Lipid Peroxidation of Muscle Food As Affected by NaCl†

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The prooxidant effect of NaCl on minced turkey muscle lipid peroxidation was demonstrated by using two methods that determine accumulation of TBA-RS and conjugated dienes. The prooxidant effect of NaCl was inhibited by EDTA and ceruloplasmin. This indicated that the effect of NaCl is to enhance the activity of iron ions toward lipid peroxidation. In a model system of washed muscle residue in the presence of ascorbic acid and iron, NaCl decreased lipid peroxidation. However, if muscle cytosol was added to the model system, NaCl enhanced lipid peroxidation. The effects of NaCl on both model systems and in minced turkey muscle seem to derive from the capability of NaCl to displace iron ions from binding macromolecules.

INTRODUCTION

Lipid peroxidation has a detrimental effect on the quality of muscle foods, as it leads to the development of off-flavors (St. Angelo and Bailey, 1987).

The mechanism of lipid peroxidation in muscle foods was studied by several researchers utilizing model systems of linoleate emulsion (Lin and Watts, 1970), microsome membranes (sarcosomes), or water-extracted muscle residue (insoluble muscle fraction) (Sato and Hegarty, 1971; Kanner and Harel, 1985; McDonald and Hultin, 1987; Asghar et al., 1988). These model systems were used to understand better the catalysis of lipid peroxidation by heme or non-heme iron compounds.

Recently it was demonstrated that the initiation of lipid peroxidation in turkey muscle tissue is affected especially by "free" chelatable iron ions (Kanner et al., 1988a-c). This could be partially prevented by removing the iron supplement from the diet fed prior to slaughtering (Kanner et al., 1989).

Sodium chloride, which is an important additive in the meat industry, has been reported to act as a prooxidant (Lea, 1937; Chang and Watts, 1950; Tappel, 1952; Banks, 1961; Ellis et al., 1968; Powers and Mast, 1980; Kanner and Kinsella, 1983) or as an antioxidant (Chang and Watts, 1950; Mabrouk and Dugan, 1960). Ellis et al. (1968) postulated that salt may activate a component in the lean meat which results in a change in the oxidation characteristics of adipose tissue.

It is well recognized that sodium chloride may accelerate muscle lipid peroxidation, but its action is poorly understood.

We will demonstrate in this paper that the catalytic effect of NaCl is derived from enhancement of the prooxidant effect of chelatable iron ions.

MATERIALS AND METHODS

Materials. Ascorbic acid, trichloroacetic acid, sodium chloride, and chloroform were purchased from Merck (Darmstadt, FGR). Thiobarbituric acid (TBA), ammonium acetate, sodium acetate, Ferrozine, Neocuproine, and ceruloplasmin type VII were obtained from Sigma Chemical Co. (St. Louis, MO). Ethylenediaminetetraacetic acid disodium salt (EDTA) and ferric chloride were purchased from BDH Chemicals Ltd. (Poole, U.K.), and Chelex 100 was purchased from Bio-Rad Laboratories (St. Albans, Herts, U.K.).

Methods. Fresh dark and light turkey muscle was minced in a Moulinex food processor equipped with a plastic work bowl and stainless steel blades for 30 s. Minced tissues were mixed with tetracycline (100 ppm) and different amounts of NaCl and stored at 4 °C in polyethylene bags.

Isolation of washed muscle residue fraction (MW) from fresh turkey muscle was carried out by mincing the tissue with a Moulinex food processor for 30 s. The minced tissue (10 g) was further homogenized in 30 mL of 0.03 M (pH 6.0) sodium acetate with a Polytron homogenizer. The homogenate was centrifuged for 30 min at 100000*g* at 4 °C. The resultant supernatant fraction was used as the soluble cytosolic extract. The pellet was washed and centrifuged two more times with acetate and used as the MW.

Chelatable iron ions were assayed by using $FeNH_4(SO_4)\cdot 12H_2O$ for standard calibration. Minced muscle tissue was mixed with double-distilled water treated with Chelex 100 and homogenzied in the Polytron for 30 s. The homogenate was centrifuged at 100000*g* at 4 °C for 30 min. The supernatant was determined for chelatable iron ions by a method developed by Stookey (1970) using a Ferrozin reagent.

Lipid peroxidation was determined with TBA according to the procedure of Bidlack et al. (1973), and conjugated dienes were determined as described previously (Kanner et al., 1988b). The results are means of triplicates; in the figures, each error bar denotes one standard deviation.

