Maxwell (1981). The lipid extracts, in dichloromethane/methanol (90:10) containing 0.005% butylated hydroxytoluene (BHT), were evaporated to dryness on a rotary evaporator (Brinkmann Instruments Inc., Westbury, NY) and redissolved in 5 mL of 9:1 hexane/ethyl acetate. Cholesterol oxides were separated from cholesterol and other muscle lipids following the sample cleanup procedure of Park and Addis (1985). Prior to sample cleanup, 6-ketocholesterol was incorporated into the hexane/ethyl acetate extracts as an internal standard. Acetone extracts were evaporated to dryness by rotary evaporation and redissolved in 4 mL of ethyl acetate. Prior to GC analysis, 1 mL of each ethyl acetate extract was evaporated to dryness under nitrogen and trimethylsilyl (TMS) derivatives of cholesterol oxides were prepared by redissolving the COPS in 100 μL of BSTFA and holding at room temperature for 30 min. The TMS ether sterols were then evaporated to dryness under nitrogen and redissolved in 100 μL of ethyl acetate. A Hewlett-Packard 5890 gas chromatograph with flame ionization detection equipped with a HP 5673A automatic injector and a HP 3392A integrator was used to quantify cholesterol oxidation products. COPS were separated on a fused silica capillary column DB-1 (15 m × 0.25 mm i.d., film thickness 0.25 μm, J&W Scientific, Folsom, CA). Carrier gas (helium) was delivered at 1.2 mL/min. Oven temperature programming was as follows: 170 to 220 °C at 10 °C/min and held isothermally at 220 °C for 5 min; 220 to 234 °C at 0.4 °C/min; 234 °C to 255 °C at 1.5 °C/min and held isothermally at 255 °C for 40 min. Injector port and detector temperatures were 275 and 330 °C, respectively. Four microliters of the TMS derivatives of the COPS were injected onto the column with a split ratio of 11:1.

Determination of α-Tocopherol. α-Tocopherol was extracted from feed, plasma, and muscle samples following the methods of Brubacher et al. (1985), Bieri et al. (1979), and Buttriss and Diplock (1984), respectively. In the case of feed and muscle, α-tocopherol was extracted, following saponification, into diethyl ether and hexane, respectively. Both solvents contained 0.005% BHT. Percentage recoveries were determined by the addition of known quantities of α-tocopherol to samples prior to extraction. Following extraction, the resulting peak areas on chromatograms were compared with those obtained by direct injection onto the column of standard α-tocopherol solutions. In the determination of plasma α-tocopherol, α-tocopherol acetate was incorporated into samples as an internal standard. Percentage recovery of α-tocopherol from plasma samples was determined by comparison of peak areas of α-tocopherol acetate recovered with those obtained by direct injection of α-tocopherol acetate onto the column. HPLC quantitation of α-tocopherol was carried out with a Waters Model 6000A pump equipped with a μBondapak reversed-phase C_{18} column (25 cm × 4 mm) and UV detection at 294 nm (Waters Model 440 UV detector). The eluting solvent was methanol/water (97:3) at a flow rate of 2.5 mL/min.

Statistical Analysis. The data were subjected to analysis of variance utilizing a completely randomized split plot design (Steel and Torrie, 1980). Fisher's LSD test was applied to determine the significance of differences between mean values.

RESULTS AND DISCUSSION

Concentrations of α-Tocopherol in Feed, Plasma, and Muscle Tissue. The α-tocopherol contents of the pig diets were generally found to be higher than the levels at which α-tocopherol acetate was incorporated into the diets during diet preparation. The additional α-tocopherol may be attributed to the α-tocopherol naturally present in the feed ingredients (Baurenfeind, 1980). The inclusion of oxidized oil in the diets had a pronounced effect on the α-tocopherol content of diets containing the basal level of α-tocopherol where oxidized oil-containing diets had almost 50% less α-tocopherol than diets prepared with the fresh oil (Table II). This suggests that native α-tocopherol in the corn oil may have been destroyed during the oxidation process, thus diminishing its contribution to the overall α-tocopherol content of the diet. In the supplemented diets, the relative contributions of α-tocopherol from feed ingredients were lower and hence the effect of the oxidized oil on total α-tocopherol content was less pronounced.

