# Influence of Dietary Fat and Vitamin E Supplementation on Free Radical Production and on Lipid and Protein Oxidation in Turkey Muscle Extracts

Philippe Gatellier,[†] Yves Mercier,[†] Edmond Rock,[‡] and Michel Renerre*[,†]

Station de Recherches sur la Viande, INRA, Theix, 63122 St Genès-Champanelle, France, and CRNH, Laboratoire des Maladies Métaboliques et des Micronutriments, INRA, Theix, 63122 St Genès-Champanelle, France

The objectives of this study were to investigate the effects of dietary fat (6% soy oil or rapeseed oil or tallow) and α-tocopheryl acetate supplementation at two levels (30 or 200 ppm) on radical production, measured by ESR spectroscopy, and on lipid and protein oxidation in turkey muscle extracts oxidized by an enzymic system (NADPH, ADP, $FeSO_4$/cytochrome P450 reductase). Two muscles were tested: pectoralis major (glycolytic) and sartorius (oxidative) muscles. Radical production measured by ESR was higher in pectoralis major muscle than in sartorius muscle, whereas lipid and protein oxidation was more important in sartorius muscle, showing the importance of the pro-/antioxidant ratio in oxidative processes in muscular cells and of the measurement methodology to appreciate the free radical production. Dietary fat had no effect on the level of ESR signals, whereas feeding of animals with soy oil induced higher oxidation of lipids. Protein oxidation was less sensitive to the nature of the dietary fat than lipid oxidation. Vitamin E supplementation significantly decreased radical production, as measured by ESR spectroscopy. Vitamin E also decreased lipid and protein oxidation, but the effect of vitamin E on protein oxidation was less pronounced than on lipid oxidation.

**Keywords:** *Turkey; meat; dietary fat; dietary vitamin E; ESR; lipid oxidation; protein oxidation; chemical induction*

## INTRODUCTION

Feeding different dietary unsaturated fats to monogastrics such as pigs, broilers, or turkeys, and particularly *n*−3 fatty acids with protective effects against human diseases (Mercier et al., 1999), affects the composition of triglycerides and, to a lesser extent, that of phospholipids (Genot et al., 1997). However, increasing the degree of polyunsaturation accelerates oxidative processes, leading to deterioration in meat flavor, meat color, and nutritional composition in pig (Asghar et al., 1988) as has been seen in poultry (Lauridsen et al., 1997). Turkey meat is also particularly prone to these oxidative damages during refrigerated storage (Mercier et al., 1998a; Renerre et al., 1999). The rate of lipid oxidation is dependent not only on the presence of prooxidants, especially heme- and nonheme-iron (Rhee and Ziprin, 1987; Monahan et al., 1993a; Decker et al., 1993) and ferritin (Decker and Welch, 1990) but also on the presence of antioxidants (Renerre et al., 1996, 1999). Some authors, such as Kanner et al. (1986), have shown that iron in the presence of ascorbic acid stimulated lipid peroxidation and that an enzymatic system (NADPH, cytochrome P450, ADP-$Fe^{3+}$) was present in turkey to stimulate lipid peroxidation. Lipid oxidation and protein oxidation are also interdependent phenomena (Renerre and Labadie, 1993; Mercier et al., 1995, 1998a), leading to decreases of functional properties of muscular proteins (Decker et al., 1992). However, the effect of dietary lipid on protein oxidation is largely unknown.

Free radicals, such as hydroxyl radical and other oxygenated radicals, are closely evolved in these oxidative processes. The oxidation chain may be broken by the addition of antioxidants which, when present in trace amounts, will intercept radicals before they react with unsaturated lipids. Many phenolic compounds can act as antioxidants; among them, one of nature's best known antioxidants, vitamin E (α-tocopherol), acts principally by scavenging peroxyl radicals.

Many studies have indicated that dietary supplementation of vitamin E decreases lipid oxidation during meat storage, in beef (Faustman et al., 1989; Arnold et al., 1993a; Lanari et al., 1994), in pork (Monahan et al., 1992; Buckley et al., 1995), in chicken (Sheehy et al., 1993), and in turkey after a storage at 4 °C (Mercier et al., 1998a; Renerre et al., 1999) or at −20 °C (Mercier et al., 1998b). The effect of vitamin E on lipid oxidation is well documented, but much less is known about its effect on protein oxidation.

ESR spectroscopy is a powerful method to investigate free radical production in oxidative media. In bovine muscle we have previously demonstrated that lipids can be oxidized by a metmyoglobin−$H_2O_2$ activated system and that this oxidation is mediated by a radical which can be detected by ESR spectroscopy (Gatellier et al., 1995). Formation of this radical is completely inhibited by the addition of trolox, a water-soluble form of vitamin E. In porcine muscle, Monahan et al. (1993b) measured

* Author to whom correspondence should be addressed [fax 33 (0)4 73 62 42 68; e-mail renerre@clermont.inra.fr].

[†] Station de Recherches sur la Viande.

[‡] Laboratoire des Maladies Métaboliques et des Micronutriments.

10.1021/jf990943j CCC: $19.00 
Published on Web 04/14/2000

the effect of dietary fat and vitamin E on iron-induced free radical production by ESR spectroscopy.

The aim of this work was to study the effect of a supranutritional administration of vitamin E, in turkeys fed with different sources of fat, on oxidative processes occurring in a muscle extract after an enzymatic induction. For this purpose, radical production occurring during oxidation of lipids was investigated by ESR spectroscopy. At the same time, lipid and protein oxidations were also determined.

## MATERIALS AND METHODS

**Reagents.** All reagents were purchased from Sigma Chemical Co. (Sigma-Aldrich, St Quentin Fallavier, France).

**Animals and Diets.** Thirthy-six male turkeys of BUT strain were divided into three groups, which received, during 16 weeks, a basal diet enriched with 6% of the following fat sources: rapeseed or soy oil or tallow fat. For each diet, animals were divided in two subgroups that received 30 ppm (control) or 200 ppm (supplemented) of α-tocopheryl acetate (Hoffman-La Roche, France). At 16 weeks of age, animals were slaughtered. Two muscles, different from the oxidative capacity viewpoint, pectoralis major muscle (the less oxidative) and sartorius muscle (the more oxidative), were removed from the left side of the carcasses at slaughter and stored in ice. At 6 h post-mortem muscles were frozen at −80 °C until use.

