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# Lipid Hydroperoxidase Activity of Myoglobin and Phenolic Antioxidants in Simulated Gastric Fluid

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Our recent study demonstrated the potential of gastric fluid at pH 3.0 to accelerate lipid peroxidation and cooxidation of dietary constituents in the stomach medium. Metmyoglobin is known to catalyze the breakdown of lipid hydroperoxides to free radicals, a reaction that could enhance the propagation step and general lipid peroxidation. During this reaction, a part of the free radicals is autoreduced by metmyoglobin. At pH 3.0, metmyoglobin at low concentration was almost $7 \times 10^4$ times as effective as at pH 7.0 in enhancing the rate of lipid peroxidation. Our study demonstrated that metmyoglobin, at a low concentration (∼1:30), as compared with that of the hydroperoxides in the lipid system, worked prooxidatively increasing the amounts of linoleate hydroperoxides. However, at a high concentration (∼1:3), metmyoglobin acted antioxidatively and decomposed hydroperoxides, whose concentrations then remained at zero for a long time. Catechin, a known polyphenol, supports the inversion of metmyoglobin catalysis, from prooxidation to antioxidation. The antioxidative activity of the couple metmyoglobin−catechin was better at pH 3.0 than at pH 7.0, indicating that this reaction is more dependent on metmyoglobin than on catechin. During this reaction, catechin or quercetin not only donates reducing equivalents to prevent lipid peroxidation but also prevents the destruction and polymerization of metmyoglobin. The results of this research highlighted the important and possible reactions of heme proteins and polyphenols as couple antioxidants, working as hydroperoxidases or as pseudo-peroxidases. We hypothesize that the occurrence of these reactions in the stomach could have an important impact on our health and might help to better explain the health benefits of including foods rich in polyphenol antioxidants in the meal, especially when consuming red meat.

**KEYWORDS: Lipid peroxidation; hydroperoxides; myoglobin; catalysis; hydroperoxidase activity; phenolic antioxidant; simulated gastric fluid; red meat**

## INTRODUCTION

Lipid peroxidation has a detrimental effect on the quality of various foods and especially muscle foods (*1, 2*). The consumption of oxidized fat on repeated occasions may pose a chronic threat to human health. Epidemiological studies and experimental data suggest that diets high in fat and red meat are risk factors, contributing to the development of atherogenesis (*3*−*6*) and several kinds of cancer (*7*−*10*). Our recent study (*11*) demonstrated the potential of gastric fluid to accelerate reactions, which generate lipid hydroperoxides and cooxidize dietary constituents in stomach medium.

Muscle foods contain large amounts of endogenous catalysts, such as free iron ions and myoglobin, which accelerate lipid peroxidation (*1, 12*−*16*). Myoglobin can act prooxidatively or antioxidatively, depending on the concentration of $H_2O_2$ hydroperoxides, reducing agents, and their compartmentalization in the food material (*17*−*20*).

The aim of this study was to evaluate the myoglobin hydroperoxidase activity, at the low pH of simulated gastric fluid (SGF), in the presence and without phenolic antioxidants.

## MATERIALS AND METHODS

Metmyoglobin (metMb, from horse skeletal muscle), soybean lipoxygenase (type I-B), linoleic acid, Tween 20, butylated hydroxytoluene (BHT), catechin, pepsin (A, from porcine stomach mucosa), ferrous ammonium sulfate, xylenol orange, and triphenylphosphine were obtained from Sigma Chemical Co. (St. Louis, MO). Sodium acetate (Frutarom, Haifa, Israel), sodium chloride, hydrogen peroxide (30%), and L-(+)-ascorbic acid (AA) were obtained from Merck (Darmstadt, Germany). Ferric chloride (Fe) was obtained from Riedel-de-Haen (Hannover, Germany). Sodium borohydride was from BDH (Poole, Dorset, England). Solvents were all high-performance liquid chromatography grade, acetic acid (J. T. Baker, Phillipsburg, NJ). Soybean oil was bought at commercial stores in Israel.

**Hydroperoxides Measurement in Linoleic Acid Emulsions. Spectroscopic Measurement.** Hydroperoxides were determined by means of the ferrous ion oxidation−xylenol orange (FOX2) method (*21*), including spectral analysis at 560 nm with a $H_2O_2$ standard curve.