Plasma and muscle α-tocopherol levels were significantly influenced by dietary α-tocopherol (Table I), in agreement with previous studies (Jensen et al., 1988; Monahan et al., 1990a,b). The results of several earlier studies suggest that oxidized dietary lipids may promote vitamin E deficiency symptoms in pigs (Thafvelin, 1960; Grant, 1966; Simesen et al., 1979). In the present study, α-tocopherol concentrations in plasma or muscle of pigs receiving the

Table I. Mean α-Tocopherol Content of Pig Diets and of Plasma and Muscle from Pigs Fed Diets Containing Oxidized or Fresh Corn Oil with 10, 100, or 200 mg of α-Tocopherol Acetate/kg of Diet

group	dietary treatment	α-tocopherol concn		
		diet, mg/kg	plasma,[a] μg/mL	muscle,[a] μg/g
1	oxidized oil + α-tocopherol (10 mg/kg)	12.7	0.20[a]	0.45[a]
2	fresh oil + α-tocopherol (10 mg/kg)	23.5	0.53[a]	0.78[a]
3	oxidized oil + α-tocopherol (100 mg/kg)	135.0	2.01[b]	2.98[b]
4	fresh oil + α-tocopherol (100 mg/kg)	140.6	1.47[b]	2.58[b]
5	oxidized oil + α-tocopherol (200 mg/kg)	226.3	3.10[c]	4.19[c]
6	fresh oil + α-tocopherol (200 mg/kg)	214.7	3.54[c]	4.07[c]

[a] Means in the same column bearing different superscripts are significantly different ($P < 0.05$).

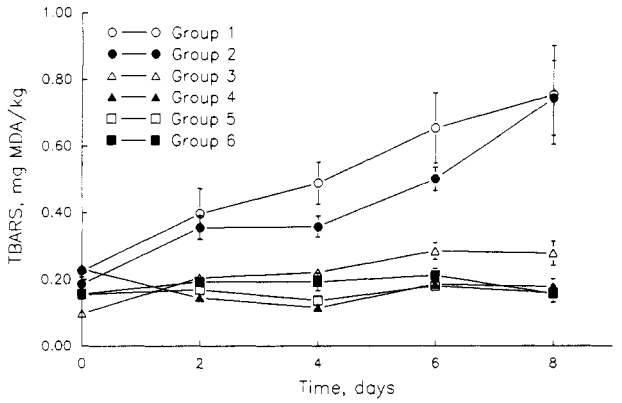

Figure 1. Effect of dietary oil and α-tocopherol supplementation on TBARS (mean ± SEM) of fresh pork chops during storage at 4 °C for 8 days. See Table I for description of dietary treatments (groups).

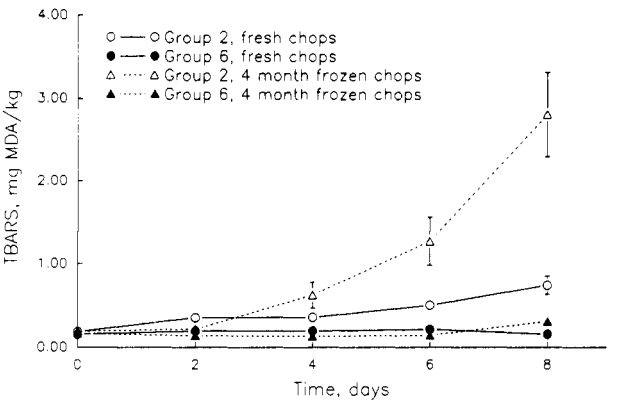

Figure 2. Effect of freezer storage (−20 °C) and dietary α-tocopherol supplementation on TBARS (mean ± SEM) of pork chops during storage at 4 °C for 8 days. See Table I for explanation of groups.

basal level of α-tocopherol were lower in pigs fed the oxidized oil compared to concentrations in pigs fed the fresh oil. However, differences due to dietary oil were not significant. Thode-Jensen et al. (1983) also reported that plasma vitamin E was not influenced by feeding oxidized fat to pigs from 4 to 11 weeks of age. In previous studies, differences in the type of oxidized oil fed to pigs, and in the degree to which it had been oxidized (Kubow, 1990), may account for some of the variation in reports pertaining to the effect of dietary oxidized lipid on tissue vitamin E levels. Simesen et al. (1979) reported, for example, that feeding oxidized herring oil appeared to enhance the vitamin E requirement of pigs but oxidized lard did not.