**Vitamin E Content of Muscles.** Vitamin E content was determined according to the method of Buttriss and Diplock (1984); the samples were analyzed by normal phase HPLC fitted with a fluorometer detector (Renerre et al., 1999).

**Muscle Extract Preparation and Oxidation.** One gram of muscle was ground with a Waring blender in 10 mL of 20 mM Hepes, 150 mM KCl, pH 7.4, buffer. Extract was then homogenized with a Polytron for 30 s (half of the maximum speed) and centrifuged for 5 min at 3500*g* to eliminate myofibrillar proteins. Supernatant was then oxidized at 20 °C by an oxidative system composed of 2.19 mM NADPH, 2.63 mM ADP, and 0.11 mM $FeSO_4$ (C. Astier, E. Rock, A. Lacourt, A. M. Rock, P. Grolier, A. Mazur, and Y. Rayssiguier, personal communication).

**ESR Signal Measurement.** ESR spectra were recorded on a Bruker ECS 106 spectrometer (operating at 9.79 GHz) using pyridyl oxide *N*-*tert*-butylnitrone (POBN) spin-trapping reagent. Experimental conditions were as follows: microwave power, 20 mW; modulation frequency, 100 kHz; modulation amplitude, 1.016 G; time constant, 61.92 ms; gain, $5 \times 10^5$. Signals were recorded just after mixing of all reagents and after an incubation period of 1 h in the oxidative system with six acquisitions averaged. A quantitative estimation of the concentration of the formed radicals was obtained by evaluating the increase of the ESR signal in arbitrary units between time 0 and a 1 h incubation.

**Lipid Oxidation Measurement.** Lipid oxidation was measured relative to the TBARS content according to the method of Lynch and Frei (1993). Samples of 0.5 mL (1 g of muscle homogenized in 10 mL of a solution containing 0.15 mM KCl and 0.1 mM BHT) were incubated with 1% (w/v) 2-thiobarbituric acid in 50 mM NaOH (0.25 mL) and 2.8% (w/v) trichloroacetic acid (0.25 mL) in a boiling water bath for 10 min. After the mixture had cooled to room temperature, the pink chromogen was extracted with *n*-butanol (2 mL), and its absorbance was measured at 535 nm (Kontron). TBARS concentration was calculated using 1,1,3,3-tetraethoxypropane as standard. The analysis was performed on samples after 0, 1, and 5 h of oxidation, and the results were expressed as nanomoles of MDA per milliliter of sample.

**Protein Oxidation Measurement.** Protein oxidation was measured by an estimation of carbonyl groups formed during the incubation using the method of Oliver et al. (1987). Muscle extract obtained as previously was divided into two equal aliquots of 0.5 mL. Proteins were precipitated in both aliquots by 10% TCA (w/v final concentration) and centrifuged for 10 min at 2000*g*. One pellet was treated with 2 N HCl, and the other was treated with an equal volume of 0.2% (w/v) 2,4-dinitrophenylhydrazine (DNPH) in 2 N HCl. Both samples were incubated for 1 h at room temperature and stirred regularly. The samples were then reprecipitated with 10% TCA and washed twice with ethanol/ethyl acetate (1:1). The pellets were dissolved in 6 M guanidine-HCl with 20 mM sodium phosphate buffer, pH 6.5. Samples were centrifuged for 10 min at 2000*g* to remove insoluble fragments. Protein concentration was calculated at 280 nm in the HCl control using BSA in 6 M guanidine as standard. Measurement of phenyl hydrazone at 370 nm against the HCl control, using an average absorption of 21.0 $mM^{-1}$ $cm^{-1}$, gave the content of DNPH incorporated, and results were expressed as nanomoles per milligram of protein. Carbonyl content was measured after 0, 1, and 5 h of incubation in the oxidative medium.

**Table 1. Vitamin E Content (Parts per Million) in Control (C) and Supplemented (E) Muscles of Turkeys Fed Different Fats[a]**

| treatment | pectoralis major muscle | | sartorius muscle | |
|---|---|---|---|---|
| | C | E | C | E |
| rapeseed | 1.00 ± 0.09ax | 3.43 ± 0.56bxy | 2.03 ± 0.45ax | 6.68 ± 0.75bx |
| tallow | 0.53 ± 0.10ay | 4.01 ± 0.76bx | 1.07 ± 0.16ay | 7.58 ± 1.08bx |
| soy | 0.37 ± 0.05az | 2.90 ± 0.24by | 0.85 ± 0.19ay | 5.18 ± 0.50by |

[a] Values are means (SD of $n = 6$ animals. Values in the same row (a,b, for the vitamin E supplementation) or in the same column (x,y,z, for type of fat) not bearing common superscripts differ significantly ($p < 0.05$).

**Statistical Analysis.** Unpaired Student's *t* test was performed to ascertain levels of statistical significance. All values are reported as mean ± standard deviation (SD) ($n = 6$) of the mean. Data, obtained after a 1 h incubation, were analyzed by a three-way analysis of variance (SAS) to assess the effect of muscle, fat, and vitamin E dietary supplementation. Mean values were compared using the Newman−Keuls test.

## RESULTS AND DISCUSSION

**Vitamin E Content.** Vitamin E content of muscle was significantly influenced by supplementation in the diet (Table 1). Even with a 200 ppm supplementation, the level of vitamin E in supplemented animals was almost 5 times greater than in control animals. In sartorius muscle, vitamin E content was about twice that in pectoralis major muscle but with differences with the nature of fat. The muscles of turkeys fed soy oil had always a lower vitamin E content than those fed rapeseed oil or tallow. These results are in good accordance with those previously published by our laboratory (Mercier et al., 1998a) with animals supplemented with 400 ppm of vitamin E. Moreover, the vitamin E content of muscles reflects the vitamin E content of the diet, and the results obtained previously with a 400 ppm supplementation (Mercier et al., 1998a) were ∼2-fold greater than those measured in this study with a 200 ppm supplementation.

