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Oxidized lipid depresses canine growth, immune function, and bone formation

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Abstract

Dietary oxidized lipids can increase oxidative stress and potentially contribute to a variety of disease syndromes. This research describes the first use of a canine model to assess the effects of dietary oxidized lipids on growth, antioxidant status, and some immune functions. Three groups of eight, two-month old coon-hound puppies were pair fed diets for 16 weeks. The control diet contained <50 ppm aldehydes, and two additional diets contained thermally oxidized lipids targeted to contain 100 ppm aldehydes (medium-oxidation) and 500 ppm aldehydes (high-oxidation). Dogs fed the high-oxidation diet weighed less than those from the medium-oxidation ($P < 0.05$) and control groups ($P < 0.001$) at the end of the study. Oxidized lipids reduced serum vitamin E levels, total body fat content, and bone appositional rate. At different time points of the study, peripheral blood neutrophils and monocytes from dogs fed the HO diet had reduced oxidative burst capacity and produced less superoxide and hydrogen peroxide when stimulated with phorbol esters compared to the control group. Lymphocyte blastogenesis in response to concanavalin A was suppressed by dietary oxidized lipid. This study indicates that dietary oxidized lipids negatively affect the growth, antioxidant status, and some immune functions of dogs. Importantly, some effects are evident at 100 ppm aldehydes in the diet, which is a moderate level of oxidation. The rapid growth and weight gain of the dog during the first 6 months of life may also provide a better model for assessing the risks of dietary oxidized lipid in children and adolescents than previously used rodent models. © 2003 Elsevier Science Inc. All rights reserved.

Keywords: Oxidative stress; Oxidized lipid; Immune function; Bone; Canine

1. Introduction

Oxidative stress in an organism occurs when the production of oxidants exceeds antioxidant capacity. The resulting free radicals can damage tissues by reacting with cellular proteins, nucleic acids, and lipids. A variety of environmental and normal and pathological metabolic conditions can contribute to oxidative stress via the formation of reactive oxygen and nitrogen species [1]. Currently, free radical damage to cellular components is thought to be a contributing factor in a variety of human diseases or conditions such as aging, cancer, atherosclerosis, arthritis, inflammatory bowel disease, neurodegenerative disease, and some eye diseases [2].

Lipid oxidation yields a very complex group of by-products that include hydroxy and dihydroxy fatty acids, hydroperoxides, volatile aldehydes, and alkyl and olefinic radicals [3]. Ingestion of oxidized lipids can influence oxidative stress because they are capable of being absorbed through the digestive tract [4] and incorporated into membrane phospholipids where they alter membrane fluidity [5,6]. In humans, there has been concern that dietary oxidized lipids may contribute to atherosclerosis, especially in diabetic patients [4] and also confound in vivo measurements of oxidative stress [7]. Studies in chickens have demonstrated that feeding products with a low level of oxidation can suppress growth in broilers [8,9], and highly oxidized lipids can cause a nutritional encephalopathy [10]. Another study in chickens demonstrated that feeding ther-

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Table 1
Basal diet composition[a]

Ingredient	Percent
Ground Corn	40.5
Chicken Protein	32.4
Poultry Fat	12.2
Fish Meal	5.0
Beet Pulp	5.0
Mineral Mix[a]	2.2
Dried Whole Egg	1.0
Chicken Digest	1.0
Vitamin Premix[b]	0.4
Ground Flax	0.35
Choline Chloride	0.05

The lipid portion of the control diet contained <50 ppm aldehydes, and the medium-oxidation and high-oxidation diets were targeted to contain 100 ppm aldehydes and 500 ppm aldehydes respectively.

[a] Diet formulated to contain: 1.23% Ca; 0.9% P; 0.33% Na; 0.54% K; 54 mg/kg Mn; 230 mg/kg Zn; 430 mg/kg Fe; 36 mg/kg Cu; 0.5 mg/kg Co; 3.6 mg/kg I; 3.6 mg/kg Mg; 0.3 mg/kg Se.