Lipid Oxidation in Raw and Cooked Pork. Analysis of variance of the lipid oxidation data for raw and cooked pork revealed that the TBARS were significantly influenced by dietary α-tocopherol ($P < 0.01$) but not by the type of dietary lipid fed to the pigs. The extent of lipid oxidation was significantly higher ($P < 0.01$) in uncooked pork chops from pigs fed the basal diet after 2, 4, 6, and 8 days of storage at 4 °C (Figure 1) when compared to each of the supplemented groups. With regard to these latter groups, lipid oxidation tended to be lower in chops from pigs fed 200 mg of α-tocopherol acetate/kg of diet compared to pigs fed 100 mg/kg, but the differences were not statistically significant. In the case of uncooked pork chops placed in refrigerated storage after 4 months of frozen storage, TBARS were higher in chops from all groups compared to fresh chops (Figure 2). Again, significant protection against lipid oxidation during refrigerated storage was observed in chops from pigs fed the supplemented diet compared to the basal diet. The antioxidative effect of α-tocopherol during frozen storage was demonstrated by the increased tendency to oxidize of chops from pigs fed the basal diet compared to the supplemented group after the pork had been frozen for 4 months (Figure 2). These results confirm the findings of previous researchers relative to the antioxidative effect of α-tocopherol in uncooked pork (Astrup, 1973; Buckley et al., 1989) and other meats (Marusich et al., 1975; Shorland et al., 1981; Frigg et al., 1990).

In previous studies (Monahan et al., 1990a,b, 1992), we have observed variability in the tendency of raw pork to oxidize. It is likely that postslaughter handling, particularly the effectiveness of maintaining pork in a chilled state after removal from the slaughterhouse chiller, influences the susceptibility of raw pork to oxidize during subsequent refrigerated storage. The occurrence of microbiological spoilage during refrigerated storage is also related to the effectiveness of postslaughter chilling. Thus, the practical significance of retarding lipid oxidation in raw pork during prolonged aerobic refrigerated storage is questionable, since microbiological spoilage may be at least as significant as lipid oxidation in contributing to deterioration in meat quality. Further investigations are currently underway in this area. In addition, the degree to which raw meat has been processed influences its propensity to undergo oxidative deterioration (Buckley et al., 1989). Increasing the α-tocopherol content of raw meats may therefore be beneficial in situations where factors such as grinding during processing increase the tendency of the muscle lipids to oxidize.

The susceptibility of muscle tissue from different species to oxidize has been shown to be related to the degree of unsaturation of the muscle lipids (Allen and Foegeding, 1981; Tichivangana and Morrissey, 1985). In meats held at refrigeration temperatures, increased muscle α-tocopherol may therefore have a more beneficial antioxidative effect in those meats containing a higher proportion of unsaturated lipids (Rhee et al., 1988; Frigg et al., 1990; Monahan et al., 1992) and, hence, having an increased tendency to oxidize.

In cooked ground pork, samples oxidized rapidly over the 4-day storage period and particularly over the first 48 h (Figure 3). The formation of lipid oxidation products was significantly higher ($P < 0.01$) in pork from pigs fed the basal level of α-tocopherol acetate (10 mg/kg) compared to pigs fed the supplemented levels (100 and 200 mg/kg

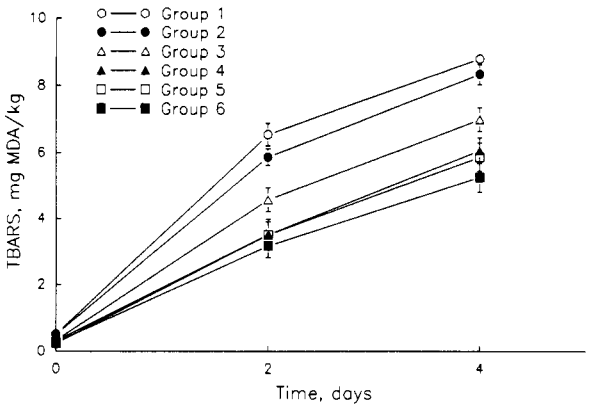

Figure 3. Effect of dietary oil and α-tocopherol supplementation on TBARS (mean ± SEM) of cooked ground pork during storage at 4 °C for 4 days. See Table I for explanation of groups.

of feed). Differences in TBARS between the two supplemented groups were only significant ($P < 0.05$) after 4 days of storage. These results concur with those of Faustman et al. (1989) and Monahan et al. (1990a,b; 1992) in studies with beef and pork, respectively. Sensory evaluation studies are necessary to determine if the significant differences in TBARS (Figure 3) elicit differences in off-flavor intensity of pork from pigs fed the various diets. Previous research indicates that above a TBARS threshold value of 1.0 (Tarladgis et al., 1960) oxidized flavors are detectable by experienced panelists. These threshold values suggest that, in this study, pork from all groups had considerable oxidized flavor after 48 h of refrigerated storage.