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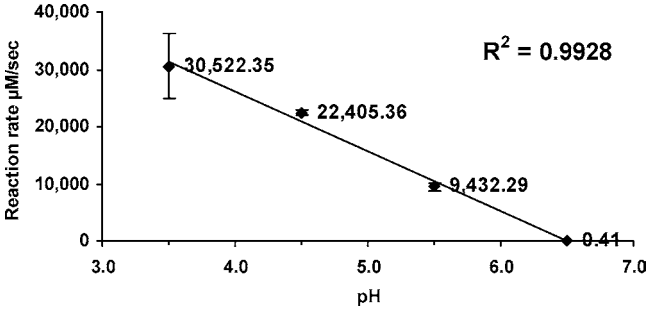


**Figure 1.** pH effect on linoleate peroxidation by metMb. The oxidation rate of linoleate was measured by the change at 234 nm for the first 30 s at 25 °C with a rapid kinetic accessory (SFA-12, Hi-TECH scientific) connected to a UV–vis diode array spectrophotometer. The system contains at different pH values acetate buffer (0.2 M), linoleic acid (2.7 mM), linoleic hydroperoxides (13.7 $\mu$M), Tween 20 (0.18%), ethanol (0.14%), and metMb (2.5 $\mu$M). Data are means ± SD ($n = 3$).

The reaction mixtures contained 2.7 mM linoleic acid, 0.14% (v/v) Tween 20, and 0.14% (v/v) ethanol as emulsifiers, in various solutions: 0.01 M acetate buffer (pH 7.0−3.0), HCl solutions (pH 3.0−1.5), SGF, and human gastric fluid. The catalysts (metMb, Fe-AA, or metMb-Fe-AA) and the antioxidants (quercetin or catechin) were added to the reaction mixtures, as indicated in the Results section. Catechin was dissolved in 10% ethanol in water; thus, the ethanol concentration in the reactions was 1−2%. The reaction tubes were incubated in triplicate in a shaking bath at 37 °C for 180 min. Samples of 25 or 50 $\mu$L were taken from the tubes during the incubation and added to 475 or 950 $\mu$L, respectively, of FOX2 reagent. Before each experiment, an old stock solution of linoleic acid was mixed with fresh solution in order to maintain an accurately consistent initial level of hydroperoxides. It had been confirmed in preliminary experiments that the presence of metMb, Fe, AA, quercetin or catechin themselves, concentration used, did not interfere with the FOX2 assay.

**Kinetics Measurement on Linoleic Acid Peroxidation.** The determination of rapid changes of diene concentration due to linoleic acid oxidation by metMb was also evaluated by a rapid kinetic accessory (SFA-12, Hi-TECH Scientific, Salisbury, United Kingdom) connected to a HP8452A UV−vis diode array spectrophotometer. The rapid kinetic accessory allowed us to mix the reagents with the catalyzer at a very short period of time and to read the changes spectrophotometrically from the 0.01 of the second.

**Hydroperoxides Measurement in Soybean Oil Emulsions.** Soybean oil was peroxidized by stirring and heating at 60 °C for 3 days. The reaction mixtures contained oil at 0.7 mg/mL, with 0.05% (v/v) Tween 20 and 3% (v/v) ethanol. The assay was similar to that of linoleic acid, except for the addition of a centrifugation step for 2 min at 20800*g* prior to the spectral analysis at 560 nm.

**Separation of Myoglobin by Gel Electrophoresis.** Lipid peroxidation was determined at pH 3.0 as described above. Myoglobin (25 $\mu$M), linoleic acid (2.7 mM), and hydroperoxides (455 ± 7 $\mu$M) with or without catechin (500 $\mu$M) were incubated at 37 °C. During incubation, samples of 20 $\mu$L were collected, 20 $\mu$L of buffer was added, and the solution was heated to 100 °C for 5 min. After they were heated, the samples were cooled in ice until applied to the gels of sodium dodecyl sulfate (SDS) polyacrylamide (10%). Electrophoresis was performed on a Bio Rad (Hercules, CA) system. After elution, the gels were developed by Coomassie Blue.

**Statistical Analysis.** Results (means ± SD) are expressed as percentage or weight or molar basis. Statistical significance was assayed using one-way analysis of variance, followed by a ranking procedure using Student−Newman−Keuls test (SAS software, SAS Institute Inc., Cary, NC). Results are the means of triplicate, and in the figures, each error bar (I) denotes the standard deviations.

## RESULTS

The effect of pH on myoglobin as a catalyzer of linoleate peroxidation is summarized in **Figure 1**. The results showed that at pH 3.0 the reaction rate is ~$7 \times 10^4$ higher than at pH 7.0. The effect of metMb concentration on linoleate peroxidation is demonstrated in **Figure 2**. In the pH 3.0 of SGF, at a low concentration, metMb (2.5 $\mu$M) acts prooxidatively by increasing the concentration of linoleate hydroperoxides by almost 10-fold. Increasing the metMb concentration decreases the hydroperoxide accumulation, and at a concentration of 30 $\mu$M, metMb works antioxidatively to decompose hydroperoxides to zero concentration and to keep the system for a long period of time (180 min) without propagation. The same antioxidant trend by metMb was achieved using free iron ions (10 $\mu$M) and AA (100 $\mu$M) as additive catalyzers (**Figure 3**). The inversion of catalysis by metMb from prooxidation to antioxidation was tested also in a model system containing soybean oil (**Figure 4**). In this system, a high concentration of metMb works antioxidatively; however, 30 $\mu$M metMb and even 120 $\mu$M metMb did not succeed to decrease the hydroperoxides to zero concentration. A similar effect by working with triglycerides was achieved in our previous articles (*11*), also by using a polyphenol antioxidant. The addition of a polyphenolic antioxidant such as catechin on the inversion catalysis of different concentrations of metMb is demonstrated in **Figure 5**. MetMb at a concentration of 5 or 10 $\mu$M works prooxidatively in the linoleate system that contained 100 $\mu$M hydroperoxides. Catechin at a concentration of 100 or 250 $\mu$M changes the reaction from prooxidative to antioxidative of 10 and 5 $\mu$M metMb, respectively. The concentration of catechin needed to inverse catalysis of metMb from prooxidation to antioxidation is also dependent on the initial concentration of the lipid hydroperoxides (**Figure 6**). The pH level affects the activity of the couple metMb−phenolic-