[b] Diet formulated to contain: 30.5 KIU/kg vitamin A; 20 IU/kg vitamin E; 1855 IU/kg vitamin D; 18 mg/kg thiamin; 44 mg/kg riboflavin; 73 mg/kg niacin; 38 mg/kg pantothenate; 13 mg/kg pyridoxine; 0.62 mg/kg biotin; 2.0 mg/kg folic acid.

mally oxidized lipids lowered vitamin E levels in tissues, and contributed to tissue susceptible to peroxidation with iron-ascorbate [11]. Studies in rats indicate that dietary oxidized lipids can lead to peroxidized tissue proteins [12], upregulation of peroxisome proliferator-activating receptor alpha [13] and an increased number of aberrant crypts in the intestine, indicative of a precancerous cellular change [14].

Dietary oxidized lipid presents a potential health hazard for both companion animals and humans. The goal of the present study was to determine the acute effects of feeding dietary oxidized lipid on the growth, antioxidant status, and some immune functions of dogs.

2. Materials and methods

2.1. Reagents

All reagents were purchased from Sigma Chemical Co. unless otherwise specified.

2.2. Dogs and diet

Twenty-four, two-month old purpose-bred coonhounds (mean weight of 5.7 kg) were obtained from Covance Research Products (Kalamazoo, MI). The dogs were randomly assigned to one of three groups and were pair-fed one of three test diets (Table 1) for a period of 16 wk. The primary lipid source was unpreserved poultry fat (Pfau Oil, Jeffersonville, IN). The control diet was measured to contain <50 ppm aldehydes. The medium-oxidation (MO) diet was targeted to contain 100 ppm aldehydes and the high-oxidation (HO) diet was targeted to contain 500 ppm aldehydes. To generate the oxidized lipid, the poultry fat was placed in a stainless steel jacketed tank and heated to 180°F and stirred by a paddle mixer operating at 100 rpm. Oxygen (150 mL/h) from a pressurized bottle was fed through a diffuser placed at the bottom of the fat tank. It was necessary to maintain the treatment for 24 h for the MO diet, and 48 h for the HO diet. All of the diets met or exceeded the NRC requirements for the dog and were isocaloric and isonitrogenous. The Purdue University Animal Care and Use Committee approved the research protocol.

The diet oxidation level was determined by measuring total aldehydes by the GC/MS-headspace method. Briefly, a representative subsample of ground food was weighed (0.04 g) into a headspace vial along with 1 μL internal standard/surrogate mix (10 μL chloroform and 20 μL 1-octanol in 10 mL of methanol). The capped vial was heated to 130°C for 60 min, agitated for last 12 min, then pressurized with helium to 15 psi and allowed to equilibrate. Headspace gas was drawn from the vial, super-cooled with liquid nitrogen to −200°C for cryo-focusing, and injected onto the gas chromatograph at 1 ml/min. Aldehyde separation was performed by a Hewlett-Packard 5890 Series II gas-liquid chromatograph equipped with a Resteck #10655 Stabilewax column (0.25 mm i.d. × 30 m; film thickness 0.25 μm); and with a programmed temperature gradient change of 40°C for 4 min then 7°C/min up to 160°C, then 9°C/min to 230°C and held constant for 1 min. The GC analyte was injected onto a Hewlett-Packard 5972 Mass Spectrometer for quantification of individual aldehydes. Total aldehydes were computed as the sum of peak areas for n-pentanal, n-hexanal, n-heptanal, n-octanal, n-nonanal, and benzaldehyde and expressed as ppm relative to initial sample weight and the 1-octanol peak was quantified for determination of recovery.

2.3. Collection of blood and cell isolation

Peripheral blood was collected by venapuncture into sterile heparinized and nonheparinized vacutainer tubes (for serum) at the start of the study and after 8 and 16 wks on the test diets. Heparinized blood was layered onto Ficoll-hypaque and white blood cells isolated by centrifugation. Monocytes were isolated by cytoadherence to plastic culture dishes containing RPMI 1640 medium with 7.6×10^{-5} mol/L bovine serum albumin (BSA), 1×10^{5} U/L penicillin, 6.9×10^{-5} mol/L streptomycin, and 2×10^{-3} mol/L L-glutamine. Total peripheral blood white cells were utilized to measure lymphocyte blastogenesis in response to concanavalin A, and oxidative burst capacity (superoxide and peroxide production).

2.4. Fatty acid analysis

The fatty acid analysis of the diet, serum, and bone biopsies followed a method described previously [15].