Effect of Oxidized Lipid on TBARS Formation. Data show that, while pork from pigs fed oxidized oil at each dietary α-tocopherol level tended to have higher TBARS than pork from pigs fed fresh oil (Figures 1 and 3), differences were not significant. Earlier studies indicate that lipid hydroperoxides are not absorbed in the digestive system (Andrews et al., 1960; Bunyan et al., 1968). In contrast to these results, Buckley et al. (1989) found that pork from pigs fed a diet containing 3% oxidized corn oil [peroxide value (PV) = 300 mequiv/kg of oil], was significantly more susceptible to oxidation than pork from pigs fed fresh corn oil. Buckley et al. (1989) suggested that the animal's natural defense system, namely the glutathione peroxidases, may have been overwhelmed by the high dietary peroxides and, hence, allowed the passage of lipid peroxides through the intestinal mucosa. In the present study, the oxidized oil had a lower peroxide value (PV = 150) than that used in the study of Buckley et al. (1989) (PV = 300) and the pigs may have been able to deal more effectively with the lower dietary peroxide level. In the study of Buckley et al. (1989), the effect of dietary oxidized oil on muscle α-tocopherol levels was not determined. However, the more highly oxidized lipids fed in the latter study may have had a more pronounced effect on tissue α-tocopherol levels and, hence, on the susceptibility of tissue lipids to oxidation than the less oxidized lipid fed in the present study.

Cholesterol Oxidation in Raw and Cooked Pork. In raw pork chops initial cholesterol oxidation products were nondetectable (limit of detection was 5–10 ng of COPS), and even after 8 days of refrigerated storage detectable COPS were only present in some samples (data not shown). Park and Addis (1987) also reported that the COPS content of raw ground beef and turkey was essentially zero at day 0 of storage. The rate of lipid oxidation in raw samples was low (Figures 1 and 2) compared to that found in other studies (Monahan et al., 1990a; Park and Addis, 1987) and may account for the low rate of cholesterol oxidation. Smith et al. (1981) suggest that the hydroperoxides of polyunsaturated fatty acids formed during lipid oxidation may be necessary to initiate cholesterol oxidation.

The mean cholesterol content of ground pork from all groups, immediately after cooking, was 0.63 mg/g of cooked meat. Pie et al. (1991) detected 0.99 mg of cholesterol/g of cooked ground pork. The cooking method used in the present study ensured low evaporative losses compared to those with pan-frying (Pie et al., 1991) and may partly explain the difference in values. In addition, differences in the extramuscular fat content of ground meats may account for the variation in the reported cholesterol values (Sweeney and Weihrauch, 1976; Eichhorn et al., 1986). In cooked ground pork, analysis of variance of the data revealed that COPS formation was significantly ($P < 0.05$) influenced by dietary α-tocopherol but not by dietary oil. Three COPS were consistently identified and present in detectable quantities in all samples: 5β,6β-epoxycholestan-3β-ol (β-epoxide), cholest-5-ene-3β,7β-diol (7β-OH), and 7-oxocholest-5-en-3β-ol (7-keto). On day 0 of refrigerated storage, detectable quantities of these COPS, particularly 7β-OH, were found in some, but not all, samples (data not shown). Table II shows the effect of dietary α-tocopherol supplementation on COPS formation in cooked pork after 2 and 4 days of refrigerated storage. In this study, total COPS were calculated as the sum of the concentrations of the major COPS detected, i.e., β-epoxide, 7β-OH, and 7-keto. After 2 days of storage at 4 °C, cooked pork from pigs fed the supplemented diets had significantly lower ($P < 0.05$) levels of β-epoxide, 7β-OH, 7-keto, and total COPS than pork from pigs fed the basal diet. Only in the case of β-epoxide and total COPS of cooked pork stored for 2 days from pigs fed oxidized corn oil was cholesterol oxide formation significantly lower in pigs fed 200 mg of α-tocopherol acetate/kg of diet compared to pigs fed 100 mg/kg of diet. In all other groups, COPS formation in pork from pigs fed 200 mg/kg of diet was not significantly different from that of pigs fed 100 mg/kg of diet, even though COPS tended to be higher in pork from pigs fed the latter diet. After 2 days of refrigerated storage, the total COPS formed in cooked pork from pigs fed the basal level of α-tocopherol acetate represented 2.7% of the total cholesterol present at day 0 of storage. In the case of pork from pigs fed diets containing 200 mg of α-tocopherol acetate, total COPS accounted for 1.6% of total cholesterol. Similarly, in cooked comminuted beef and turkey, Park and Addis (1987) reported that total COPS formed after 3 days of refrigerated storage at 4 °C represented 0.45 and 1.20% of total cholesterol, respectively. COPS levels were significantly higher ($P < 0.05$) in cooked pork from all groups after 4 days of refrigerated storage with the exception of β-epoxide levels of pork from pigs fed fresh oil with 10 mg of α-tocopherol acetate/kg of diet and oxidized oil with 100 mg of α-tocopherol acetate/kg of diet. The effects of dietary treatment on cholesterol oxide levels after 4 days of refrigerated storage were similar to those reported after 2 days (Table II).

