

The Stomach as a “Bioreactor”: When Red Meat Meets Red Wine

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To determine the stomach bioreactor capability for food oxidation or antioxidation, rats were fed red turkey meat cutlets (meal A) or red turkey meat cutlets and red wine concentrate (meal B). The hydroperoxides (LOOH) and malondialdehyde (MDA) levels of the stomach contents were evaluated during and after digestion; the postprandial plasma MDA level was also evaluated. In independently fed rats, the stomach LOOH concentration fell substantially 90 min following the meal, and the addition of red wine polyphenols enhanced LOOH reduction 3-fold. A similar trend was obtained for MDA. After pyloric ligation, the stomach contents of rats fed red meat homogenate showed >2-fold increases in LOOH and MDA accumulation. The postprandial plasma MDA level increased significantly by 50% following meal A and was maintained or even fell by 34% below basal level following meal B. The findings show that consumption of partially oxidized food could increase lipid peroxidation in the stomach and the absorption of cytotoxic lipid peroxidation products into the body. The addition of antioxidants such as red wine polyphenols to the meal may alter these outcomes. These findings explain the potentially harmful effects of oxidized fats in foods and the important benefit of consuming dietary polyphenols during the meal.

KEYWORDS: Stomach; bioreactor; red meat; red wine; oxidation; cytotoxic compounds; malondialdehyde; absorption; antioxidants

INTRODUCTION

Both epidemiological studies and experimental data suggest that populations on Western-style diets, characterized by high intakes of high-fat, red meat, processed meat, and processed and fried foods, but low intakes of fruits and vegetables, are at high risk for the development of atherosclerosis and several kinds of cancer, especially colon cancer (1). The accumulation of lipid peroxidation products in the body is known to present risks to human health (2) and to correlate closely with serious diseases such as atherosclerosis (3, 4), cancer (5, 6), diabetes mellitus (7), Parkinson's disease (8), and others (9). The diet seems to play a role in the presence of lipid peroxidation products in the body. The Western diet contains large quantities of oxidized fatty acids (10–15), phospholipids (5, 16–18), oxidized cholesterol (10, 12, 14), and cytotoxic aldehydes, because a large proportion of the diet is often consumed in fried, heated, processed, and long-stored forms. Malondialdehyde (MDA) is one of the most abundant lipid peroxidation cytotoxins formed in foods, especially in meat products (19), or endogenously in vivo (20, 21). MDA arises largely from peroxidation

of PUFAs with more than two double bonds such as linolenic, arachidonic, and docosahexaenoic acids; however, biological membranes, including those found in turkey muscle, contain other oxidizable fatty acids that do not generate MDA during their peroxidation (22, 23). Furthermore, other reactions could produce MDA (23). In addition, peroxide decomposition during the lipid peroxidation process forms multiple aldehydes and other carbonyl compounds, in which MDA is only one product. Therefore, MDA is not a stoichiometric marker for lipid peroxidation; however, its accumulation reflected the intensity of the lipid peroxidation process, as compared to LOOH accumulation (24–26). Recently, a broad comparison study from 10 different laboratories throughout the world concluded that measurements of MDA and isoprostanes in plasma and urine are potential candidates for general biomarkers of biological oxidative stress (20). We have recently demonstrated that the MDA level in human plasma increased significantly following the consumption of red turkey meat, but the elevation was completely eliminated when red wine polyphenols were included in the meal (26). The stomach is a primary temporary location for food storage, and it enables chemical and biochemical interactions between food constituents. We demonstrated, in a model system, that the stomach acts as a “bioreactor” and the gastric fluid as a medium for further dietary lipid peroxidation and/or antioxidation (27). Studies have shown that in the

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stomach dietary hydroperoxides were decomposed to aldehydes, which were partly absorbed into the body (28). Furthermore, other lipid peroxidation products can be absorbed from food and detected in the body: the lipid hydroperoxide levels in human plasma were found to be elevated following a fatty meal (29). High-fat, high-cholesterol foods were found to enhance plasma levels of cytotoxic chylomicron remnants and of advanced lipid oxidation end products (14, 29, 30), and humans have been shown to excrete increased amounts of malondialdehyde in their urine after ingestion of oxidized fats (16, 26).