Briefly, total lipids were extracted with chloroform/methanol (2:1, vol/vol), saponified and fatty acid methyl esters (FAME) prepared using boron trifluoride. FAME were analyzed by gas-liquid chromatography using a capillary column [16].

2.5. Prostaglandin E_2 production

Monocytes isolated by cytoadherence were stimulated with 10 μg/mL of O55:B5 *Eschericia coli* lipopolysaccharide in growth medium for 24 hrs. After 24 hours the cell free supernatant fluid was collected and the adherent cells washed in HBSS and digested with 1 mol/L ammonium hydroxide and 0.2% Triton X-100 to measure total cellular DNA [17]. The concentration of PGE_2 in the monocyte culture medium was measured by radioimmunoassay as previously described [18].

2.6. Lymphocyte blastogenesis

Peripheral blood mononuclear cells isolated by centrifugation on Ficoll-hypaque were suspended in growth medium (RPMI 1640 containing 1×10^5 U/L penicillin, 6.9×10^{-5} mol/L streptomycin, 2×10^{-3} mol/L L-glutamine, and 10% fetal calf serum) at a concentration of 1×10^6 cells/mL. Two hundred microliters of cell suspension was dispensed in triplicate into 96 well culture plates with 3 wells for each sample containing growth medium and 3 wells containing growth medium plus 9.62×10^{-14} mol/L concanavalin A. After 72 hours, the wells were pulsed with 1 μCi of [^{3}H]thymidine (50 μl volume) and incubated an additional 24 hours. The cells were harvested on glass fiber filters (PHD cell harvester, Cambridge, MA), and the amount of [^{3}H]thymidine incorporation determined by measuring decays per min (dpm) in a scintillation counter.

2.7. Peroxide assay

Peripheral blood white cells isolated by centrifugation on Ficoll-hypaque were washed and resuspended in Hank's balanced salt solution containing 1.8 μmol/L of calcium chloride and 0.01 mol/L of dextrose, and adjusted to 2×10^6 cells per mL. One microliter of 0.02 mol/L 2′7′-dichlorofluorescin diacetate (DCFH-DA) (Molecular Probes, Eugene, OR) per mL of cell suspension was added to the cell suspension and the cells incubated at 37°C for 15 min [19]. The cells were stimulated with phorbol myristate acetate (PMA) (100 μL of PMA to 900 μL of cell suspension for a final PMA concentration of 2×10^{-3} mol/L). Samples were maintained at 37°C and stimulated and unstimulated samples measured on an EPICS Elite flow cytometer (Coulter Cytometry, Hialeah, FL) (488 nm excitation, 525 nm emission filters) after 15 and 30 min.

2.8. Superoxide assay

Samples were prepared in the same manner as described for the peroxide assay. One microliter of a 0.01 mol/L solution of hydroethidine (Molecular Probes, Eugene, OR) in N,N,dimethylformamide was added per mL of cell suspension [20]. Cells were incubated for 15 min at 37°C and stimulated with PMA. Samples were maintained at 37°C and stimulated and unstimulated samples measured on the flow cytometer (488 nm excitation, 590 nm emission filters) after 15 and 30 min.

2.9. Clinical pathology

A standard small animal blood chemistry panel and hematology analysis was performed on all dogs.

2.10. Serum vitamin E

The vitamin E concentration in serum was measured by HPLC. Ethanol was used to precipitate the proteins in the serum and hexane used to extract the vitamin E. The hexane extract was evaporated to dryness under nitrogen gas and the sample reconstituted in methanol:$CHCl_3$ (2:1). The amount of vitamin E was determined by HPLC using a fluorescence RF-530 detector with excitation at 293 nm and emission at 331 nm.

2.11. Body composition

Total body fat was measured at the end of the feeding trial using a Lunar DPX-IQ dual energy x-ray absorptiometer (Lunar Corporation, Madison, WI). The Lunar human pediatric software was used to provide an x-ray energy level suitable to the size and weight of the dogs.