Relationship between Lipid and Cholesterol Oxidation. Lipid oxidation, estimated by the TBA method, and COPS formation were shown to be linearly related (Figure 4). The data indicated a significant correlation ($r = 0.88$, $P < 0.01$) between TBARS value and total COPS concentration in cooked pork stored over 4 days. The correlation coefficients for β-epoxide, 7β-OH, and 7-keto with TBARS were 0.75 ($P < 0.01$), 0.78 ($P < 0.01$), and 0.84 ($P < 0.01$), respectively.

Our results demonstrate that the rate of cholesterol oxidation in pork is greatly accelerated during storage following cooking and appears to follow the same trend as lipid oxidation in general. Cholesterol molecules function

Table II. Effect of Dietary Oil and α-Tocopherol Supplementation on Cholesterol Oxide Content (Micrograms per Gram) of Cooked Pork during Storage at 4 °C[a]

dietary oil	dietary α-tocopherol, (mg/kg of diet)	day 2				day 4			
		β-epoxide	7β-OH	7-keto	total COPS	β-epoxide	7β-OH	7-keto	total COPS
oxidized	10	5.29[c]	4.49[b]	7.70[b]	17.47[c]	7.21[b]	5.35[b]	10.92[b]	23.48[b]
	100	4.20[b]	3.21[a]	5.37[a]	12.77[b]	4.93[a]	4.41[a]	9.31[a]	18.64[a]
	200	3.22[a]	2.57[a]	4.03[a]	9.82[a]	5.65[a]	4.28[a]	8.41[a]	18.34[a]
fresh	10	5.05[b]	3.94[b]	7.17[b]	16.15[b]	5.67[a]	5.07[b]	9.79[b]	20.53[b]
	100	3.65[a]	3.10[a]	5.09[a]	11.76[a]	5.64[a]	4.54[ab]	7.85[a]	18.03[a]
	200	3.42[a]	2.86[a]	4.06[a]	10.33[a]	5.15[a]	4.05[a]	8.79[ab]	17.99[a]

[a] For each oil type, means in the same column bearing different superscripts are significantly different ($P < 0.05$).

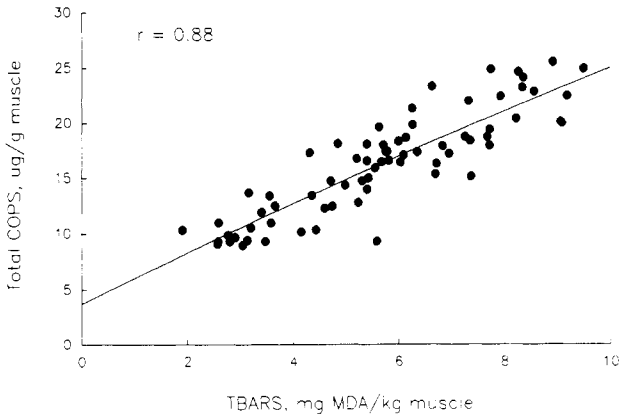

Figure 4. Relationship between TBARS and total COPS formation in cooked ground pork stored for up to 4 days at 4 °C.