Both epidemiological and experimental studies have demonstrated that daily consumption of foods rich in polyphenols, for example, red wine, has health benefits (31–34). Varied ameliorative properties are attributed to polyphenols; they include antioxidant activity (35), amelioration of cardiovascular diseases (31), improvement of endothelial function (36), modulation of γ -glutamylcysteine synthetase expression (37), inhibition of the intestinal absorption of dietary lipids (38, 39), and improvement of health and survival of mice fed a high-fat diet (32). However, the absorption of polyphenols is very limited (33, 40), and the proposed mechanism of action of these molecules in the human body (26) is yet to be well-established.

In this study we tested the hypothesis that the stomach can act as a bioreactor, in which further lipid peroxidation of partially oxidized food, such as red meat, could take place, resulting in the accumulation of lipid peroxidation products. The relatively high sensitivity to lipid peroxidation of heated turkey red muscle is mostly due to its high level of "free" iron ions and relatively low levels of vitamin E (19, 41). We further tested whether the addition of dietary antioxidants, such as red wine polyphenols, to the meal could convert these peroxidation processes to antioxidation. The present findings support this hypothesis and, furthermore, they demonstrate the beneficial effect of polyphenolic antioxidants in general and indicate that the stomach and the gastrointestinal tract might constitute the main biological sites of action for these compounds.

MATERIALS AND METHODS

Chemicals and Food Products. 2-Thiobarbituric acid (TBA) was obtained from Sigma Chemical Co., St. Louis, MO. Potassium hydroxide and potassium dihydrogen phosphate were obtained from Merck, Darmstadt, Germany. The triglyceride reagent set was obtained from Pointe Scientific, Inc., Canton, MI. High-performance liquid chromatography (HPLC) grade methanol was obtained from J. T. Baker, Phillipsburg, NJ. Red wine (Israeli Shiraz and Cabernet Sauvignon) and fresh red turkey thigh meat were purchased at commercial stores in Israel.

Animals and Diets. The study plan was approved by the Ethics Committee of the Hebrew University Authority for Animal Facilities. Twenty-five mature Hebrew University Sabra strain (derived from Wistar) male rats weighing 220–250 g were used in the study. The animals were housed in individual metabolic cages 2 days prior to the experiment day and were nourished on a regular laboratory diet. On the experiment day, following an overnight fast, blood samples were collected, and the rats were returned to their cages for a further 2 h. Then the rats were presented with a test meal comprising either red turkey meat cutlets or red turkey meat cutlets with concentrated dealkoholized red wine (containing 3 μ mol of polyphenols as equivalent catechin per gram of meat) for 30 min. Food remnants were collected, weighed, and immediately frozen, pending further analysis of MDA and LOOH concentrations. After an average period of 90 min, the rat stomach was removed and blood was drawn under general anesthesia, and the rats were sacrificed by exsanguination. The stomach contents were collected, weighed, and immediately frozen in liquid nitrogen,

pending assay for MDA and hydroperoxides (LOOH). Blood samples were centrifuged, and the plasma was frozen immediately at -80°C , pending assay for MDA.

Pyloric Ligation. The rat ($n = 3$) was anesthetized, a canola was attached to the end of its stomach at the pyloric sphincter, and a syringe was connected to the outlet of the canola to collect all of the stomach content during the experiment. Rats were gavaged fed by 2 mL of red meat homogenate.

Test Meals. *Preparation of Meat Cutlets.* Red turkey thigh meat was cut into pieces, ground for 45 s in a domestic food processor (Magimix, Robot Corp., Burgundy, France), cooked as small cutlets, shaped by cooking mold (10 g each), on an electric grill at a high temperature (190°C) on both sides (2 min on one side, and 1.5 min on the other side) until "well done". The cutlets were immediately cooled on ice, vacuumed packed, and frozen at -80°C in a laboratory freezer pending the experiment day. Meal A contained meat cutlets; meal B contained meat cutlets and red wine concentrate (3 μ mol of polyphenols as equivalent catechin per gram of meat).