2.12. Trabecular bone volume and bone appositional rate

The bones of the dogs were labeled with intramuscular (IM) oxytetracycline injections (10 mg/kg) on two successive days and the injection series repeated 10 d later. Three days after the final injection, the dogs were anesthetized with medetomidine (0.1 mg/kg, IM) and given butorphanol tartrate (0.1 mg/kg, IM) as an analgesic. A 10 gauge bone biopsy needle was used to obtain biopsies from the iliac crest. The biopsies were preserved in ethanol, and were embedded in polymethymethacrylate resin. The polymerized specimen blocks were sectioned at 12 μm using a Poly-cut™ (Leica, Deerfield, IL) and sections stained with toluidine blue for determination of trabecular bone volume and unstained sections used for fluorescence microscopic determination of bone appositional rate. Image analysis software (Optimas 6.1, Media Cybernetics, Silver Spring, MD) was used to calculate the trabecular bone volume and bone appositional rate. Trabecular bone volume [(volume of trabeculae/volume of trabeculae + marrow) × 100] was

Table 2
Fatty acid analysis of diet (mol/100 mol)

Fatty Acid	Oxidation level of diet		
	Control	100 ppm aldehydes	500 ppm aldehydes
14:0	0.636[a]	0.843[b]	0.90[c]
16:0	21.4	21.16	22.42
16:1n-7	6.67[a]	5.18[b]	5.17[b]
18:0	5.43[a]	6.92[b]	7.44[c]
18:1n-9	37.85	37.25	37.65
18:1n-7	2.23	2.36	2.41
18:1 total	40.08	39.61	40.06
18:2n-6	19.34[a]	17.81[b]	15.21[c]
18:3n-3	1.27[a]	1.21[b]	1.05[b]
20:1n-9	0.67[a]	0.80[b]	0.78[b]
20:4n-6	0.51	0.50	0.52
22:6n-3	0.496[a]	0.556[b]	0.67[c]

Mean values for diet fatty acid composition within a row having different superscripts ([a, b, c]) are significantly different by one-way ANOVA and Tukey's studentized range test ($p < 0.05$).

Table 3
Fatty acid analysis of serum (mol/100 mol)

Fatty acid	Oxidation level of diet				
	Control	100 ppm aldehydes	500 ppm aldehydes	Pooled SD	Pr > F
16:0	14.96	15.15	14.96	1.4	0.87
16:1n-7	1.31[a]	1.17[a,b]	1.11[b]	0.2	0.016
18:0	16.81	17.57	17.72	1.5	0.10
18:1 total	14.08	14.06	14.92	1.7	0.18
18:2n-6	24.00[a]	23.94[a]	21.92[b]	1.8	0.0002
18:3n-3	ND	ND	ND	ND	—
20:3n-6	19.34	17.81	17.22	2.9	0.44
20:4n-6	21.26	19.9	19.84	3.4	0.32
22:4n-6	1.14	0.83	1.18	0.5	0.05
22:5n-6	0.17[b]	0.19[b]	0.73[a]	0.6	0.002
22:5n-3	1.57[a]	0.76[b]	0.55[b]	0.9	0.0009
22:6n-3	2.62[b]	3.28[a,b]	3.98[a]	1.8	0.04

Mean values for serum fatty acid composition (n = 8) within a row having different superscripts ([a, b, c]) are significantly different by two-way ANOVA and Tukey's studentized range test ($p < 0.05$). ND = not detected.

measured from digital images acquired by systematic sampling of the biopsies. The bone appositional rate was determined by the line intercept method on digital images acquired by systematic sampling [21].

2.13. Statistical analysis

Data were analyzed using SAS (SAS Institute Inc., Cary, NC) and Prism (GraphPad Software, San Diego, CA). Data were analyzed by one-way ANOVA and Tukey's studentized range test. Additionally, Prism software was used to test for linear trend in some data groups by using a one-way ANOVA and a non-parametric test for linear trend.

3. Results

3.1. Effect of lipid oxidation on diet, serum and bone fatty acids

Fatty acid analysis of the diets indicated that the oxidation process significantly decreased the concentration of 16:1n-7, 18:2n-6, and 18:3n-3 fatty acids and increased the concentration of 14:0, 18:1n-7, 18:0, 20:1n-9 and 22:6n-3 fatty acids (Table 2). Analysis of the serum indicated that animals fed oxidized lipids had decreased concentrations of 16:1n-7, 18:2n-6 and 22:5n-3 fatty acids and increased 22:5n-6 and 22:6n-3 (Table 3). Bone biopsies from the iliac crest contained lower concentrations of the essential fatty acids 18:2n-6 and 18:3n-3 in the MO and HO group compared to the control group and higher levels of 14:0 and 16:0 fatty acids (Table 4).