as an integral part of the lipid bilayer of cell membranes and are closely associated with membranal phospholipids. The high polyunsaturated fatty acid content of phospholipids and their considerable vulnerability to attack by oxidizing species generated within and close to the cell membrane have led to the belief that lipid oxidation is initiated at the subcellular membrane level (Igene and Pearson, 1979; Gray and Pearson, 1987). It is conceivable, therefore, that cholesterol oxidation should proceed in a way analogous to lipid oxidation. Smith et al. (1981) postulated that intermolecular free-radical processes, involving hydrogen extraction from cholesterol by peroxy or oxy radicals of polyunsaturated fatty acids, may promote cholesterol oxidation. Alternatively, intramolecular oxidation between the oxidized fatty acyl portion and the cholesterol portion of cholesterol esters may occur. Both mechanisms could operate within muscle cell membranes. Dietary α-tocopherol supplementation has been shown to significantly increase the α-tocopherol content of muscle mitochondrial and microsomal cell membranes in pigs (Monahan et al., 1990b) and chickens (Asghar et al., 1989). The localization of α-tocopherol at the proposed site of initiation of lipid oxidation thus provides a means of retarding lipid and cholesterol oxidation through dietary supplementation.

The risks associated with dietary intake of lipid and cholesterol oxidation products at the levels present in meats and other food remain to be defined (Addis and Park, 1989). Nevertheless, it is evident from this and other studies (Park and Addis, 1987; Pie et al., 1991) that products of lipid oxidation increase during storage of meats and especially after cooking. The results of animal studies, particularly those in which levels of COPS representative of those present in foods that have been fed (Jacobson et al., 1985), suggest that reducing the intake of dietary COPS may be beneficial. The results of the present study indicate that increasing the α-tocopherol content of muscle by dietary means may provide an effective way of reducing COPS formation in muscle foods.

ACKNOWLEDGMENT

This study was supported in part by the Michigan Agricultural Experiment Station and by the National Live Stock and Meat Board. This latter support is gratefully appreciated.

LITERATURE CITED

Adams, M. L.; Sullivan, D. M.; Smith, R. L.; Richter, E. F. Evaluation of direct saponification method for determination of cholesterol in meats. *J. Assoc. Off. Anal. Chem.* **1986**, *69*, 844–846.

Addis, P. B.; Park, S. W. Role of lipid oxidation products in atherosclerosis. In *Food Toxicology: A Perspective on the Relative Risks*; Taylor, S. L., Scanlan, R. A., Eds.; Dekker: New York, 1989; pp 297–330.

Allen, C. E.; Foegeding, E. A. Some lipid characteristics and interactions in muscle foods—a review. *Food Technol.* **1981**, *35* (5), 253–257.

Ames, B. N.; Hollstein, M. C.; Cathcart, R. Lipid peroxidation and oxidative damage to DNA. In *Lipid Peroxides in Biology and Medicine*; Yagi, K., Ed.; Academic Press: New York, 1982; pp 339–351.

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

AOAC. *Official Methods of Analysis*, 14th ed.; Association of Official Analytical Chemists: Arlington, VA, 1984; p 507.

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.

Astrup, H. N. Vitamin E and the quality of pork. *Acta Agric. Scand. Suppl.* **1973**, *19*, 152–157.

Baurenfeind, J. Tocopherols in foods. In *Vitamin E: A Comprehensive Treatise*; Machlin, L. J., Ed.; Dekker: New York, 1980; pp 99–167.

Bieri, J. G.; Tolliver, T. J.; Catignani, G. L. Simultaneous determination of α-tocopherol and retinol in plasma or red cells by high pressure liquid chromatography. *Am. J. Clin. Nutr.* **1979**, *32*, 2143–2149.

Brubacher, G.; Muller-Mulot, W.; Southgate, D. A. Vitamin E (alpha-tocopherol) in foodstuffs: HPLC method. In *Methods for the Determination of Vitamins in Foods*; Elsevier Applied Science Publishers: London, 1985; pp 97–106.

Buckley, D. J.; Gray, J. I.; Asghar, A.; Price, J. F.; Crackel, R. L.; Booren, A. M.; Pearson, A. M.; 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.

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.

Buttriss, J. L.; Diplock, A. T. High-performance liquid chromatography methods for vitamin E in tissues. *Methods Enzymol.* **1984**, *105*, 131–138.

Csiky, I. Trace enrichment and separation of cholesterol oxidation products by adsorption high-performance liquid chromatography. *J. Chromatogr.* **1982**, *241*, 383–389.

Eichhorn, J. M.; Wakayama, E. J.; Blomquist, G. J.; Bailey, C. M. Cholesterol content of muscle and adipose tissue from crossbred bulls and steers. *Meat Sci.* **1986**, *16*, 71–78.