Preparation of Meat Homogenate for Gavage Feeding. Shortly prior to the experiment, heated red turkey thigh meat was ground with 3 volumes of water in a laboratory blender (Waring, New Hartford, CT) for 15 s and filtered through a gauze cloth.

Preparation of Concentrated Wine. Alcohol was evaporated from red wine, under vacuum at 37°C , in an R-200 Rotavapor (Buchi, Flawil, Switzerland), and the alcohol-free wine was freeze-dried. The red wine concentrate was diluted with water to a polyphenol concentration of 50 mM, as equivalent catechin, and frozen in 1.5 mL aliquots at -80°C pending use in the experiment.

Determination of Red Wine Polyphenols. The polyphenol contents of the wine and of the concentrated wine were determined with Folin–Ciocalteu reagent and calculated as catechin equivalent as described previously (27).

Determination of Malondialdehyde. MDA was extracted from meat cutlets and plasma samples as described previously (42). The supernatant was reacted with TBA and filtered through a 0.2 μm membrane. A 10 μL sample was injected into an SCL-10A VP HPLC (Shimadzu, Kyoto, Japan), separated with a Lichrocart column, model RP-18, 125-4S (Merck), and monitored with an RF-10AXL HPLC fluorescence detector (Shimadzu) that was set at excitation and emission wavelengths of 532 and 553 nm, respectively. The mobile phase was a 35:65 (v/v) mixture of methanol and 0.05 M potassium phosphate buffer, pH 7, and the flow rate was 1 mL/min. Standard MDA solutions were used to generate a calibration curve and to spike plasma samples for determination of the recovery.

Determination of Hydroperoxides. Hydroperoxides were determined by means of the ferrous ion oxidation–xylenol orange (FOX2) method (43), which included spectral analysis at 560 nm by comparison with an H_2O_2 standard curve, as was done previously (26). The stomach contents and meat samples were each homogenized with 2 volumes of double-distilled H_2O , and the hydroperoxides in the samples were extracted by 10-fold dilution in methanol under slow stirring for 15 min and centrifuging at 20800g for 2 min. Ninety microliters of the upper layer was subjected to the FOX2 assay, which included TPP reagent controls, to prevent potential interference.

Determination of Triglycerides. Triglycerides were analyzed by an enzymatic method with the triglyceride (GPO) (liquid) reagent set (Pointe Scientific).

Statistical Analysis. The results were subjected to repeated-measures analysis of variance (ANOVA) as a three-period crossover design with SAS version 9.1 (SAS Institute Inc., Cary, NC) software, followed by application of the Student–Newman–Keuls Test.

RESULTS

To determine whether the stomach could act as a "bioreactor" and the gastric fluid as a medium for further dietary lipid peroxidation and/or antioxidation, rats were fed two test meals: red turkey meat cutlets (meal A) or red turkey meat cutlets and red wine concentrate (meal B).

The amounts of meat consumed by the rats in the two groups were similar (group A, 2.4 ± 1.3 g; group B, 2.1 ± 1.0 g). The