The weak ability of turkey to store dietary vitamin E compared to that of other species such as broilers or pigs has been previously described (Bartov et al., 1983; Sklan et al., 1983); therefore, to avoid this problem, a supplementation for a long period such as in this study (16 weeks) is needed. For example, with a 400 ppm supplementation for only 4 weeks, the increase in vitamin E level in turkey muscles was only ∼2-fold (Marusich et al., 1975). With a lower supplementation (250 ppm/40 ppm basal) for only 3 weeks, the difference in vitamin E level between control and supplemented turkeys was only 1.2 (Sante and Lacourt, 1994).

**ESR Spectroscopy.** The NADPH, ADP, and $FeSO_4$ system is a powerful oxidant system often used in the

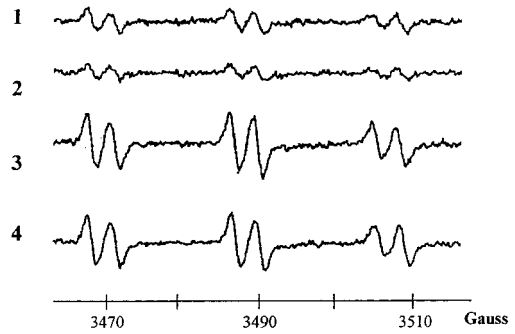


**Figure 1.** ESR spectra recorded with POBN spin-trapping agent, after 1 h of incubation with the oxidative system, in muscle extracts of turkeys fed rapeseed oil: (1) pectoralis major muscle of supplemented animal; (2) sartorius muscle of supplemented animal; (3) pectoralis major muscle of control animal; (4) sartorius muscle of control animal.

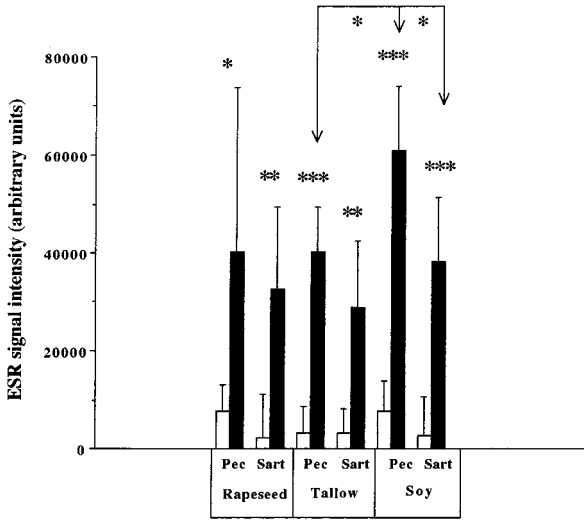


**Figure 2.** Effect of dietary fat and vitamin E supplementation (control, black bars; supplemented, white bars) on ESR signal intensity in pectoralis major (Pec) muscle and in sartorius (Sart) muscle of turkey (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

oxidation of crude extract or biological membranes (Arthur et al., 1988). This system can activate the NADPH cytochrome P450 reductase, an enzyme present in microsomal membranes, which can produce superoxide anion $O_2^{\bullet -}$ and hydrogen peroxide $H_2O_2$. These oxygenated substances can react with iron to produce $OH^{\bullet}$ and other oxidants able to initiate lipid oxidation and myoglobin oxidation in biological systems (Anton et al., 1993).

Radicals formed during the oxidation of the different meat extracts were trapped by POBN molecules. Identification of radicals trapped was performed by comparison of hyperfine splitting constants with similar constants previously discribed in the literature. The ESR spectra consisted of a triplet of doublets (Figure 1), and the hyperfine splitting constants ($a^N = 15.7$ G and $a^H = 2.6$ G) of the free radicals were typical of the POBN spin adduct of alkyl radicals: ethyl and pentyl radicals (Iwahashi et al., 1992). These lipid radicals come from oxidation of membranal lipid fatty acids: linolenic acid for ethyl radical and linoleic and arachidonic acid for pentyl radical (Iwahashi et al., 1992).

Figure 2 shows the effect of vitamin E on radical accumulation in muscle extracts of turkeys fed different fat sources, after a 1 h incubation with the enzymatic

**Table 2. Influence of Vitamin E Supplementation on Mean Values (Newman−Keuls Test) of Muscle Characteristics[a]**

| treatment | ESR | TBARS | carbonyl | vitamin E |
|---|---|---|---|---|
| | (A) Pectoralis Major Muscle | | | |
| control | 47231a | 8.92a | 4.10a | 0.63a |
| vitamin E | 6238b | 1.63b | 3.53b | 3.45b |
| | (B) Sartorius Muscle | | | |
| control | 33293a | 12.99a | 5.96a | 1.31a |
| vitamin E | 2770b | 2.35b | 4.29b | 6.48b |

[a] Values in the same column not bearing common superscripts differ significantly ($p < 0.05$).

**Table 3. Influence of Type of Fat on Mean Values (Newman−Keuls Test) of Muscle Characteristics[a]**

| treatment | ESR | TBARS | carbonyl | vitamin E |
|---|---|---|---|---|
| | (A) Pectoralis Major Muscle | | | |
| rapeseed | 24159a | 3.08a | 2.21a | 2.22a |
| tallow | 21748a | 2.28a | 4.61b | 2.27a |
| soy | 34295a | 10.46b | 4.63b | 1.63b |
| | (B) Sartorius Muscle | | | |
| rapeseed | 17494a | 6.71a | 3.90a | 4.36a |
| tallow | 16039a | 5.76a | 5.59b | 4.32a |
| soy | 20561a | 10.54b | 5.88b | 3.02b |

[a] Values in the same column not bearing common superscripts differ significantly ($p < 0.05$).

oxidative system. Vitamin E strongly inhibited free radical production. ESR signals were from 5 to 8 times greater in control than in supplemented extracts, and the vitamin E effect was always significant ($p < 0.05$). These results are confirmed by Newmans−Keuls test (Table 2), showing a significant effect of vitamin E supplementation whatever the muscle, and by variance analysis (Table 4), which indicates a strong effect of vitamin E and muscle type on ESR signal intensity ($p < 0.001$) without interaction between the different variables.