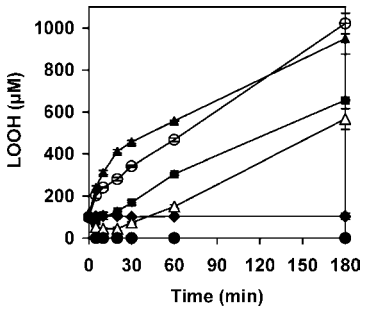


**Figure 2.** Effect of metMb concentration on linoleic acid peroxidation at pH 3.0. Hydroperoxides were measured by the FOX2 method. The system contains acetate buffer at pH 3.0 (0.2 M), linoleic acid (2.7 mM), linoleic hydroperoxides (100 $\mu$M), Tween 20 (0.18%), ethanol (0.14%), and metMb at different concentrations. Data are means ± SD ($n = 3$). Key: 0 $\mu$M (◆); 2.5 $\mu$M (▲); 5 $\mu$M (×); 10 $\mu$M (■); 20 $\mu$M (△); and 30 $\mu$M (●).

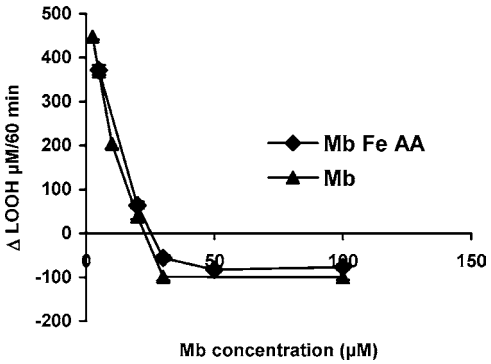


**Figure 3.** Effect of myoglobin concentration with and without iron–AA on linoleic acid peroxidation at pH 3.0 after 60 min. The system contains the same compound as described in **Figure 2** and free iron ions (10 $\mu$M) and AA (100 $\mu$M). Data are means ± SD ($n = 3$).

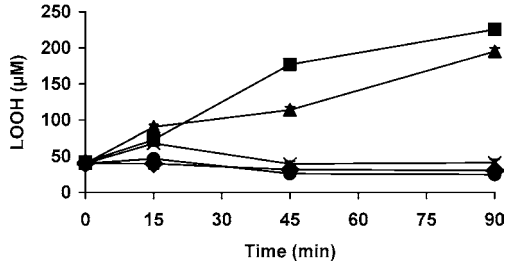


**Figure 4.** Effect of myoglobin concentration on soybean oil peroxidation at pH 3.0. The system contains soybean oil (0.7 mg/mL), Tween 20 (0.18%), acetate buffer at pH 3.0 (0.2 M), hydroperoxides (40 $\mu$M), ethanol (3%), and metMb at different concentrations, incubated at 25 °C. Data are means ± SD ($n = 3$). Key: 0 $\mu$M (◆); 3 $\mu$M (■); 6 $\mu$M (▲); 30 $\mu$M (▼); and 120 $\mu$M (●).

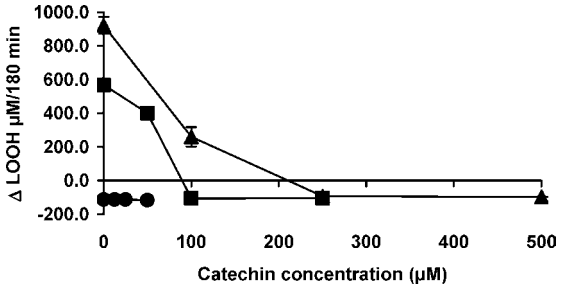


**Figure 5.** Linoleic acid peroxidation by metMb at different concentrations, affected by catechin, at pH 3.0. The system contains reactants such as those in **Figure 2**; the initial hydroperoxide concentration was 100 $\mu$M. Data are means ± SD ($n = 3$). Key: 5 $\mu$M (▲); 10 $\mu$M (■); and 30 $\mu$M (●).