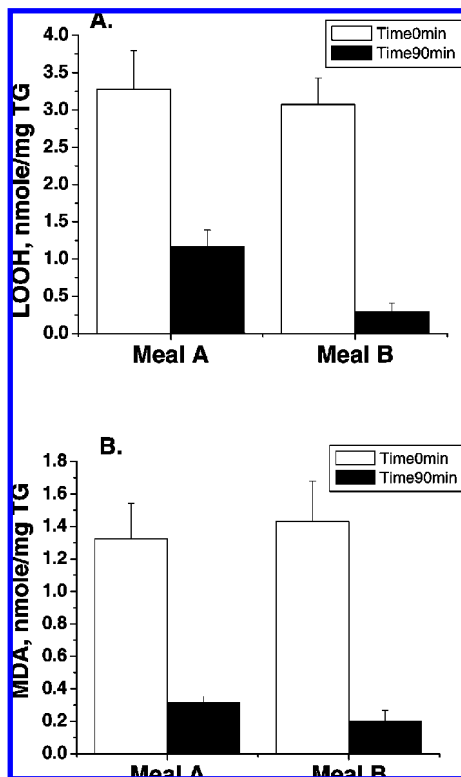


Figure 1. Lipid peroxidation levels of rat food before eating and (in the rat stomach) 90 min after eating, as indicated by LOOH concentration (A) or MDA concentration (B). Data are means \pm SD (control, $n = 14$; wine, $n = 11$).

initial levels of lipid peroxidation in the two test meals, as indicated by the levels of LOOH and MDA, were also similar: in group A the concentration of LOOH was 3.28 ± 0.52 nmol/mg TG and that of MDA was 1.32 ± 0.22 nmol/mg TG; in group B the respective concentrations were 3.07 ± 0.35 and 1.43 ± 0.25 nmol/mg TG. There were no statistically significant differences between the two groups (Figure 1), as expected.

Following consumption of meal A there was a significant reduction in hydroperoxides concentration. Their concentration in the rat lumen 90 min after the consumption of meal A fell to 1.17 ± 0.22 nmol/mg TG (equal to $77.0 \mu\text{M}$), which is still an appreciable level of LOOH. Although the initial levels of LOOH in the two test meals were similar, the reduction following consumption of meal B was >3 times that following meal A, which resulted in a much lower LOOH concentration of 0.29 ± 0.11 nmol/mg TG in the former case. The difference between the two study groups was statistically significant ($P < 0.05$) (Figure 1A). A similar trend was obtained for MDA concentration (Figure 1B).

To determine whether a lipid peroxidation process occurs in the rat stomach during food digestion, a canola was attached to the end of the rat stomach at the pyloric sphincter. Rats were gavage fed with red meat homogenate, and the stomach contents were collected every 15–20 min to monitor the lipid peroxidation process. The LOOH concentration increased 2.2-fold, from 2.04 nmol/mg TG (in the food) to 4.43 nmol/mg TG (in the stomach contents) following 120 min of digestion (Figure 2A). Figure 2B shows the progress of lipid peroxidation in the rat stomach, as evaluated according to the accumulation of MDA. During the first 15 min after feeding, there was a slight reduction in MDA concentration that was followed by a steady increase in its concentration during the next 105 min, which resulted in a

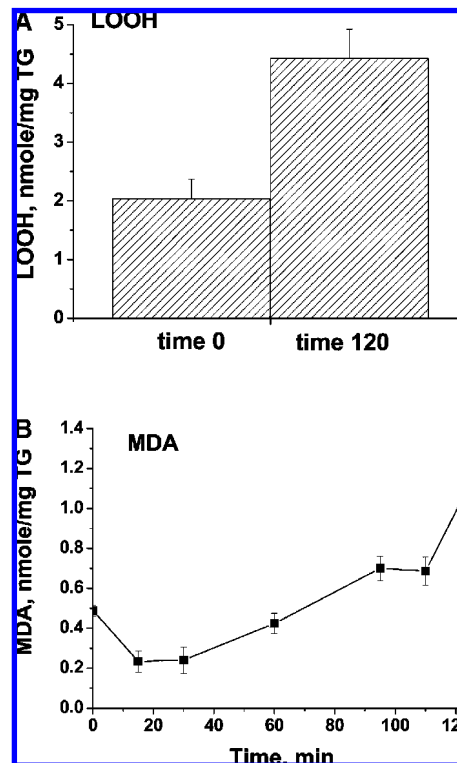


Figure 2. Lipid peroxidation in rat stomach following a gavage feeding with 2 mL of heated red meat homogenate (see Materials and Methods), as indicated by accumulation of LOOH (A) or MDA (B) in rat stomach lumen. Data are means \pm SD ($n = 3$).