Surprisingly, fat source had little effect on the level of radical production (Figure 2; Tables 3 and 4), even if polyunsaturated fatty acids (PUFA), which are well-known to present greater oxidation, are in highest proportion in turkeys fed soy oil (Genot et al., 1997). For the pectoralis major muscle, fat source affected the radical production, with soy oil inducing a higher value compared to tallow ($p < 0.05$) (Figure 2). Soy oil and tallow are, respectively, the more (63% PUFA) and the less unsaturated (19% PUFA) fat sources tested here, rapeseed oil (40% PUFA) being intermediate (Genot et al., 1997). ANOVA analysis does not show any fat source effect on radical production (Table 4).

Figure 2 also shows that ESR signal level was always lower in sartorius muscle than in pectoralis major muscle, but this effect was significant only with animals fed soy oil. ANOVA analysis (Table 4) confirms this muscle effect on ESR signal ($p < 0.01$). Sartorius muscle is more prone to oxidative processes because it contains more myoglobin and free iron (Kanner et al., 1990; Mercier et al., 1998a; Renerre et al., 1999) than pectoralis major muscle, but it also contains about twice as much vitamin E as pectoralis major muscle (Table 1) and certainly other antioxidants that are not examined in this experiment. Sartorius muscle is also more protected by antioxidant enzymes such as catalase, SOD, GPX, and GR than pectoralis major muscle and presents a higher global antioxidant status as measured by the TEAC method (Renerre et al., 1999). Radical production in meat extract is a compromise between pro-

**Table 4. Effect of Type of Fat, Muscle, and Vitamin E Supplementation on ESR, TBARS, Carbonyl Level, and Vitamin E Content**[a]

| | ESR | | | TBARS | | | carbonyl | | | vitamin E | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| source | df | ms | sig | df | ms | sig | df | ms | sig | df | ms | sig |
| fat | 2 | 4.80E+08 | NS | 2 | 296.47 | *** | 2 | 36.02 | *** | 2 | 7.50 | *** |
| muscle | 1 | 1.36E+09 | ** | 1 | 103.39 | *** | 1 | 30.76 | *** | 1 | 62.16 | *** |
| vitamin | 1 | 2.30E+10 | *** | 1 | 1449.01 | *** | 1 | 22.58 | *** | 1 | 286.80 | *** |
| fat × muscle | 2 | 1.15E+08 | NS | 2 | 24.11 | ** | 2 | 0.76 | NS | 2 | 1.02 | * |
| fat × vitamin | 2 | 3.44E+08 | NS | 2 | 122.19 | *** | 2 | 2.39 | ** | 2 | 4.59 | *** |
| muscle × vitamin | 1 | 4.93E+08 | NS | 1 | 50.50 | ** | 1 | 5.44 | ** | 1 | 24.85 | *** |
| fat × muscle × vitamin | 2 | 9.55E+07 | NS | 2 | 5.24 | NS | 2 | 6.32 | *** | 2 | 0.59 | NS |

[a] Values are degrees of freedom (df), mean squares (ms), and significance (sig) from three-way variance analysis (NS, nonsignificant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

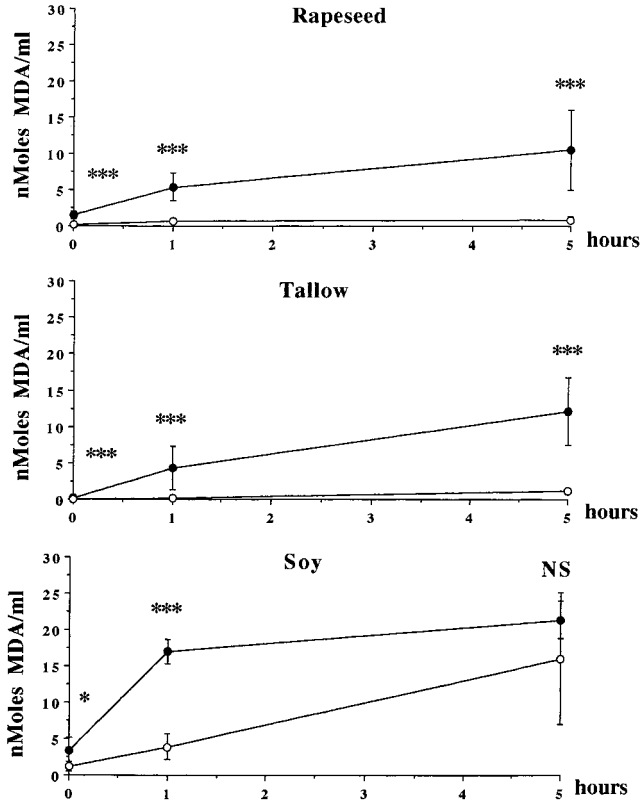


**Figure 3.** Effect of dietary fat and vitamin E supplementation (control, solid symbols; supplemented, open symbols) on lipid oxidation in pectoralis major muscle (NS, nonsignificant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

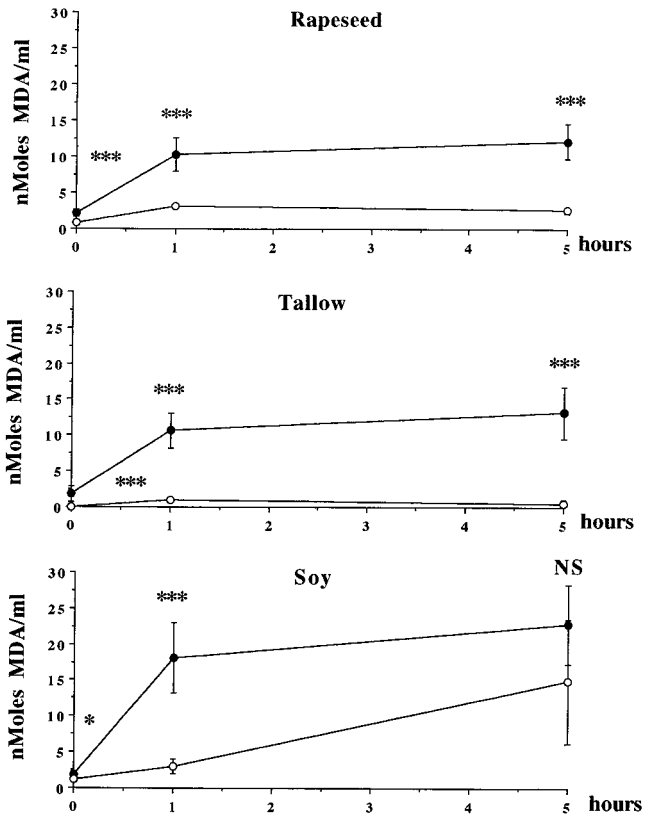


**Figure 4.** Effect of dietary fat and vitamin E supplementation (control, solid symbols; supplemented, open symbols) on lipid oxidation in sartorius muscle (NS, nonsignificant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