3.2. Serum chemistry and hematology

All blood chemistry and hematology data were within the normal value range for dogs, except the HO fed dogs had significantly decreased triglyceride levels compared to the control dogs (Table 5). There was a significant linear trend at 8 weeks ($P < 0.0004$) and at 16 weeks ($P < 0.0001$) for vitamin E levels to decrease as oxidation levels in the diet increased. At 8 weeks the vitamin E level was greater in the control group compared to the HO group ($P < 0.05$), and at 16 weeks the vitamin E level of the control group was greater than both the MO group ($P < 0.01$) and the HO group ($P < 0.001$) (Table 5).

3.3. Effect of oxidized lipids on weight gain

At the start of the experiment, the mean weight all dogs was 5.72 ± 0.15 kg and there were no significant differ-

Table 4
Fatty acid analysis of iliac crest bone biopsy (mol/100 mol)

Fatty acid	Oxidation level of diet				
	Control	100 ppm aldehydes	500 ppm aldehydes	Pooled SD	Pr > F
14:0	1.44[c]	2.04[b]	2.64[a]	0.2	0.001
16:0	21.07[c]	22.63[b]	25.56[a]	1.1	0.0001
16:1n-7	7.17	6.28	6.77	1.1	0.24
18:0	7.63	8.77	7.87	1.2	0.17
18:1 total	38.54	38.68	39.29	3.9	0.92
18:2n-6	13.71[a]	11.46[b]	9.29[c]	0.8	0.0001
18:3n-3	0.57[a]	0.43[a,b]	0.38[c]	0.1	0.0083
20:1n-9	0.63	0.73	0.70	0.2	0.59
20:4n-6	3.77	3.55	2.45	2.0	0.39
22:4n-6	0.79	0.74	0.48	0.4	0.29
22:5n-3	0.20	0.18	0.17	0.1	0.89
22:6n-3	0.45	0.45	0.42	0.2	0.95

Mean values for bone biopsy fatty acid composition (n = 8) within a row having different superscripts ([a, b, c]) are significantly different by one-way ANOVA and Tukey's studentized range test ($p < 0.05$).

Table 5
Serum chemistry

	8 wks			16 wks		
	Oxidation level			Oxidation level		
	Control	100 ppm aldehydes	500 ppm aldehydes	Control	100 ppm aldehydes	500 ppm aldehydes
Vitamin E (serum ppm)	0.96 ± 0.22^{a}	0.56 ± 0.1^{a}	0.12 ± 0.06^{b}	2.2 ± 0.26^{a}	1.3 ± 0.1^{b}	0.52 ± 0.05^{c}
Triglycerides (mg/dL)	100 ± 9^{a}	45.8 ± 4^{b}	$30 \pm 3^{b,c}$	62 ± 5^{a}	60 ± 10^{a}	30 ± 1.4^{b}

Mean values (± S.E.M.; n = 8) within a row for each time point (8 or 16 wks) having different superscripts ($^{a, b, c}$) are significantly different by one way ANOVA and Tukey's multiple comparison test.

ences in the mean body weight of the dogs of each group (Fig. 1). However, after seven wks there was a significant difference ($P < 0.05$) in body weight between the control dogs and the MO and HO fed groups. This was essentially maintained through the end of the study, and after 16 wks the mean weights were: control, 20.43 ± 1.2 kg; MO, 19.35 ± 1.3 kg; HO, 17.7 ± 1.1 kg. Control > High-Ox, $P < 0.001$; Medium-Ox > High-Ox, $P < 0.05$.

3.4. Body composition

At the end of the study, the percent body fat of the HO group was 15.3% ± 1.1 which was significantly less ($P < 0.05$) than that of the control group (21.1% ± 1.8). The percent body fat of the MO group was 19.6% ± 1.6, and was not significantly different from the other two groups.

3.5. Trabecular bone volume and appositional rate

Histomorphometric analysis of biopsies from the iliac crest revealed a significant linear trend ($P = 0.034$) for decreased trabecular bone volume with increasing oxidation levels in the diet. Trabecular bone volume (%) was 42.8 ± 3.6 in control dogs, 36.3 ± 3.3 in MO dogs and 32.9 ± 2.1 in HO dogs. In addition, dogs fed the MO and HO diet had significantly reduced bone appositional rate compared to the control fed dogs. The appositional rate for control dogs was 3.81 ± .003 μm/day, 2.71 ± .004 μm/day in MO dogs, and 2.5 ± .002 μm/day in HO dogs (Control > Medium-Ox; $P < 0.05$; Control > High-Ox; $P < 0.01$).