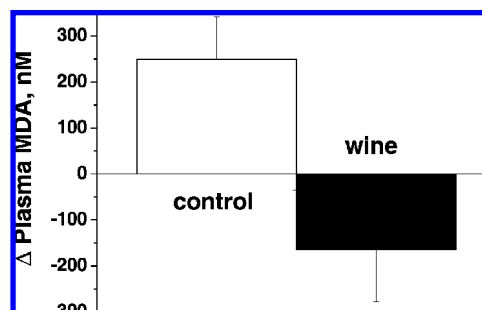


Figure 3. Postprandial levels of rat plasma MDA, following consumption of control and wine test meals. Data are means \pm SD (control, $n = 8$; wine, $n = 6$, a subgroup of Figure 1). Fasting plasma MDA level = 500 ± 280 .

2.3-fold increase in MDA concentration, as determined after the rat had been sacrificed and its stomach contents collected.

Because MDA was found to be absorbed by rats (44), it was important to determine whether the consumption of meals A and B, which contained similar initial levels of MDA, could affect the postprandial plasma levels of MDA. The fasting MDA plasma levels of rats in the two study groups were similar, 500 ± 280 nM. Ninety minutes after the consumption of red turkey meat cutlets (meal A), the postprandial plasma MDA concentration increased significantly by 50%, whereas the consumption of the same meat cutlets simultaneously with concentrated wine (meal B) totally prevented the elevation in plasma MDA concentration and even reduced it by 34% below basal level. The difference between the two study groups was found to be statistically significant ($P < 0.05$) (Figure 3).

DISCUSSION

Oxidative damage by reactive oxygen species appears to be involved in the pathogenesis of various diseases. In the present

study we evaluated the putative lipid peroxidation process in the rat stomach during food digestion and its consequence as reflected in the rat plasma, that is, postprandial changes in the plasma MDA level. We also addressed the possible beneficial role of dietary polyphenols in preventing lipid peroxidation processes and their deleterious outcomes.

Partially oxidized food, for example, heated red meat, might undergo further oxidation under simulated stomach conditions, yielding deleterious compounds such as hydroperoxides (25, 27) and reactive carbonyls (16, 18, 25), for example, 4-hydroxynonenal and MDA. However, evaluation of the lipid peroxidation level in the rat stomach contents 90 min after the consumption of a meal revealed decreases in the levels of LOOH and MDA. The reduction in hydroperoxides levels found in our study is consistent with previous findings that dietary hydroperoxides were decomposed in the stomach, to yield advanced lipid peroxidation end products (ALEs) (39, 40). The breakdown of LOOH under the low-pH conditions of the stomach can be enhanced by metmyoglobin, which is found in muscle foods (45). Hydroperoxides and also aldehydes, for example, MDA, are more hydrophilic compounds than the intact triglyceride molecule; therefore, it is most likely for these compounds to be found in the aqueous phase of the lumen. Thus, we consider that the reductions in LOOH and MDA concentrations that were detected 90 min after the meal was ended could be attributed largely to the continuous transfer of soluble LOOH and MDA into the duodenum and to the gut, together with the liquid aqueous phase of the stomach contents, during the dynamic process of digestion, in which the rate of stomach emptying increases more rapidly than that of lipid peroxidation. Furthermore, during the dynamic process of lipid peroxidation, while LOOHs are being formed as a result of a free radical chain reaction sequence, they are simultaneously being decomposed; therefore, it would be difficult to determine the extent of lipid peroxidation in the rat stomach by sampling at a single time point.

Therefore, to evaluate lipid peroxidation in the rat stomach quantitatively, unlike the procedures in other studies, a canola was attached to the pyloric end of the stomach and all of the food was collected during 120 min of digestion time. This revealed a >2-fold enhancement in the accumulation of both LOOH and MDA during digestion of red muscle meat, indicating that the stomach can actually serve as a bioreactor in which lipid peroxidation processes can take place.