and antioxidant effect. In rat heart extract, Arthur et al. (1988) showed an ESR signal with an oxidation induced by ADP/$Fe^{2+}$/NADPH. This signal was enhanced by a vitamin E deficiency. In porcine microsomal fractions, Monahan et al. (1993b) showed an ESR signal, similar to the signal observed in our study, induced by $FeCl_2$. This radical formation, the nature of which was not clearly identified by the authors, appeared to be higher in muscle microsomes from pigs receiving oxidized corn oil compared to a control with fresh corn oil. Vitamin E supplementation (200 ppm/10 ppm basal) completely suppressed this ESR signal.

**TBARS Measurement.** Figures 3 and 4 show the time course of lipid oxidation, as measured by TBARS production, during the enzymic oxidation of different muscle extracts. Whatever the incubation time, vitamin E significantly lowered (Figures 3 and 4) the level of TBARS whatever the muscle or nature of fat. After a 1 h incubation, TBARS production was ~5 times greater in control than in supplemented muscles (Table 2). These results are in good accordance with ESR spectroscopy measurements showing a higher radical production in control animals. After a 5 h incubation, differences in TBARS concentration between control and supplemented animals were always highly significant in animals fed rapeseed oil or tallow, but differences were not significant in animals fed soy oil. This result shows that in animals fed soy oil, the vitamin E scavenging effect is probably overwhelmed by the amount of radicals formed from PUFAs.

Whatever the muscle, TBARS production was more pronounced in soy oil fed animals than in rapeseed oil and tallow fed animals with significant differences (Figures 3 and 4 and Table 3) showing an increase of lipid oxidation in relationship with the degree of unsaturation of dietary fats. Nevertheless, by ANOVA analysis, interactions between factors are noted (Table 4).

These results are consistent with previous reports on broiler meat (Asghar et al., 1990) or on turkey meat (Mercier et al., 1998a). In porcine muscle microsomal

**Table 5. Correlation Coefficients between Different Parameters (ESR, TBARS, Carbonyl, and Vitamin E)**

| | ESR | TBARS | carbonyl | vitamin E |
|---|---|---|---|---|
| (A) Pectoralis Major Muscle | | | | |
| ESR | 1.000 | 0.697 | 0.204 | −0.774 |
| TBARS | 0.697 | 1.000 | 0.407 | −0.683 |
| carbonyl | 0.204 | 0.407 | 1.000 | −0.274 |
| vitamin E | −0.774 | −0.683 | −0.274 | 1.000 |
| (B) Sartorius Muscle | | | | |
| ESR | 1.000 | 0.783 | 0.552 | −0.754 |
| TBARS | 0.783 | 1.000 | 0.732 | −0.832 |
| carbonyl | 0.552 | 0.732 | 1.000 | −0.544 |
| vitamin E | −0.754 | −0.832 | −0.544 | 1.000 |

fractions, Monahan et al. (1993b, 1994a,b) showed that TBARS values of pigs fed with oxidized oil, compared to controls fed with fresh oil, were not significantly different but that vitamin E supplementation considerably decreased lipid oxidation. In turkey muscle extracts, Genot et al. (1997) showed that lipid oxidation induced by iron/ascorbate system was more pronounced in animals fed soy and rapeseed oil than tallow. In a previous work (Gatellier et al., 1996), we have demonstrated that vitamin E supplementation (400 ppm/30 ppm basal) strongly decreased TBARS in turkey muscle extract when oxidized by $Fe^{3+}$/ascorbate and that oxidation in tallow-fed animals was lower than in animals fed rapeseed or soy oil.

Essentially within 1 h of induction time, TBARS accumulation was more important in sartorius muscle than in pectoralis major muscle, showing that lipid oxidation is largely dependent on Fe catalyst such as myoglobin and free iron (Figures 3 and 4). The same results have been obtained in fresh turkey muscles after a refrigerated storage (Mercier et al., 1998a; Renerre et al., 1999) and in muscle extracts after oxidation by $Fe^{3+}$/ascorbate (Gatellier et al., 1996).

ANOVA analysis (Table 4) shows an effect of fat source, muscle, and supplementation on TBARS level, after a 1 h incubation, even if interactions between parameters are always significant, thus limiting the significance of these results. Correlation analysis (Table 5) confirms that TBARS production was correlated with ESR signal intensity ($r = 0.70$ for pectoralis major muscle and $r = 0.78$ for sartorius muscle; $p < 0.001$), showing that lipid oxidation is directly linked with alkyl radical production. Significant negative correlations can be noted between oxidation parameters, as measured by ESR signal and TBARS content, and vitamin E content of muscles (Table 5), confirming that vitamin E decreases lipid oxidation by limiting the extent of alkyl radical production.

**Carbonyl Group Measurement.** Figures 5 and 6 show that carbonyl groups were formed in a time-dependent manner during the enzymic oxidation of muscle extracts. Carbonyl concentration largely increased after a 1 h incubation and remained relatively stable between 1 and 5 h.