3.6. Oxidative burst

There was no difference in oxidative burst activity between the groups at the start of the experiment. Monocyte production of superoxide was not affected after 8 wks of feeding, but by 16 wks the HO group had significantly reduced production 15 min after PMA stimulation compared to the control group (Fig. 2). There was also a significant linear trend ($P = 0.04$) 30 min after PMA stimulation for decreasing superoxide production with increasing oxidation level in the diet. After 8 wks on the test diets there was no difference in monocyte production of hydrogen peroxide 15 min after PMA stimulation. However, by 30 min, H_2O_2 production by monocytes from the MO and HO dogs declined but monocyte H_2O_2 production continued to increase in the control dogs. After 16 wks on the diets, there was no difference in monocyte production of H_2O_2 between the groups.

After 8 wks, there was no difference in superoxide production by neutrophils 15 min after PMA stimulation. However, by 30 min, the MO and HO group had declining production of superoxide, and the neutrophils from the control group produced significantly more superoxide (Fig. 3). By 16 wks, the decline in fluorescence due to superoxide generation observed at 8 weeks was not evident but superoxide was lower in the MO and HO fed groups but not significantly. Similar to what was observed for superoxide production, by 8 wks of feeding the neutrophils had declining H_2O_2 production after the initial measurement taken at 15 min post PMA stimulation. The neutrophils from control dogs produced significantly more H_2O_2 than those from the MO and HO group. By 16 wks, H_2O_2 production capacity was similar in all groups.

3.7. Monocyte prostaglandin E_2 production

Prostaglandin E_2 production by peripheral blood monocytes was 1691 ± 891 pg/μg of DNA in control dogs, 5151 ± 3995 pg/μg of DNA in MO dogs, and 5690 ± 2768 pg/μg of DNA in HO dogs. The greater amount of PGE_2 produced by monocytes from dogs fed the MO or HO diet was not statistically significant.

3.8. Lymphocyte blastogenesis

At the start and after 8 wks there was no significant difference in the amount of ^{3}[H]-thymidine incorporation in response to concanavalin A stimulation (control = 32.1 ± 7.9, MO = 22.7 ± 7.1, HO = 28.8 ± 12; measured as percent increase in dpm of ^{3}[H]-thymidine incorporation). However, after 16 wks, there was a significant reduction ($p < 0.05$) in ^{3}[H]-thymidine incorporation in the HO group compared to the control group (control = 38.1 ± 6.5, MO = 26.2 ± 6.3, HO = 11.14 ± 14.8; measured as percent increase in dpm of ^{3}[H]-thymidine incorporation).