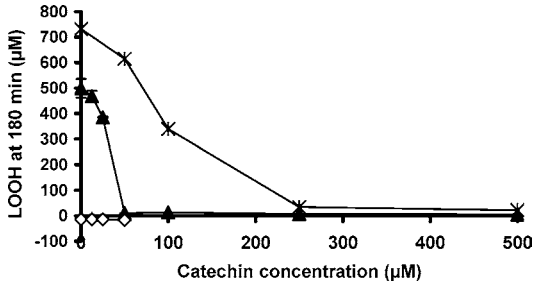


**Figure 6.** Effect of catechin concentration on the peroxidation or antioxidation by metMb at three linoleic acid hydroperoxide concentrations. The system contained acetate buffer at pH 3.0 (0.2 M), linoleic acid (2.7 mM), linoleic hydroperoxides (100, 300, and 600 $\mu$M), Tween 20 (0.18%), ethanol (0.14%), and metMb (30 $\mu$M). Data are means ± SD ($n = 3$). Key: 100 $\mu$M (◇); 300 $\mu$M (■); and 600 $\mu$M (*).

coupled compound (**Figure 7A**,**B**). At pH 7, the couples metMb (10 $\mu$M) and catechin (50, 75, and 100 $\mu$M) work only in part antioxidatively, but at pH 3.0, metMb (10 $\mu$M) coupled with catechin (100 $\mu$M) acted antioxidatively and decomposed hydroperoxides to a concentration of zero. The heme structure of metMb (Soret band at 408 nm) was disrupted during the catalysis of linoleate hydroperoxide breakdown, most probably by free radicals. This effect is prevented by quercetin (**Figure 8A**,**B**). During this reaction quercetin is oxidized, which is observed by the decrease at 370 nm.

The oxidation and polymerization of metMb during catalysis of lipid peroxidation are demonstrated at **Figure 9**. The results showed that metMb is partly polymerized to the 34 kDa dimer protein, but this dimerization is prevented if catechin (500 $\mu$M) is included in the model system.

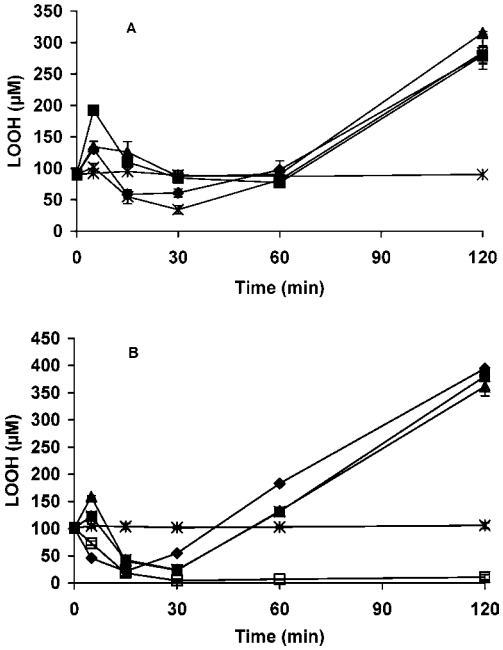


**Figure 7.** Effect of pH on linoleic acid peroxidation by metMb and catechin. (**A**) pH 7.0; (**B**) pH 3.0. The system contains the same reactants such as those in **Figure 2**. Data are means ± SD ($n = 3$). Cat = catechin. Key: cat (*); metMb (◆); metMb + cat (50 $\mu$M) (▲); metMb + cat (75 $\mu$M) (■); and metMb + cat (100 $\mu$M) (□).

## DISCUSSION

The involvement of heme protein, as catalyzers of lipid peroxidation, was first described by Robinson (*22*). The reaction between heme proteins such as myoglobin, hemoglobin, and lipid hydroperoxides and their contribution to oxidative damage has been extensively researched over many decades (*12*−*19*, *23*).

MetMb is known to catalyze the breakdown of lipid hydroperoxides to free radicals, a reaction that enhances the propagation step and general peroxidation (*12*, *25*−*27*). Several studies that involved working with linoleic acid have demonstrated that those reactions (1 and 2) are relevant (*12*, *26*, *27*).

$$MbFe^{3+} + LOOH \rightarrow MbFe^{4+}{-}OH + LO^{\bullet} \quad (1)$$

$$MbFe^{4+}{-}OH\ LOOH \rightarrow MbFe^{3+}LOO^{\bullet} + H_2O \quad (2)$$

However, other studies (*28*) with phospholipids have found a different mechanism (3 and 4) to be the relevant one and that no ferryl species was produced.

$$MbFe^{3+} + LOOH \rightarrow MbFe^{2+} + LOO^{\bullet} + H^{+} \quad (3)$$

$$MbFe^{2+} + LOOH \rightarrow MbFe^{3+}LO^{\bullet} + HO^{-} \quad (4)$$

where $MbFe^{3+}$ = metMb; $MbFe^{4}{-}OH$ = ferryl myoglobin; $MbFe^{2+}$ = deoxymyoglobin; and LOOH = lipid hydroperoxide.