RESULTS

The prooxidant effect of NaCl on minced turkey muscle lipid peroxidation is demonstrated in Figures 1 and 2. Evidence of accelerating lipid peroxidation in minced muscle was obtained by two methods which determine accumulation of TBA-RS and conjugated dienes, respectively. Increasing NaCl concentration in both raw and frozen-thawed minced muscle enhanced lipid peroxidation (Figure 3).

The addition of $FeCl_3$ to turkey minced muscle enhanced lipid peroxidation, which was further accelerated by the addition of 0.3 M NaCl (Figure 4). The prooxidant effect of NaCl was prevented by adding EDTA (Figure 5) or ceruloplasmin (Figure 6).

Washed muscle residue was interacted with the cytosol (first supernatant extracted from minced muscle; Table I). Results show that with washed muscle residue lipid peroxidation in the presence or absence of cytosol is

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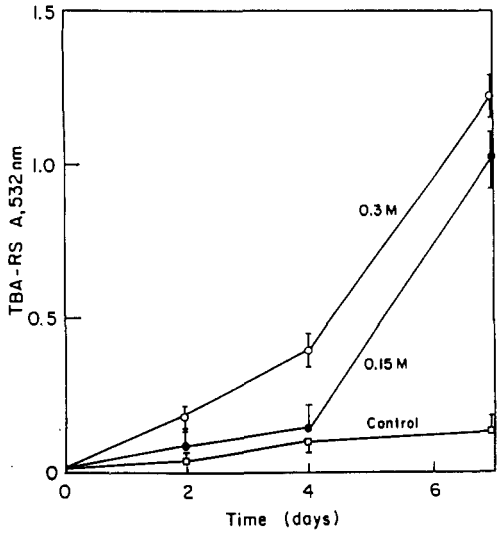

Figure 1. Effect of NaCl on lipid peroxidation (TBA-RS) of minced turkey dark muscle stored at 4 °C, 0.1*A* = 7.7 nmol of MDA/g of tissue.

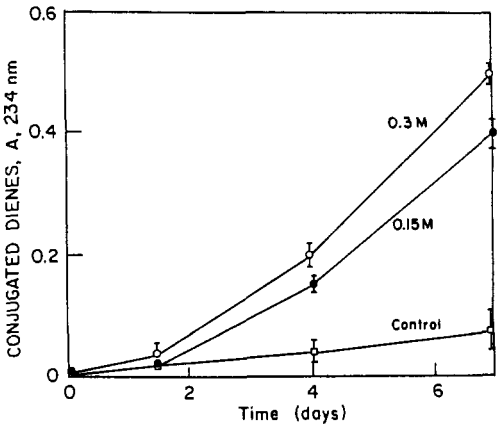

Figure 2. Effect of NaCl on lipid peroxidation (accumulation of conjugated dienes) of minced turkey dark muscle stored at 4 °C.

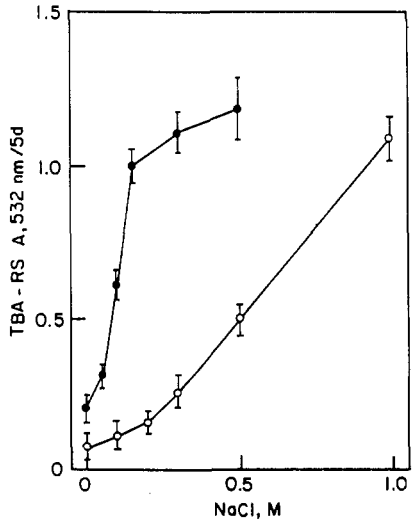

Figure 3. Effect of NaCl concentration on lipid peroxidation of turkey minced dark muscle stored at 4 °C. (○) Control; (●) freeze-thawed. 0.1*A* = 7.78 nmol of MDA/g of tissue.

affected by NaCl in a different manner. Lipid peroxidation of washed muscle residue was accelerated by ascorbic acid and by iron, especially in the presence of both compounds. The addition of 0.3 M NaCl to this model system decreased lipid peroxidation. If washed

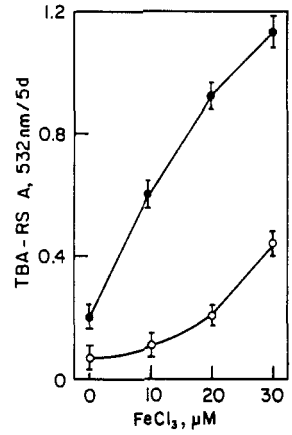

Figure 4. Effect of iron ions and added NaCl on minced muscle lipid peroxidation at 4 °C. (○) Control; (●) 0.3 M NaCl. 0.1*A* = 7.7 nmol of MDA/g of tissue.