Vitamin E supplementation significantly decreased carbonyl group content (Figures 5 and 6). This effect was confirmed by Newman−Keuls analysis (Table 2), but differences in carbonyl content between control and supplemented animals were less pronounced than those in TBARS, notably in the first hour of oxidation. These differences were more significant after a 5 h incubation, except in pectoralis major muscle of turkeys fed soy oil (Figure 5). The positive effect of vitamin E supplementation by lowering carbonyl content was more important

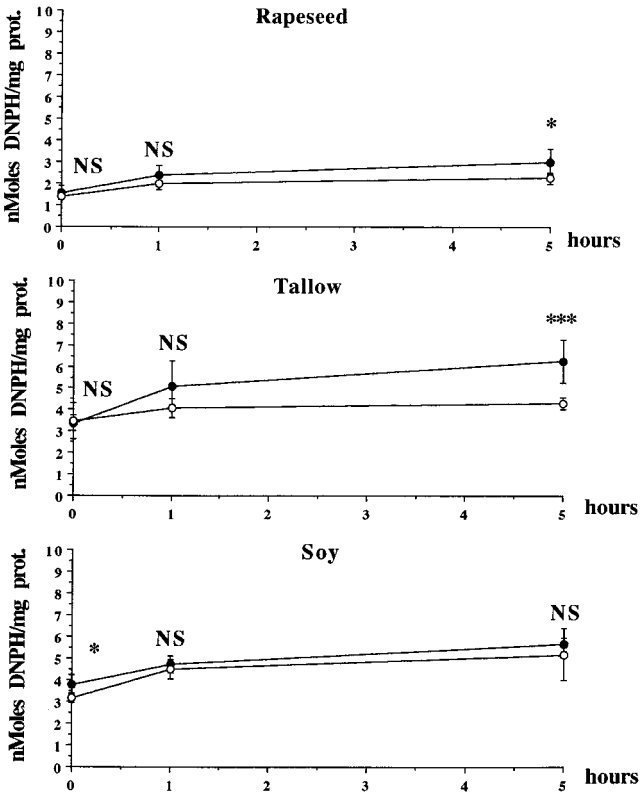


**Figure 5.** Effect of dietary fat and vitamin E supplementation (control, solid symbols; supplemented, open symbols) on protein oxidation in pectoralis major muscle (NS, nonsignificant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

in oxidative sartorius muscle than in glycolytic pectoralis major muscle (Figures 5 and 6).

By Newman−Keuls analysis (Table 3) it was shown that when animals were fed rapeseed oil, a significant decrease in carbonyl formation was found in both muscles. More surprisingly, no difference was noted between animals fed soy oil and tallow.

As for TBARS values, carbonyl formation was more pronounced in sartorius than in pectoralis major muscle, especially in control animals fed soy and rapeseed oil. These results can be compared with previous results, obtained in our laboratory, when turkey muscle extracts were oxidized by $Fe^{3+}$/ascorbate (Gatellier et al., 1996). A similar effect of vitamin E, on carbonyl production, was observed with this chemical oxidative system but, in this experiment, animals fed tallow showed a lower protein oxidation than animals fed soy or rapeseed oil. In fresh turkey meat, we have previously shown that feeding soy oil induced significantly higher oxidation of proteins than rapeseed oil or tallow and that vitamin E supplementation decreased the carbonyl content after 9 days of storage but only in sartorius muscle (Mercier et al., 1998a). In rats, after a dietary vitamin E supplementation, Reznick et al. (1992) also found a reduction in carbonyl groups from supplemented animals and showed that red muscles were more prone to oxidation than white muscles.

As for TBARS measurements, ANOVA analysis shows a great effect of muscle, fat source, and vitamin E supplementation on carbonyl production with a significant interaction between parameters (Table 4).

Correlation analysis (Table 5B) shows, in oxidative sartorius muscle, that carbonyl production was significantly correlated with lipid oxidation ($r = 0.73$; $p <$

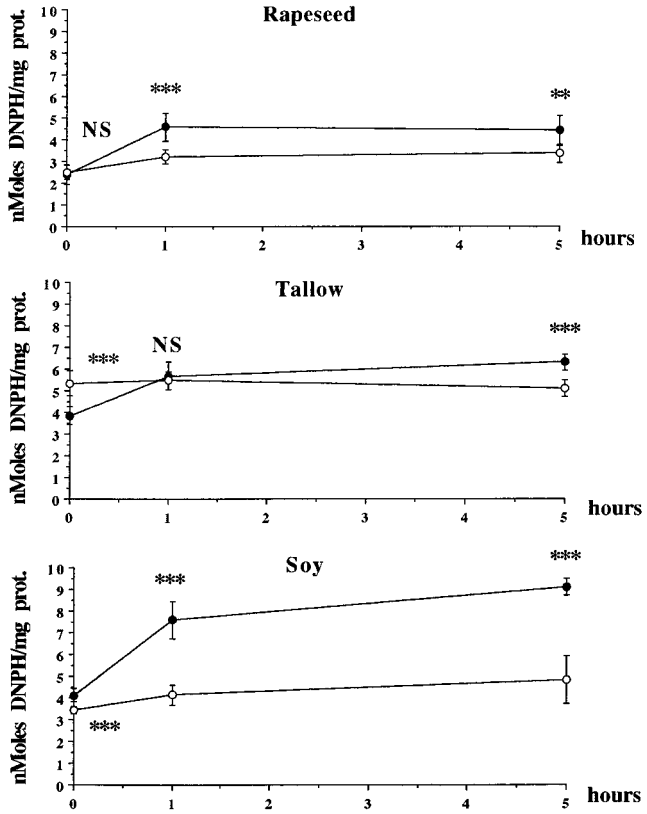


**Figure 6.** Effect of dietary fat and vitamin E supplementation (control, solid symbols; supplemented, open symbols) on protein oxidation in sartorius muscle (NS, nonsignificant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

0.001) and, to a lesser extent, with alkyl radical production ($r = 0.55$; $p < 0.001$). In pectoralis major muscle, a correlation was observed only between carbonyl production and TBARS formation ($r = 0.41$; $p < 0.01$) (Table 5A). These results demonstrate that lipid oxidation is directly linked with alkyl radical production, whereas linkage between protein oxidation and radical production is less evident. Alkyl radical formation reflects lipid peroxidation, and lipid peroxidation is probably a prerequisite step in protein oxidation. Correlation analysis also confirms that vitamin E had less of an effect on protein oxidation than on lipid oxidation. Significant negative correlations can be observed between carbonyl formation and vitamin E content in sartorius muscle but not in pectoralis major muscle (Table 5).

Carbonyl production is a general measure of protein oxidation. Oxidation of lysine, arginine, proline, and threonine residues leads to formation of carbonyl derivatives (Stadtman, 1990; Berlett and Stadtman, 1997). In addition, carbonyl groups may be introduced into proteins by reaction with aldehydes produced during lipid oxidation, such as malonaldialdehyde and 4-hydroxynonenal (Esterbauer et al., 1991; Berlett and Stadtman, 1997), leading to the formation of fluorescent products.