An early investigation on ferryl species found that they decayed more rapidly at acidic pH and that their reactions with reducing agents were faster under acidic conditions (*29*−*31*). Skibsted's group (*30*) working on the same problem used electron spin resonance spectroscopy to determine the autoreduction of ferryl myoglobin and noted a sharp $g = 2.003$ formed at pH 5.0, suggesting the formation of a free radical intermediate, $+^{\bullet}MbFe^{3+}{-}OH$. The rate constants for ferryl formation and autoreduction showed different pH dependences, but the pH

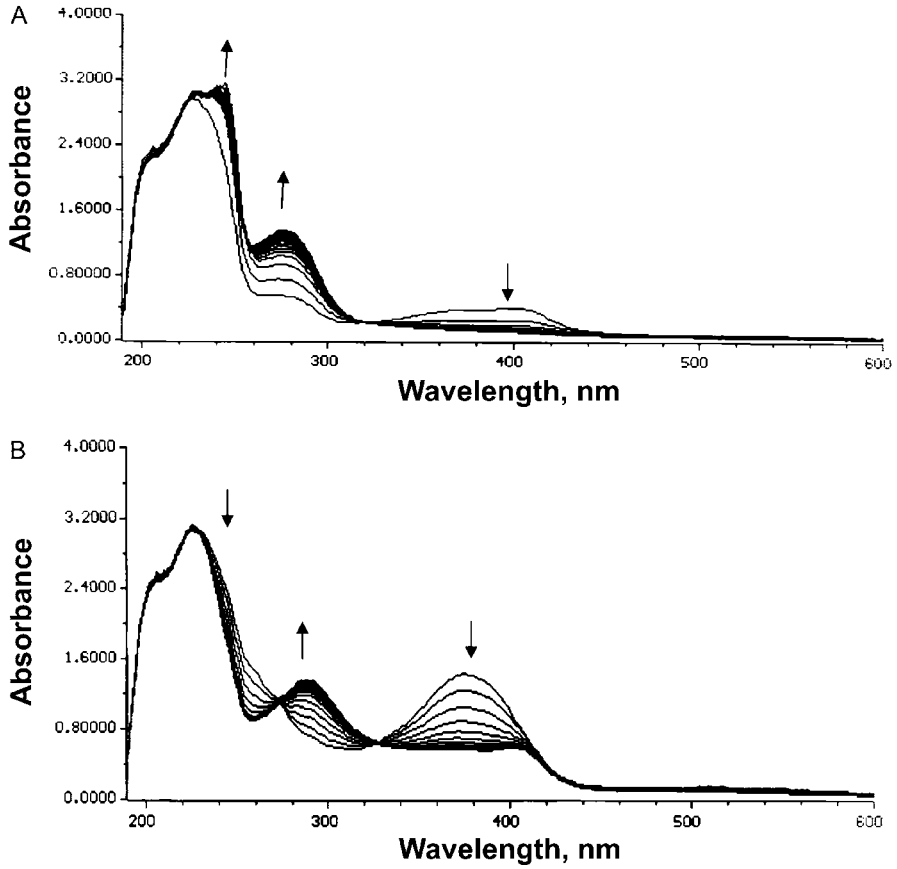


**Figure 8.** Changes on the spectrum of metMb, quercetin, and linoleic acid during lipid peroxidation at pH 4.0. The system was scanned each 10 s for 210 s without quercetin (**A**) and with quercetin (50 $\mu$M) (**B**). The concentrations of linoleic acid (2.7 mM), hydroperoxides (90 $\mu$M), and metMb (10 $\mu$M) in buffer acetate, pH 4.0 (0.01 M), at 25 °C. Data are means ± SD ($n = 3$).

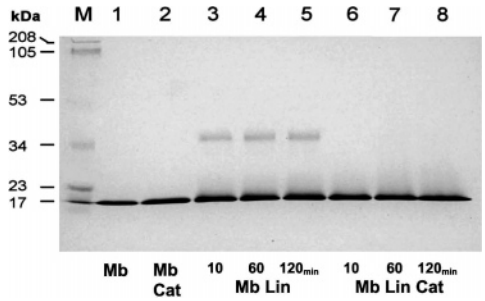


**Figure 9.** Separation of myoglobin by SDS–polyacrylamide gel electrophoresis during linoleic acid peroxidation with and without catechin at pH 3.0. The system contains acetate buffer (0.01 M) at pH 3.0, linoleic acid (2.7 mM), linoleic hydroperoxides (455 $\mu$M), Tween 20 (0.18%), ethanol (0.14%), metMb (25 $\mu$M), and catechin (500 $\mu$M).

dependence of the autoreduction was the same as that for metMb-catalyzed breakdown of linoleate hydroperoxides (*31*).