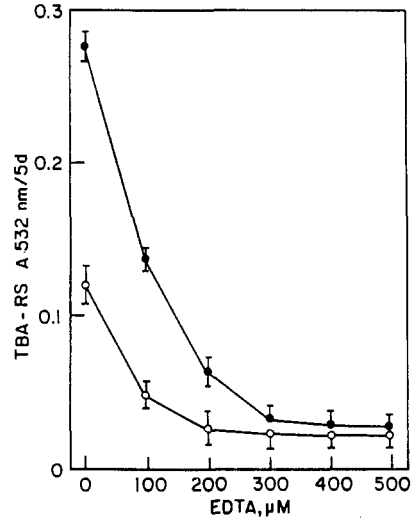

Figure 5. Inhibition of minced turkey dark muscle lipid peroxidation by EDTA in the presence and absence of NaCl, stored at 4 °C. (○) Control; (●) 0.3 M NaCl. 0.1*A* = 7.7 nmol of MDA/g tissue.

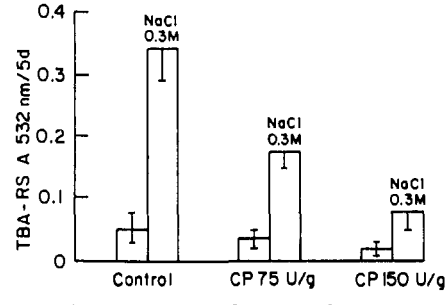

Figure 6. Inhibition of minced turkey dark muscle lipid peroxidation by ceruloplasmin (CP) in the presence and absence of NaCl, stored at 4 °C. Dark muscle stored at 4 °C. 0.1*A* = 7.7 nmol of MDA/g of tissue.

muscle residue was interacted with the cytosol, it decreased the prooxidant effects of ascorbic acid. The addition of 0.3 M NaCl to the model system containing washed muscle residue and cytosol increases lipid peroxidation compared with the control, which does not contain NaCl. The cytosol alone showed no specific lipid peroxidation, but the addition of iron and ascorbic acid to the cytosol, especially in the presence of NaCl, increased lipid peroxidation slightly but significantly (Table I).

We determined the effect of NaCl on the extraction of iron compounds from muscle tissues. Muscle was first washed with 0.3 M NaCl and then twice with distilled

Table I. Effect of NaCl on Lipid Peroxidation of a Turkey Dark Washed Muscle Residue (MW) in the Presence or Absence of the Cytosol Fraction[a]

treatment	TBA-RS as nmol of MDA (g of tissue)$^{-1}$ 30 min^{-1}			
	H_2O	AH_2[b]	Fe[c]	AH_2–Fe
MW	4.03 ± 1.8	44.80 ± 2.1	13.44 ± 1.9	134.40 ± 2.8
MW + 0.3 M NaCl	2.68 ± 1.5	20.16 ± 1.8	11.20 ± 1.4	116.4 ± 3.2
MW + cytosol	3.36 ± 1.2	6.72 ± 1.2	22.40 ± 2.4	33.60 ± 1.5
MW + cytosol + 0.3 M NaCl	35.84 ± 1.9	29.12 ± 2.2	62.72 ± 2.0	67.20 ± 2.1
cytosol	0.89 ± 0.9	4.03 ± 1.6	8.96 ± 1.8	13.44 ± 1.9
cytosol + 0.3 M NaCl	4.48 ± 1.5	8.96 ± 1.5	17.92 ± 2.0	17.92 ± 1.4

[a] Turkey dark muscle washed residue was prepared by homogenizing the tissues with 0.03 M acetate, pH 6.5 (1:4 w/v). The homogenate was separated by ultracentrifugation at 100000*g*. The supernatant (cytosol) was kept for reconstitution. The pellet was washed a further two times in distilled water treated with Chelex 100. Washed muscle residue was diluted by 0.03 M acetate or cytosol at a ratio of 1:4 (w/v) in the presence or absence of 0.3 M NaCl and incubated at 37 °C for 30 min. [b] AH_2, ascorbic acid (200 μM). [c] Fe, $FeCl_3$ (10 μM).