Emanuel, H. A.; Hassel, C. A.; Addis, P. B.; Bergman, S. D.; Zavoral, J. H. Plasma cholesterol oxidation products (oxys-

terols) in humans fed a meal rich in oxysterols. *J. Food Sci.* **1991**, *56*, 843–847.

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.

Finocchiaro, E. T.; Lee, K.; Richardson, T. Identification and quantification of cholesterol oxides in grated cheese and bleached butter oil. *J. Am. Oil Chem. Soc.* **1984**, *61*, 877–883.

Frigg, M.; Prabucki, A. L.; Ruhdel, E. U. Effect of dietary vitamin levels on oxidative stability of trout fillets. *Aquaculture* **1990**, *84*, 145–158.

Grant, C. A. Diseases associated with auto-oxidation of diet fat (pig). *Proc. Nutr. Soc.* **1966**, *25*, 18–24.

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

Halliwell, B.; Gutteridge, J. M. C. The importance of free radicals and catalytic metal ions in human disease. *Mol. Aspects Med.* **1985**, *8*, 91–193.

Higley, N. A.; Taylor, S. L.; Herian, A. M.; Lee, K. Cholesterol oxides in processed meats. *Meat Sci.* **1986**, *16*, 175–188.

Igene, J. O.; Pearson, A. M. Role of phospholipids and triglycerides in warmed-over flavor development in meat model systems. *J. Food Sci.* **1979**, *44*, 1285–1290.

Imai, H. Arterial wall injury and repair due to oxygenated sterols and possible precursors. In *Autoxidation in Food and Biological Systems*; Simic, M. G., Karel, M., Eds.; Plenum Press: New York, 1980; pp 613–637.

Jacobson, M. S.; Price, M. G.; Shamoo, A. E.; Heald, F. P. Atherogenesis in White Carneau pigeons. Effects of low level cholestane-triol feeding. *Athersclerosis* **1985**, *57*, 209–217.

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

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.

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

Loudenslager, M. J.; Ku, P. K.; Whetter, P. A.; Ullrey, D. E.; Whitehair, C. K.; Stowe, H. D.; Miller, E. R. Importance of diet of dam and colostrum to the biological antioxidant status and parenteral iron tolerance of the pig. *J. Anim. Sci.* **1986**, *63*, 1905–1914.

Lundberg, W. O. Mechanisms. In *Symposium on Lipids and Their Oxidation*; Schultz, H. W., Day, E. A., Sinnhuber, R. O., Eds.; AVI: Westport, CT, 1962; pp 31–50.

Marmer, W. N.; Maxwell, R. J. Dry column method for the quantitative extraction and simultaneous class separation of lipids from muscle tissue. *Lipids* **1981**, *16*, 365–371.

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.; Gray, J. I.; Morrissey, P. A.; Asghar, A.; Hanrahan, T. J.; Lynch, P. B. Effect of dietary vitamin on the stability of raw and cooked pork. *Meat Sci.* **1990a**, *27*, 99–108.

Monahan, F. J.; Buckley, D. J.; Morrissey, P. A.; Lynch, P. B.; Gray, J. I. Effect of dietary α-tocopherol supplementation on α-tocopherol levels in porcine tissues and on susceptibility to lipid peroxidation. *Food Sci. Nutr.* **1990b**, *42F*, 203–212.

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.* **1992**, *31*, 229–241.

Morgan, J. N.; Armstrong, D. J. Formation of cholesterol-5,6-epoxides during spray drying of egg yolk. *J. Food Sci.* **1987**, *52*, 1224–1227.

Morrissey, P. A.; Apte, S. Influence of species, haem and non-haem iron fractions and nitrite on hexanal production in cooked muscle systems. *Sci. Aliments* **1988**, *8*, 3–14.

Mottram, D. Lipid oxidation and flavor in meat and meat products. *Food Sci. Technol. Today* **1987**, *1* (3), 159–162.

Mukai, F. H.; Goldstein, B. D. Mutagenicity of malonaldehyde, a decomposition product of peroxidised polyunsaturated fatty acids. *Science* **1976**, *191*, 868–869.

Nourooz-Zadeh, J.; Appelqvist, L. A. Cholesterol oxides in Swedish foods and food ingredients: milk powder products. *J. Food Sci.* **1988**, *53*, 74–87.

O'Brien, P. J. Peroxide-mediated metabolic activation of carcinogens. In *Lipid Peroxides in Biology and Medicine*; Yagi, K., Ed.; Academic Press: New York, 1982; pp 317–338.