Most recently, our study (*11*) demonstrated that the acid pH of gastric fluid enhanced metMb-catalyzed lipid peroxidation: A fast kinetics accessory was used to show that metMb accelerated the oxidation of 2.7 mM linoleic acid (containing 13.7 $\mu$M linoleate hydroperoxides) by almost $7 \times 10^4$ times more at pH 3.0 than at pH 7.0. The protonated form of ferryl has been hypothesized to regulate the peroxidatic activity of myoglobin (*31*). Our results for the first time highlighted the high potential of metMb to act as a catalyst at the low pH 3.0 of the gastric fluid.

At a low concentration in the lipid system, metMb acted prooxidatively and so increased the concentration of linoleate hydroperoxides, but at a high concentration, it acted antioxidatively and so decomposed the hydroperoxides, which then remained at zero concentration for a long time. Similar findings have been published by many authors (*17*, *19*, *20*, *28*, *32*); the so-called antioxidative activity of heme proteins was been found at linoleate−heme ratios ranging from 50 to 200, but the prooxidative activity of the heme protein has been observed only when the linoleate heme ratio exceeded 250 (*33*). Our present study showed that the inversion from antioxidation to prooxidation was dependent not only on the linoleate−heme protein ratio but, most importantly, on the ratio between the concentration of linoleate hydroperoxides and heme proteins. Decreasing the concentration of linoleate hydroperoxides increases the antioxidative effects of metMb. Most interesting, it was found recently that the antioxidant peroxidase activity of myoglobin contributes significantly to attenuate the ischemia/reperfusion injury of the heart by oxygen reactive species (*34*). In systems without hydrogen peroxide or lipid hydroperoxides, heme proteins are silent compounds.

The interaction of metMb with linoleate hydroperoxides leads to the generation of ferryl myoglobin and alkoxyl and peroxyl radicals (*12*). All of these species could be autoreduced by the heme proteins, which include the porphyrin ring and amino acids as electron donors, and such autoreduction would produce free radical proteins and cause intramolecular rearrangement and cross-linkage of the protein (*19*, *23*−*25*, *30*, *35*). In our present study, the autoreduction through the porphyrin ring was manifested in the bleaching of the Soret band at 408−421 nm (**Figure 8A**). The generation of protein radicals, intramolecular rearrangement, and cross-linking of the globin protein were also exhibited in the dimerization of metMb to the 34 kDa dimer. If the autoreduction process is very efficient, the antioxidative tone

of heme proteins will dominate in the system. The antioxidative effect has been found to be very strongly supported by phenolic antioxidants such as catechin (*11*, *20*). Phenolic compounds work better as antioxidants, electron donors, or radical scavengers, when they are in the form of phenolates or when the pH is 7−8 (*36*, *37*). However, the antioxidative activity of the metMb−catechin couple is more effective at pH 3.0 than at pH 7.0. This antioxidative reaction is more dependent on metMb than on catechin, but catechin supports the inversion of metMb catalysis from prooxidation to antioxidation.

The interaction between metMb and linoleate hydroperoxides, through the generation of free radicals and autoreduction, destroys the heme protein, as illustrated below. This activity permits metMb to work as a hydroperoxidase and catalytic antioxidant by the following reactions (5−7).

$$\text{P}-\text{hemeFe}^{3+} + \text{LO}^{\bullet} \rightarrow \text{P}-{}^{\bullet}\text{hemeFe}^{3+} + \text{LO}^{-} \quad (5)$$

$$\text{P}-{}^{\bullet}\text{hemeFe}^{3+} + \text{autoreduction} \rightarrow {}^{\bullet}\text{P}-\text{hemeFe}^{3+} \quad (6)$$

$${}^{\bullet}\text{P}-\text{hemeFe}^{3+} + \text{PhOH} \rightarrow \text{P}-\text{hemeFe}^{3+} + \text{PhO}^{\bullet} + \text{H}^{+} \quad (7)$$

where P = protein; H = heme ring; Fe = heme iron; $\text{PHemeFe}^{3+}$ = metMb; $\text{LO}^{\bullet}$ = alkoxy1 radical; PhOH = polyphenol antioxidant; and $\text{PhO}^{\bullet}$ = phenoxyl radical.

The equations show that the radical on metMb could be on the heme (eq 5) or on the protein (eq 6) and both seem to be reduced by polyphenols (eq 7). Polyphenols act not only as scavengers of lipid free radicals but, most probably, as electron donors for the porphyrin and protein radicals, thus preventing the destruction of the heme and the cross-linking of the proteins (reaction 7). Our study was performed at pH 3.0, which simulated the pH of the gastric fluid without the addition of pepsin in order to prevent changes in myoglobin. Most recently, it was found that pepsin proteolysis of myoglobin decreases its peroxidases activity but increases its prooxidative effects on lipid oxidation (*38*). The effects of myoglobin proteolysis on lipid peroxidation at low pH should be further determined.