Table II. Lipid Peroxidation of Washed Muscle Residue (MW) and Cytosol Extracted by 0.3 M NaCl

treatment	TBA-RS, nmol of MDA (g of tissue)$^{-1}$ 30 min^{-1}			
	H_2O	AH_2[a]	Fe[b]	AH_2–Fe
MW (first extraction by acetate)[c]	3.36 ± 1.0	49.28 ± 1.2	15.68 ± 1.8	138.88 ± 2.8
MW (first extraction by 0.3 M NaCl in acetate)	1.79 ± 1.2	10.08 ± 1.9	4.48 ± 1.5	120.95 ± 2.5
cytosol (acetate)	2.24 ± 1.4	16.80 ± 2.1	8.96 ± 1.7	17.92 ± 2.5
cytosol (0.3 M NaCl in acetate)	20.16 ± 1.8	24.64 ± 2.5	28.00 ± 2.5	31.36 ± 1.3

[a] AH_2, ascorbic acid (200 μM). [b] Fe, $FeCl_3$ (10 μM). [c] Acetate 0.03 M, pH 6.5. The preparation of washed muscle residue was carried out under conditions similar to those described in Table I, except that in some samples muscle tissue was washed with acetate containing 0.3 M NaCl.

Table III. Effect of NaCl on the Extraction of Chelatable Iron Ions from Turkey Muscle Tissue[a]

treatment	iron, μg/g of tissue	
	dark muscle	light muscle
extraction (H_2O)	0.8 ± 0.09	0.3 ± 0.07
extraction (0.3 M NaCl)	1.5 ± 0.10	0.5 ± 0.06

[a] Iron ions were extracted from muscle tissues by homogenization with distilled water (treated with Chelex 100) or 0.3 M NaCl (1:4 w/v). The homogenate was centrifuged at 100000*g*, 4 °C. The supernatant was concentrated by freeze-drying and determined for iron ions by the Ferrozine method.

Table IV. Effect of NaCl on Cytosol Extraction and Separation of Cytosol Chelatable Iron Ions by Several Synthetic Membranes

treatment	iron, μg/g of tissue		
	Whatman 42 >300 000	Millipore[a] <300 000	Amicon[b] <10 000
extraction (H_2O)	0.60	0.37	0.15
extraction (0.3 M NaCl)	1.85	0.37	0.15

[a] Millipore membrane, 300 000 MW cutoff. [b] Amicon membrane, ~10 000 MW cutoff.

water. The results presented in Table II show that it was possible to increase the stability of the muscle washed residue to the prooxidant effect of ascorbic acid if the muscle was washed previously with 0.3 M NaCl. As ascorbic acid's prooxidant effect is dependent on metal ions, it seems that by washing the muscle treated with NaCl we decrease the metal ion content in the muscle system. If ferric chloride was added to the model system, ascorbic acid increased lipid peroxidation almost the same as the control. The cytosol (alone) extracted with 0.3 M NaCl showed a higher lipid peroxidation than that extracted with acetate (Table II).

The amount of chelatable iron ions in the eluent extracted from muscle by distilled water was 0.8 μg/g of fresh weight and almost double—1.5 μg/g of fresh weight—in the eluent extracted from muscle by 0.3 M NaCl (Table III). By washing muscle tissue with 0.3 M NaCl it was possible also to extract more efficiently the added ferric chloride from minced muscles (results not shown). Table IV shows the distribution of iron ions between molecules of different molecular weight. It was found that NaCl increased the extraction of iron ions from muscle tissue; most of it was found bound to molecules of a mass greater than 300 000 Da.

DISCUSSION

The prooxidant effect of NaCl on minced turkey muscle lipid peroxidation was demonstrated by determining the accumulation of TBA-RS and conjugated dienes. These results are in agreement with our previous work (Kanner and Kinsella, 1983) and that of others showing that NaCl accelerated muscle lipid peroxidation (Ellis et al., 1968; Powers and Mast, 1980). The mechanism by which NaCl affects muscle lipid peroxidation is poorly understood (Rhee et al., 1983; Anon., 1988; Hultin, 1988).

Increasing the concentration of NaCl enhanced lipid peroxidation in raw minced muscle, especially after freezing–thawing process. This effect seems to derive from the fusion of the intracellular compounds and the destruction of the cell structure by NaCl (Shomer et al., 1987). In addition, we suggest that NaCl acts to displace iron ions from binding sites, similar to those found for Pb^{2+} and Al^{3+} by Aruoma et al. (1989).