Park, P. B.; Addis, P. B. HPLC determination of C-7 oxidized cholesterol derivatives in foods. *J. Food Sci.* **1985**, *50*, 1437–1441.

Park, S. W.; Addis, P. B. Cholesterol oxidation products in some muscle foods. *J. Food Sci.* **1987**, *52*, 1500–1503.

Peng, S. K.; Morin, R. J.; Tham, P.; Taylor, C. B. Effects of oxygenated derivatives of cholesterol on cholesterol uptake by cultured aortic smooth muscle cells. *Artery* **1985**, *13*, 144–164.

Peng, S. K.; Phillips, G. A.; Guang-Zhi, X.; Morin, R. J. Transport of cholesterol autoxidation products in rabbit lipoproteins. *Atherosclerosis* **1987**, *64*, 1–6.

Pie, J. E.; Spahis, K.; Seillan, C. Cholesterol oxidation in meat products during cooking and frozen storage. *J. Agric. Food Chem.* **1991**, *39*, 250–254.

Quinn, M. T.; Parthasarathy, S.; Fong, L. G.; Steinberg, D. Oxidatively modified low density lipoproteins: a potential role in recruitment and retention of monocyte/macrophages during atherogenesis. *Proc. Natl. Acad. Sci.* **1987**, *84*, 2995–2998.

Rhee, K. S.; Ziprin, Y. A.; Ordonez, G.; Bohac, C. E. Fatty acid profiles of the total lipids and lipid oxidation in pork muscles as affected by canola oil in the animal diet and muscle location. *Meat Sci.* **1988**, *23*, 201–210.

Shamberger, R. J.; Andreone, T. L.; Willis, C. E. Antioxidants and cancer. IV. Initiating activity of malonaldehyde as a carcinogen. *J. Natl. Cancer Inst.* **1974**, *53*, 1771–1773.

Shorland, F. B.; Igene, J. O.; Pearson, A. M.; Thomas, J. W.; McGuffey, R. K.; Aldridge, A. E. Effects of dietary fat and vitamin E on the lipid composition and stability of veal during frozen storage. *J. Agric. Food Chem.* **1981**, *29*, 863–871.

Simesen, M. G.; Nielsen, H. E.; Danielsen, V.; Gissel-Nielsen, G.; Hjarde, W.; Leth, T.; Basse, A. Selenium and vitamin E deficiency in pigs. II. Influence on plasma selenium, vitamin E, ASAT and ALAT and on tissue selenium. *Acta Vet. Scand.* **1979**, *20*, 289–305.

Smith, L. L. *Cholesterol Autoxidation*; Plenum Press: New York, 1981.

Steel, R. G. D.; Torrie, J. H. *Principles and Procedures of Statistics—A Biometrical Approach*, 2nd ed.; McGraw-Hill Book: New York, 1980.

Sweeney, J. P.; Weihrauch, J. L. Summary of available data for cholesterol in foods and methods for its determination. *Crit. Rev. Food Sci. Nutr.* **1976**, *8*, 131–159.

Tarladgis, B. G.; Watts, B. M.; Younathan, M. T.; Dugan, L. A distillation method for the quantitative determination of malonaldehyde in rancid foods. *J. Am. Oil Chem. Soc.* **1960**, *37*, 44–48.

Taylor, C. B.; Peng, S. K.; Werthessen, N. T.; Tham, P.; Lee, K. T. Spontaneously occurring angiotoxic derivatives of cholesterol. *Am. J. Clin. Nutr.* **1979**, *32*, 40–57.

Thafvelin, B. Role of cereal fat in the production of nutritional disease in pigs. *Nature* **1960**, *188*, 1169–1172.

Thode-Jensen, P.; Nielsen, H. E.; Danielsen, V.; Leth, T. Effect of dietary fat quality and vitamin E on the antioxidant potential of pigs. *Acta Vet. Scand.* **1983**, *24*, 135–147.

Tichivangana, J. Z.; Morrissey, P. A. Metmyoglobin and inorganic metals as prooxidants in raw and cooked muscle systems. *Meat Sci.* **1985**, *15*, 107–116.

Received for review December 18, 1991. Revised manuscript received May 18, 1992. Accepted May 28, 1992.

Registry No. α-Tocopherol acetate, 58-95-7; cholesterol, 57-88-5; 7β-hydroxycholesterol, 566-27-8; 7-ketocholesterol, 566-28-9; 5β,6β-epoxycholestan-3β-ol, 4025-59-6; α-tocopherol, 59-02-9.