The results of this study and others published by us (*11*, *20*) highlight the importance of the reactions of heme proteins not only with free lipids but also with membranal lipids such phospholipid hydroperoxides (*20*) and the possible inversion of the catalysis from prooxidation to antioxidation, in the presence of polyphenol antioxidants, which causes the heme proteins to act as a pseudo-membranal "glutathione peroxidase".

Recently, we presented a study in which reactions that occur in the acidic pH environment of the stomach were simulated. These reactions affected lipid peroxidation, notably the generation and breakdown of dietary hydroperoxides by the endogenous catalysts found in foods (*11*). This present study focused on the hydroperoxidase activity of metMb, a known endogenous catalyst found in muscle tissues and in food products. The study addressed the prooxidative activity of metMb and its inversion to antioxidation, as affected and supported by polyphenol antioxidants at pH 3.0.

We hypothesize that the occurrence of these reactions in the stomach could have an important impact on our health and might help to better explain the health benefits of diets rich in polyphenol antioxidants consumed during a meal containing red meat.

## LITERATURE CITED


(1) Kanner, J. Oxidative processes in meat and meat products: Quality implications. *Meat Sci.* **1994**, *36*, 169−189.

(2) St. Angelo, A. J. *Lipid Oxidation in Food*; ACS Symposium Series 500; American Chemical Society: Washington, DC, 1992.

(3) Lusis, A. J. Atherosclerosis. *Nature* **2000**, *407*, 233−241.

(4) Cohn, J. C. Oxidized fat in the diet, postprandial lipaemia and cardiovascular disease. *Curr. Opin. Lipidol.* **2002**, *13*, 19−24.

(5) Wolff, S. P.; Nourooz-Zadeh, J. Hypothesis, UK consumption of dietary lipid hydroperoxides—A possible contributory factor to atherosclerosis. *Atherosclerosis* **1996**, *119*, 261−263.

(6) Ursini, F.; Zamburlini, A.; Cazzolato, G.; Maiorino, M.; Bon, G. B.; Sevanian, A. Postprandial plasma lipid hydroperoxides: A possible link between diet and atherosclerosis. *Free Radical Biol. Med.* **1998**, *25*, 250−252.

(7) Sawa, T.; Akaike, T.; Kida, K.; Fukushima, Y.; Takagi, K.; Maeda, H. Lipid peroxyl radicals from oxidized oils and heme-iron: Implication of high-fat diet in colon carcinogenesis. *Cancer Epidemiol. Biomarkers Prev.* **1998**, *7*, 1007−1012.

(8) Ward, M. H.; Sinha, R.; Heineman, E. F.; Rothmann, M. R.; Weisenburger, D. D.; Correa, P.; Zahm, S. H.; et al. Risk of adenocarcinoma of the stomach and esophagus with meat cooking method and doneness preference. *Int. J. Cancer* **1997**, *71*, 14−19.

(9) Norat, T.; Riboli, E. Meat consumption and colorectal cancer: A review of epidemiologic evidence. *Nutr. Rev.* **2001**, *59*, 37−47.

(10) The British Government's Committee on Medical Aspects of Food and Nutrition. Department of Health (COMA). Nutrition aspects of the development of cancer. *Report on Health and Social Subjects 48*; The Stationery Office: London, 1988.

(11) Kanner, J.; Lapidot, T. The stomach as a bioreactor: Dietary lipid peroxidation in the gastric fluid and the effects of plant-derived antioxidants. *Free Radical Biol. Med.* **2001**, *31*, 1388−1395.

(12) Kanner, J.; Harel, S. Initiation of membranal lipid peroxidation by activated metmyoglobin and methemoglobin. *Arch. Biochem. Biophys.* **1985**, *237*, 314−321

(13) Kanner, J.; German, J. B.; Kinsella, J. E. Initiation of lipid oxidation in biological systems. *CRC Crit. Rev. Food Sci. Nutr.* **1987**, *25*, 317−362.

(14) Kanner, J. Mechanism of nonenzymic lipid peroxidation in muscle foods. In *Lipid Oxidation in Food*; St. Angelo, A. J., Ed.; ACS Symposium Series 500; American Chemical Society: Washington, DC, 1992; pp 55−73.

(15) Decker, E. A.; Hultin, H. O. Lipid oxidation in muscle foods via redox iron. In *Lipid Oxidation in Food*; St. Angelo, A. J., Ed.; ACS Symposium Series 500; American Chemical Society: Washington, DC, 1992; pp 33−54.

(16) Tappel, A. L. Biocatalysts: Lipoxidase and hematin compounds. In *Autoxidation and Antioxidants*; Lundberg, W. O., Ed.; Interscience Publishers: New York, 1961; pp 326−366.

(17) Kendrick, J.; Watts, B. M. Acceleration and inhibition of lipid oxidation by heme compunds. *Lipids* **1969**, *4*, 454−458.