Muscle tissue contains a small amount of "free" iron ions (Kanner et al., 1988a); addition of iron ions (10–20 μM) to minced muscle increased slightly the rate of lipid peroxidation. However, the same treatment, in the presence of NaCl, enhanced lipid peroxidation approximately 3–5-fold more than the control. We suggest that a large part of the added iron ions interacted with the proteins. This interaction seems to prevent iron ions from affecting membranous lipids and acting as catalyzers of lipid peroxidation. NaCl seems to disturb the interaction between iron ions with the proteins and therefore leaves more free iron ions to interact with the lipid fraction which enhances lipid peroxidation.

Previously it was shown that the inhibition of lipid peroxidation in model systems and muscle tissue by EDTA and ceruloplasmin is totally dependent on free iron ions and not on other potential catalyzers such as H_2O_2-activated myoglobin (Kanner et al., 1988b,c). The results showed that EDTA and ceruloplasmin inhibit the pro-

oxidant effect of NaCl. These results indicate also that the effect of NaCl is through the action of free iron ions.

To understand better the effects of NaCl on muscle tissue lipid peroxidation, experiments were designed to evaluate these effects on the washed muscle residue (membranous and the insoluble fraction) and the cytosol.

Lipid peroxidation of washed muscle residue was accelerated by the addition of ascorbic acid. Similar results were obtained by using microsomes (Kanner et al., 1986). This reaction was found to be totally dependent on traces of metal ions, especially iron ions (Thomas and Aust, 1985; Kanner et al., 1986; Buettner, 1988). The addition of 0.3 M NaCl decreased lipid peroxidation in model systems containing washed muscle residue and ascorbic acid. Thomas and Aust (1985) showed that microsomes are contaminated with iron compounds, which release iron for the catalysis of lipid peroxidation. Sodium chloride, by its possible capacity to displace iron compounds, seems to reduce the amount of iron ions inside the membranes and thus decrease the effect of ascorbic acid on lipid peroxidation. Evidence of the capacity of NaCl to displace iron compounds from the insoluble fraction was provided by the results presented in Tables II and III. The inhibitory effect of NaCl on membranous lipid peroxidation is especially affected if the amount of the iron ions is low. Increasing the concentration of iron ions in the membranous system, by addition of $FeCl_3$, reduces the inhibitory effect of NaCl from 50–75% to only 13–15%.

Sodium chloride, in particular, increased lipid peroxidation in the system containing washed muscle residue and cytosol. As the cytosol contains iron ions (Kanner et al., 1988a), most probably chelated by proteins, NaCl in this system seems to increase the amount of catalytic free iron ions, which could penetrate into the lipid phase and increase lipid peroxidation.

The cytosol of muscle tissue contains both prooxidants and antioxidants (Kanner et al., 1991). Lipid peroxidation of washed muscle residue accelerated by ascorbic acid or iron–ascorbic acid was inhibited significantly by the cytosol. The inhibitory effect of the cytosol may derive from its capacity to bind free iron ions; this antioxidant effect was suppressed by NaCl and matches results obtained in experiments with minced muscle tissue. The addition of small amounts of $FeCl_3$ to minced muscle tissue affected lipid peroxidation only slightly; however, the addition of NaCl to this system enhanced iron catalysis, most probably by preventing the binding of iron ions to macromolecules.

The incubation of the cytosol (alone), and especially that extracted in the presence of NaCl, produced a small amount of TBA-RS. The source of these compounds is not known. We suggest that these compounds may be derived in part from emulsified lipids undergoing peroxidation. However, TBA-RS could arise also from compounds other than lipids.

In conclusion, it seems that NaCl acts by displacing iron ions from binding sites and thereby affects iron ion dependent muscle lipid peroxidation. Although we do not have direct evidence, in washed muscle residue, which contains membranous lipids but also a low concentration of iron ions, NaCl seems to reduce the amount of iron ions bound to membranous fatty acids and thereby to decrease lipid peroxidation. In minced muscle tissue, which contains the insoluble fraction but also the cytosol, NaCl seems to reduce the capability of the cytosol to bind iron ions. As most of the chelatable iron is in the cytosol fraction (Kanner et al., 1988), the overall effect of NaCl, at the added concentration of 0.1–1.0 M, was in the prooxidant range.

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