In food products, few studies have shown a relationship between protein and lipid oxidation. In fish, Srinivasan and Hultin (1995) found a relationship between carbonyls and TBARS in cod when the meat was exposed to a free radical generating system. In our laboratory such a correlation had been established during beef (Mercier et al., 1995) and turkey meat storage (Mercier et al., 1998a). Otherwise, in beef, we also have previously shown an accumulation of carbonyl groups in myofibrillar proteins after a chemical oxidation during several hours or meat maturation for 10 days. This oxidation leads to fragmentation and aggregation of proteins (Martinaud et al., 1997).

In conclusion, this work shows that dietary vitamin E is able to lower free radical generation in muscle extract even in the presence of a high PUFA content. The free radical quenching ability of vitamin E demonstrated in this study may effectively lower the oxidation of lipids in muscle extracts except when the diet is too highly enriched with PUFAs such as in soy oil. In this case, supplementation with higher doses of vitamin E or combination with other antioxidants and/or micronutriments may be envisaged. Our results also clearly show that protection by vitamin E is more pronounced toward lipid oxidation than protein oxidation perhaps because, first, phospholipids are the first target of free radical attacks and, second, protein oxidation is mediated by lipid oxidation products which cannnot be trapped by vitamin E.

## ACKNOWLEDGMENT

We express our gratitude to Anne-Marie Rock, who conducted the ESR analysis.

## LITERATURE CITED

Anton, M.; Gatellier, Ph.; Renerre, M. Relationship between myoglobin and microsomal lipid oxidation: influence of muscle type and time post-mortem. *Proceedings of the International Congress on Meat Science Technology*, 39th ICoMST, Calgary, AB, Canada; 1993; pp 361−389.

Arnold, R. N.; Scheller, K. K.; Arp, S. C.; Williams, S. N.; Schaefer, D. M. Dietary α-tocopherol acetate enhances beef quality in Holstein and beef breed steers. *J. Food Sci.* **1993a**, *58*, 28−33.

Arthur, J. R.; McPhail, D. B.; Goodman, B. A. Spin trapping of free radicals in homogenates of heart from selenium and vitamin E deficient rats. *Free Radical Res. Commun.* **1988**, *4*, 311−315.

Asghar, A.; Gray, J. I.; Buckley, D. J.; Pearson, A. M.; Booren, A. M. Perspectives of warmed-over flavor. *Food Technol.* **1988**, *42*, 102−108.

Asghar, A.; Lin, C. F.; Gray, J. I.; Buckley, D. J.; Booren, A. M.; Flegal, C. J. Effects of lipid composition and α-tocopherol supplementation on membranal lipid oxidation in broiler meat. *J. Food Sci.* **1990**, *55*, 46−50.

Bartov, I.; Basker, D.; Angel, S. Effect of dietary vitamin E on the stability and sensory quality of turkey meat. *Poult. Sci.* **1983**, *62*, 1224−1230.

Berlett, B. S.; Stadtman, E. R. Protein oxidation in aging, desease and oxidative stress. *J. Biol. Chem.* **1997**, *272*, 20313−20316.

Buckley, D. J.; Morrissey, P. A.; Gray, J. I. Influence of dietary vitamin E on the oxidative stability of pig meat. *J. Anim. Sci.* **1995**, *73*, 3122−3130.

Buttris, J. L.; Diplock, A. T. HPLC methods for vitamin E in tissues. In *Methods in Enzymology*; Packer, L., Glazer, A. N., Eds.; Academic Press: New York, 1984; Vol. 105, pp 131−138.

Decker, E. A.; Welch, B. Role of ferritin as a lipid oxidation catalyst in muscle food. *J. Agric. Food Chem.* **1990**, *38*, 674−681.

Decker, E. A.; Xiong, Y. L.; Calver, J. T.; Crum, A. D.; Blanchard, S. P. Chemical, physical and functional properties of oxidized turkey white muscle myofibrillar proteins. *J. Agric. Food Chem.* **1992**, *41*, 186−189.

Decker, E. A.; Crum, A. D.; Shantha, N. C.; Morrissey, P. A. Catalysis of lipid oxidation by iron from an insoluble fraction of beef diaphragm muscle. *J. Food Sci.* **1993**, *58*, 233−236.

Esterbauer, H.; Schaur, R. J.; Zollner, H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radical Biol. Med.* **1991**, *1*, 81−128.

Faustman, C.; Cassens, R. G.; Schaeffer, D. M.; Buege, D. R.; Scheller, K. K. Vitamin E supplementation of Holstein steer diets improves sirloin steak color. *J. Food Sci.* **1989**, *54*, 485−486.

Gatellier, Ph.; Anton, M.; Renerre, M. Lipid peroxidation induced by $H_2O_2$-activated metmyoglobin and detection of a myoglobin-derived radical. *J. Agric. Food Chem.* **1995**, *43*, 651−656.

Gatellier, Ph.; Mercier, Y.; Remignon, H.; Renerre, M. Influence du mode d'alimentation sur l'oxydation des lipides et des protéines de la viande de dinde. *Viandes Prod. Carnés.* **1996**, *17*, 300−302.

Genot, C.; Meynier, A.; Viau, M.; David, E.; Metro, B.; Remignon, H.; Gandemer, G. Acides gras alimentaires, composition des lipides intramusculaires et sensibilité à l'oxydation de la viande de dinde. *Deuxiemes journées de la recherche avicole* **1997**, 231−234 (Tours, France).

Iwahashi, H.; Parcker, C. E.; Tomer, K. B.; Mason, R. P. Detection of ethyl and penthyl radical adducts of a (4-pyridyl-1-oxide)-N-tert-butylnitrone in rat liver microsomes treated with ADP, NADPH and ferric chloride. *Free Radical Res. Commun.* **1992**, *16*, 295−301.

Kanner, J.; Harel, S.; Hazan, B. Muscle membranal lipid peroxidation by an "iron redox cycle" system: initiation by oxy radicals and site-specific mechanism. *J. Agric. Food Chem.* **1986**, *34*, 506−510.