Evaluation of the postprandial levels of plasma MDA supported the major assumptions of the study hypothesis and clearly demonstrated that during 90 min following digestion of meat cutlets there was a substantial and significant increase in plasma MDA level above the base level. However, the increase in plasma MDA level over the basal level was completely prevented, and even reversed, as a result of the addition of red wine polyphenols to the same meat cutlets during the meal, although the initial MDA levels were similar in the two study meals. The results are in agreement with the findings of our recent human study that indicated the same pattern (26). In foods, MDA is bound mainly to the lysine residues of proteins, from which it is released in the course of digestion, as *N*- ϵ -(2-propenal)lysine (44). Malondialdehyde-lysine has been shown to be absorbed in rats and incorporated mostly in the liver, small intestine, and plasma (44), and MDA has been shown to be mutagenic in mammalian and human cells (46, 47) and carcinogenic in mice (48). It also seems that reactive carbonyls, for example, MDA, play a crucial role in the pathogenesis of atherosclerosis (18, 49) and in cancer-prone inflammatory

diseases (50). Lipid peroxidation processes of food, especially muscle foods, can yield a variety of reactive compounds other than MDA, such as hydroperoxides, F₂-isoprostanes, oxysterol, and 4-hydroxynonenal and also heterocyclic aromatic amines. The present study tracked the journey of one of these cytotoxins, MDA, from dietary source to the body because MDA has been shown to be absorbed from foods (26, 44) and is one of the abundant peroxidation products formed in muscle foods (51, 52). In addition, MDA accumulation is correlated with the accumulation of hydroperoxides (24, 25, 27). We assumed that other reactive carbonyls would exhibit a similar pattern. We considered that the clearance of MDA from the plasma is regulated by the enzyme aldose reductase, which was demonstrated to be an efficient catalyst for the reduction of aldehydes generated by lipid peroxidation. Conditions such as highly oxidative stress up-regulate tissue levels of aldose reductase (53). Because the diet seems to be a source of lipid peroxidation products in the body, eating could affect the regulation of aldose reductase in the body. This interpretation could explain the reduction of plasma MDA level below the basal level in rats fed red muscle food and red wine polyphenols.

Red wine polyphenols were found to have an antioxidant effect on heated red muscle tissue homogenate in simulated gastric condition (24–27). Polyphenols also facilitated the reduction of LOOH to nontoxic compounds, in the presence of catalysts such as metmyoglobin that are found in muscle food (27), and thereby promoted reductions in the concentrations of cytotoxic lipid peroxidation end products.

Beyond being effective antioxidants in the stomach during digestion, polyphenols could prevent the accumulation of cytotoxic reactive carbonyls, for example, MDA, in the plasma, by the formation of an adduct between polyphenols and aldehydes, such as those found between polyphenols and methylglyoxal (54, 55), and thereby might prevent the absorption of these compounds. An additional factor might be the interaction between polyphenols and proteolytic enzymes (56, 57) in the gastrointestinal tract, which would result in decreased release of MDA-lysine in the gut and its absorption to the plasma (44, 58).

In conclusion, it can be considered that the consumption of partially oxidized food could enhance the lipid peroxidation process in the stomach and the consequent production and absorption of cytotoxic lipid peroxidation products into the plasma (26). We suggest that the high levels of these cytotoxic compounds found in partially oxidized foods, and the further formation and absorption of these toxins during digestion, lead to the long-term development of several diseases, for example, cardiovascular diseases. However, the addition of antioxidants, especially polyphenols, to the meal may alter these outcomes.

Furthermore, we suggest that the main benefit of consuming plant polyphenols as an integral part of the human diet may arise from their many-sided ability to prevent the generation and absorption of cytotoxic ALEs such as reactive carbonyls or other reactive compounds commonly found in our foods. Diets high in fat and red meat are contributory risk factors, whereas the consumption of polyphenol-rich fruits, vegetables, and their derived beverages during the meal seems to reduce these risk factors and provide important protective benefits for human health.

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