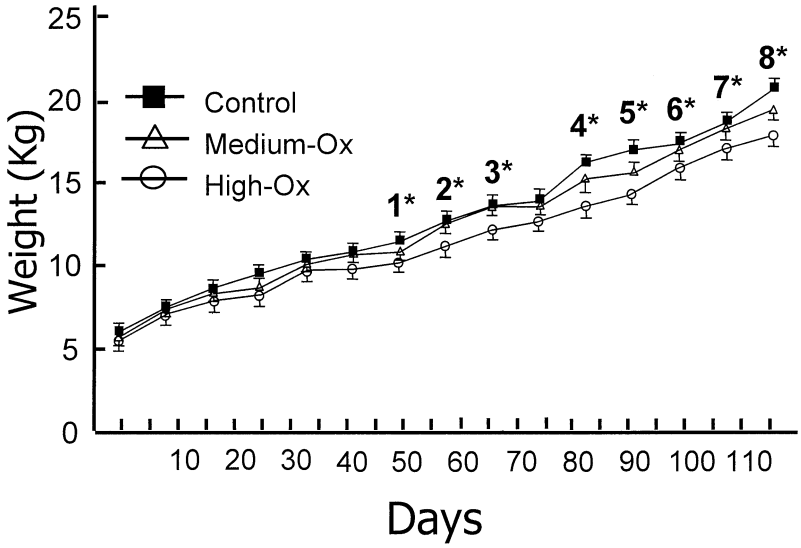

Fig. 1. Weight gain in dogs fed control (<50 ppm aldehydes), medium-oxidation (100 ppm aldehydes), and high-oxidation (500 ppm aldehydes) diets. Mean ± S.E.M. (n = 8).
1 * Control > High-Ox, $P < 0.05$
2 * Control > High-Ox, $P < 0.05$
3 * Control > High-Ox, $P < 0.01$; Medium-Ox >High-Ox, $P < 0.05$
4 * Control > High-Ox, $P < 0.01$
5 * Control > High-Ox, $P < 0.01$
6 * Control > High-Ox, $P < 0.05$
7 * Control > High-Ox, $P < 0.05$
8 * Control > High-Ox, $P < 0.001$; Medium-Ox > High-Ox, $P < 0.05$

4. Discussion

The acute systemic effects of dietary oxidized lipid on growing dogs acted to reduce weight gain, bone appositional rate, antioxidant status, and some immune functions. The body weight difference between the control and the MO group (1.65 kg) and the control and HO group (2.7 kg) at the end of the study was statistically significant. The difference in weight gain may be due in part to a difference in body composition as indicated by the lower percentage of body fat in the HO dogs compared to the control dogs. It is unknown if the MO and HO dogs would have demonstrated compensatory weight gain had they been switched to the control diet, or whether these changes in body composition reflect a change that would remain at skeletal maturity. The lower body fat content may also be a factor in the lower serum triglycerides found in dogs fed the HO diet. It is unknown if the decreased serum triglycerides are also due to decreased activity in the triglyceride biosynthetic pathway or reduced levels or activity of lipoprotein lipase.

Fatty acid analysis of the diets and bone indicated that there was a decrease in the essential fatty acid, linoleic acid (18:2n-6), with increasing oxidation level of the diet. Dogs fed the HO diet also had reduced serum concentration of linoleic acid and α-linolenic acid was not detected in the serum. It is also interesting to note that the longer chain n-3 PUFA, docosahexanoic acid (22:6n-3) was increased in both the serum and the diet. The increased level in the serum may be the result of elongation and desaturation of α-linolenic acid (18:3n-3) and sparing effect on the endogenous 22:6n-3. In iliac crest samples, both 18:2n-6 and 18:3n-3 was reduced in dogs fed the MO and HO diets which was associated with reduced rates of appositional bone formation. Although highly unsaturated fatty acids are very susceptible to oxidation, our data would suggest that perhaps other factors in the diet influence oxidation and metabolism of these PUFA in serum.

In addition to the reduced weight gain with dietary oxidized lipids, another morphological change was the reduction in trabecular bone volume and bone appositional rate. It is unknown if the reduced weight gain influenced these parameters or if other physiological parameters are a factor. The monocytes from the dogs fed the MO and HO diet had higher, though not significant, levels of LPS-induced PGE_2 production. The lower levels of vitamin E we observed could increase PGE_2 production, and increased PGE_2 could in turn contribute to a reduced bone formation rate [22].

Dietary oxidized lipids decreased the antioxidant status as measured by serum vitamin E levels. This result is consistent with other studies that report alterations of vitamin E status with dietary oxidized lipids [11,23]. Vitamin E is known to stimulate lymphocyte blastogenesis [24], and the lowered response we observed in dogs fed the HO diet could be the result of the decreased levels of this vitamin.

Dietary oxidized lipids also altered some neutrophil and monocyte functions. Neutrophils from MO and HO fed dogs exhibited impaired superoxide and hydrogen peroxide production after 8 wks, but recovered this activity 16 wks.