Kanner, J.; Bartov, I.; Salan, M. O.; Doll, L. Effect of dietary iron level in situ turkey muscle lipid peroxidation. *J. Agric Food Chem.* **1990**, *38*, 601−604.

Lanari, R. N.; Scheller, K. K.; Williams, S. N.; Scheafer, D. M. Effect of dietary vitamin E on pigment and lipid stability of frozen beef: a kinetic analysis. *Meat Sci.* **1994**, *38*, 3−15.

Lauridsen, C.; Buckley, D. J.; Morrissey, P. A. Influence of dietary fat and vitamin E supplementation on α tocopherol levels and fatty acid profiles in chicken muscle membranal fractions and susceptibility to lipid peroxidation. *Meat Sci.* **1997**, *46*, 9−22.

Lynch, S. M.; Frey, B. Mechanisms of copper- and iron-dependent oxidative modification of human low-density lipoprotein. *J. Lipid Res.* **1993**, *34*, 1745−1751.

Martinaud, A.; Mercier, Y.; Marinova, P.; Tassy, C.; Gatellier, Ph.; Renerre, M. Comparison of oxidative processes on myofibrillar proteins from beef during maturation and by different model oxidation systems. *J. Agric. Food Chem.* **1997**, *45*, 2481−2487.

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

Mercier, Y.; Gatellier, Ph.; Renerre, M. Relationships between lipid and protein oxidation in different beef muscles. *Proceedings of the International Congress on Meat Science Technology*, 41st ICoMST, San Antonio, TX, 1995; E10, pp 562−563.

Mercier, Y.; Gatellier, Ph.; Viau, M.; Remignon, H.; Renerre, M. Effect of dietary fat and vitamin E on lipid and protein oxidation in turkey meat during storage. *Meat Sci.* **1998a**, *48*, 301−317.

Mercier, Y.; Gatellier, Ph.; Viau, M.; Metro, B.; Renerre, M. Protein and lipid oxidation in turkey Sartorius muscle during frozen storage as influenced by dietary fat sources and vitamin E supplementation. *Proceedings of the International Congress on Meat Science Technology*, 44th ICoMST, Barcelona, Spain; 1998b; B72, pp 632−633.

Mercier, Y.; Gatellier, Ph.; Viau, M.; Renerre, M. Influence de la composition en acides gras alimentaires et de la teneur en vitamine E sur l'oxydation des lipides et des protéines membranaires microsomaux de muscle de dindon. *3rd journées de la recherche avicole* **1999** (St-Malo, France).

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 lipid and cholesterol oxidation in pork. *J. Agric. Food Chem.* **1992**, *40*, 1310−1315.

Monahan, F. J.; Crackel, R. L.; Gray, J. I.; Buckley, D. J.; Morrissey, P. A. Catalysis of lipid oxidation in muscle model system by haem and inorganic iron. *Meat Sci.* **1993a**, *34*, 95−106.

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

Monahan, F. J.; Gray, J. I.; Asghar, A.; Haug, A.; Strasburg, G. M.; Buckley, D. J.; Morrissey, P. A. Influence of diet on lipid oxidation and membrane stucture in porcine muscle microsomes. *J. Agric. Food Chem.* **1994a**, *42*, 59−63.

Monahan, F. J.; Asghar, A.; Gray, J. I.; Buckley, D. J.; Morrissey, P. A. Effect of oxidized dietary lipid and vitamin E on the colour stability of pork chops. *Meat Sci.* **1994b**, *37*, 205−215.

Oliver, C. N.; Alin, B. W.; Moerman, E. J.; Goldstein S.; Stadtman, E. R. Age-related changes in oxidized proteins. *J. Biol. Chem.* **1987**, *262*, 5488−5491.

Renerre, M.; Labadie, J. Fresh red meat packaging and meat quality. Review paper, *Proceedings of the International Congress on Meat Science Technology*, 39th ICoMST, Calgary, AB, Canada; 1993; pp 361−389.

Renerre, M.; Dumont, F.; Gatellier, Ph. Antioxidant enzyme activities in beef in relation to oxidation of lipid and myoglobin. *Meat Sci.* **1996**, *43*, 111−121.

Renerre, M.; Poncet, K.; Mercier, Y.; Gatellier, Ph.; Metro, B. Influence of dietary fat and vitamin E on antioxidant status of muscles of turkey. *J. Agric. Food Chem.* **1999**, *47*, 237−244.

Reznick, A. Z.; Witt, E.; Matsumoto, M.; Packer, L. Vitamin E inhibits protein oxidation in skeletal muscle of resting and exercised rats. *Biochem. Biophys. Res. Commun.* **1992**, *189*, 801−806.

Rhee, K. S.; Ziprin, Y. A. Lipid oxidation in retail beef, pork and chicken muscles as affected by concentrations of heme pigments and non heme iron and microsomal enzymic lipid peroxidation activity. *J. Food Biochem.* **1987**, *11*, 1−15.

Santé, V.; Lacourt, A. The effect of dietary α-tocopherol supplementation and antioxidant spraying on colour stability and lipid oxidation in turkey meat. *J. Sci. Food Agric.* **1994**, *65*, 503−507.

Sheehy, P. J. A.; Morrissey, P. A.; Flynn, A. Increased storage stability of chicken muscle by dietary α-tocopherol supplementation. *Irish J. Agric. Food Res.* **1993**, *32*, 67−73.

Sklan, D.; Tenne, Z.; Budowski, P. The effect of dietary fat and tocopherol on lipolysis and oxidation ip turkey meat stored at different temperatures. *Poult. Sci.* **1983**, *62*, 2017−2021.

Srinivasan, S.; Hultin, H. O. Hydroxyl radical modification of fish muscle proteins. *J. Food Biochem.* **1995**, *18*, 405−425.

Stadtman, E. R. Metal ion-catalyzed oxidation of proteins: biochemical mechanism and biological consequences. *Free Radical Biol. Med.* **1990**, *9*, 315−325.

Received for review August 23, 1999. Revised manuscript received February 15, 2000. Accepted February 23, 2000. This study was funded by the AIR program (AIR2-CT94-1577, Diet-Ox).

JF990943J