(18) O'Brien, P. J. Intracellular mechanisms for the decomposition of a lipid peroxide. 1. Decomposition of a lipid peroxide by metal ions, heme compunds, and nucleophiles. *Can. J. Biochem.* **1969**, *47*, 485−493.

(19) Harel, S.; Kanner, J. Haemoglobin and myoglobin as inhibitors of hydroxyl radical generation in a model system of iron redox cycle. *Free Radical Res. Commun.* **1989**, *6*, 1−10.

(20) Gorelik, S.; Kanner, J. Oxymyoglobin oxidation and membranal lipid peroxidation initiated by iron redox cycle. *J. Agric. Food Chem.* **2001**, *49*, 5939−5944.

(21) Nourooz-Zadeh, J. Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxides in plasma. *Methods Enzymol.* **1999**, *300*, 58−62.

(22) Robinson, M. E. Heamoglobin and methaemoglobin as oxidative catalysts. *Biochem. J.* **1924**, *18*, 255−264.

(23) Mikkelsen, A.; Sosniecki, L.; Skibsted, L. H. Myoglobin catalysis in lipid oxidation. Assay for activity with linoleic acid as substrate. *Z. Lebensm. Unters. Forsch.* **1992**, *195*, 228−234.

(24) Tew, D.; Ortiz de Montellano, P. R. The myoglobin protein radical Coupling of Tyr-103 to Tyr-151 in the $H_2O_2$-mediated cross-linking of sperm whale myoglobin. *J. Biol. Chem*. **1988**, *263*, 17880−17886.

(25) Giulivi, C.; Cadenas, E. Heme protein radicals: Formation, fate, and biological consequences. *Free Radical Biol. Med.* **1998**, *24*, 269−279.

(26) Reeder, B. J.; Wilson, M. T. Mechanism of reaction of myoglobin with the lipid hydroperoxide hydroperoxyoctadecadienoic acid. *Biochem. J.* **1988**, *330*, 1317−1323.

(27) Walters, F. P.; Kennedy, F. G.; Jones, D. P. Oxidation of myoglobin in isolated adult rat cardiac myocytes by 15-hydroperoxy-5,8,11,13 eicosatetraenoic acid. *FEBS Lett.* **1983**, *163*, 292−296.

(28) Maioriono, M.; Urisini, F.; Cadenas, E. Reactivity of metmyoglobin towards phospholipid hydroperoxides. *Free Radical Biol. Med.* **1994**, *16*, 661−667.

(29) Fantone, J.; Jester, S.; Loomis, T. Metmyoglobin promotes arachidonic acid peroxidation at acidic pH. *J. Biol. Chem.* **1989**, *264*, 9408−9411.

(30) Kroger-Ohlsen, M. V.; Andersen, M. L.; Skibsted, L. H. Acid-catalysed autoreduction of ferrylmoglobin in aqueous solution studied by freeze quenching and ESR spectroscopy. *Free Radical Res.* **1999**, *30*, 305−314.

(31) Reeder, B. J.; Wilson, M. T. The effects of pH on the mechanism of hydrogen peroxide and lipid hydroperoxide consumption by myoglobin: a role for the protonated ferryl species. *Free Radical Biol. Med.* **2001**, *30*, 1311−1318.

(32) Lewis, S. E.; Wills, E. D. Inhibition of the autoxidation of unsaturated fatty acids by haematin proteins. *Biochim. Biophys. Acta* **1963**, *70*, 336−338.

(33) Baron, C. P.; Skibsted, L. H.; Andersen, H. J. Concentration effects in myoglobin-catalyzed peroxidation of linoleate. *J. Agric. Food Chem.* **2002**, *50*, 883−888.

(34) Flogel, U.; Godecke, A.; Klotz, L. O.; Schrader, J. Role of myoglobin in the antioxidant defence of the heart. *FASEB J.* **2004**, *18*, 1156−1158.

(35) Osawa, Y.; Williams, M. S. Covalent cross-linking of the heme prosthetic group to myoglobin by $H_2O_2$; Toxicological Implications. *Free Radical Biol. Med.* **1996**, *21*, 35−41.

(36) Kumamoto, M.; Sonda, T.; Nagayama, K.; Tabata, M. Effects of pH and metal ion on antioxidative activities of catechins. *Biosci., Biothechnol., Biochem.* **2001**, *65*, 126−132.

(37) Lemanska, K.; Szymusiak, H.; Tyrankowska, B.; Zielinski, R.; Soffers, A. E. M. F.; Rietjens, I. M. C. M. The influence of pH on antioxidant properties and the mechanism of antioxidant action of hydroxyflavones. *Free Radical Biol. Med.* **2001**, *31*, 869−881.

(38) Carlsen, C. U.; Skibsted, L. H. Myoglobin species with enhanced prooxidative activity is formed during mild proteolysis by pepsin. *J. Agric. Food Chem.* **2004**, *52*, 1675−1681.

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