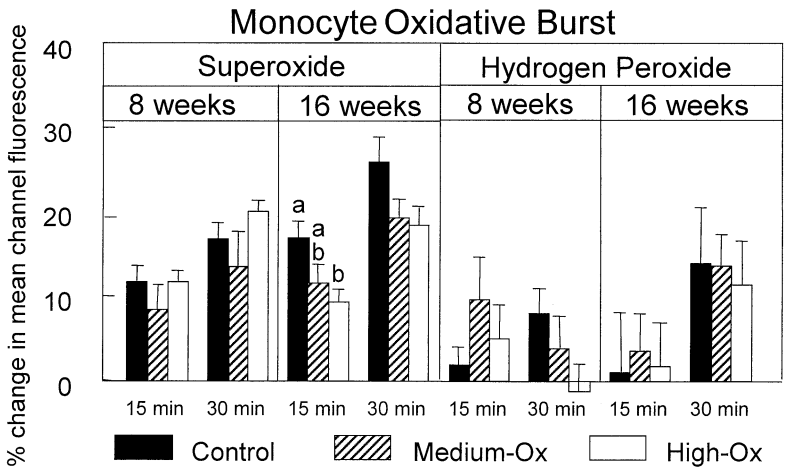

Fig. 2. Monocyte production of superoxide and hydrogen peroxide in response to 2×10^{-3} mol/L of phorbol myristate acetate after being fed experimental diets for 8 and 16 weeks. Mean values having a different letter (a, b, c) are significantly different by one way ANOVA and Tukey's multiple comparison test. Mean ± S.E.M. (n = 8).

There was some impairment of monocyte hydrogen peroxide production at 8 wks, and superoxide production at 16 wks. The fluorescent dyes used to measure oxidative burst in this study accumulate within the cells. The decreased fluorescence during the oxidative burst likely indicates leakage of fluorescent product from the cells, which indicates a loss of membrane integrity. Oxidized lipids can decrease membrane fluidity [5,6], and this may be a contributing factor to the apparent loss of membrane integrity in these cells. It is also interesting to note that there was some recovery from the impaired oxidative burst in the neutrophil and to some extent in the monocyte. There is experimental evidence that vitamin E and selenium deficiency in rats results in an upregulation of coenzyme Q in some cell membranes. Coenzyme Q has been shown to be a membrane stabilizing molecule [25] that also has antioxidant properties [26]. Our data suggests that there was a recovery of membrane stability, and upregulation of coenzyme Q could be a factor in the recovery. Further research will be necessary to determine if oxidized lipids and/or vitamin E depletion can upregulate CoQ in immune cells and if this upregulation can be sustained with chronic vitamin E depletion.

In summary, the main effects of dietary oxidized lipids were reduced weight gain and body fat content, decreased serum levels of vitamin E, alteration of some parameters of

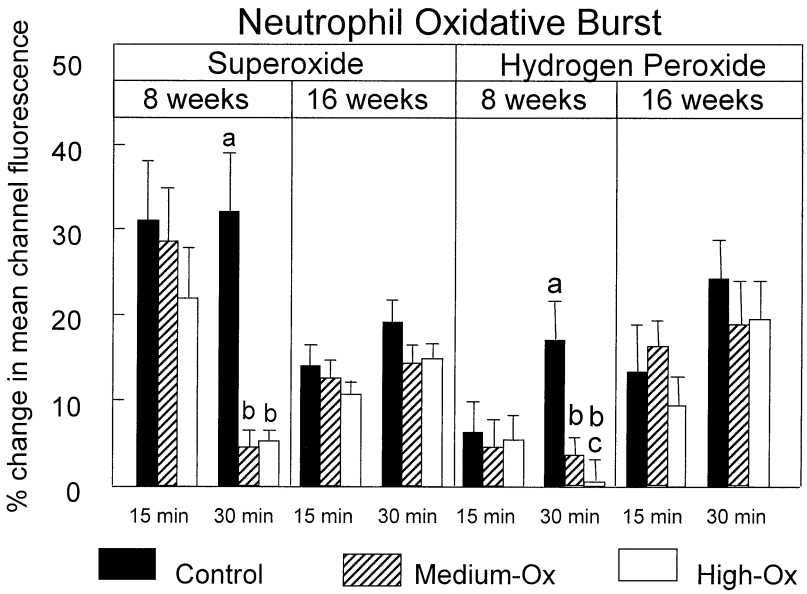

Fig. 3. Neutrophil production of superoxide and hydrogen peroxide in response to 2×10^{-3} mol/L of phorbol myristate acetate after being fed experimental diets for 8 and 16 weeks. Mean values having a different letter (a, b, c) are significantly different by one way ANOVA and Tukey's multiple comparison test. Mean ± S.E.M. (n = 8).

cellular immune function, and impaired rate of bone formation. Significantly, some effects were evident at a moderate level of oxidation (MO diet). These data have implications for both humans and companion animals and highlights the need to control oxidation products in food to insure proper growth and optimum health.

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