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Original Contribution

Antioxidant supplementation and obesity have independent effects on hepatic oxylipin profiles in insulin-resistant, obesity-prone rats

Matthew J. Picklo Sr. [a,b,*], John W. Newman [c,d,e]

[a] USDA-ARS Grand Forks Human Nutrition Research Center 2420 2nd Avenue North Grand Forks, ND 58203-9034, USA
[b] Department of Chemistry, University of North Dakota, Grand Forks, ND 58201, USA
[c] USDA-ARS Western Human Nutrition Research Center, Davis, CA 95616, USA
[d] West Coast Metabolomics Center, University of California Davis, Davis, CA 95616, USA
[e] Department of Nutrition, University of California Davis, Davis, CA 95616, USA

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ABSTRACT

Obesity-induced changes in lipid metabolism are mechanistically associated with the development of insulin resistance and prediabetes. Recent studies have focused on the extent to which obesity-induced insulin resistance is mediated through oxylipins, derived from enzymatic and nonenzymatic lipid peroxidation. Vitamin E and vitamin C are widely used antioxidant supplements, but conflicting data exist as to whether supplementation with vitamins E and C reduces insulin resistance. The purpose of this work is (1) to test the hypothesis that supplementation with vitamin E and vitamin C prevents the development of insulin resistance and (2) to determine the extent to which antioxidant supplementation modifies obesity-induced changes in hepatic oxylipins. Using obesity-prone Sprague–Dawley rats fed a high-fat, hypercaloric diet, we found that vitamin E and C supplementation did not block the development of insulin resistance, despite increased plasma levels of these antioxidants and decreased hepatic F_2-isoprostane (F_2-IsoP) concentrations. The obese phenotype was associated with increased hepatic concentrations of cytochrome P450 (CYP450)-dependent linoleic acid and α-linolenic acid-derived epoxides. Antioxidant supplementation, but not obesity, decreased levels of the lipoxygenase (LOX)-dependent, arachidonic acid-derived products lipoxin A4 (LXA_4), 8,15-dihydroxtetraenoate (8,15-DiHETE), and 5,15-DiHETE. Our data demonstrate that antioxidant supplementation and obesity impact hepatic LOX- and CYP450-dependent oxylipin metabolism.

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1. Introduction

Obesity-induced changes in oxidative stress and lipid metabolism are mechanistically associated with the development of insulin resistance and prediabetes, conditions estimated to afflict greater than one in four people in the United States [1]. In humans, urinary and plasma indices of nonenzymatic lipid peroxidation (such as isoprostanes and thiobarbituric acid-reactive substances) are positively correlated with the body mass index, and oxidative stress appears to be a mechanistic pathway to obesity-associated pathological consequences including hepatic and adipose dysfunction and insulin resistance [2–4].

The altered production of oxygenated, bioactive polyunsaturated fatty acids (PUFAs) (i.e., oxylipins) in obesity may have pathological consequences, and a number of recent studies have investigated the mediation of obesity-induced insulin resistance by oxylipins [5–9]. Oxylipins include a myriad of species derived from n6 PUFA, such as arachidonic acid (ArA; 20:4n6) and linoleic acid (LA; 18:2n6), and from n3 PUFA, such as α-linolenic acid (ALA; 18:3n3), eicosapentaenoic acid (EPA; 20:5n3), and docosahexaenoic acid (DHA; 22:6n3). Bioactivity

Abbreviations: 12(13)Ep-9-KODE, 2(13)Epoxy-9-ketooctadecadienoic acid; % en, % of energy; ALA, α-Linolenic acid; ArA, Arachidonic acid; AUC, Area under the curve; AutOx, Auto-oxidation; BHT, 2,6-Di-tert-butyl-4-methylphenol; COX, Cyclooxygenase; CYP, Cytochrome P450; DHA, Docosahexaenoic acid; DiHDoPE, Dihydroxydocosapentaenoic acid; DiHETE, Dihydroxyeicosatetraenoic acid; DiHETrE, Dihydroxyeicosatrienoic acid; DiHODE, Dihydroxyoctadecadienoic acid; DiHOME, Dihydroxyoctadecaenoic acid; DTPA, Diethylenetriaminepentaacetic acid; EPA, Eicosapentaenoic acid; EpDoPE, Epoxydocosapentaenoic acid; EpETrE, Epoxyeicosatrienoic acid; EpETE, Epoxyeicosatetraenoic acid; EpODE, Epoxyoctadecadienoic acid; EpOME, Epoxyoctadecaenoic acid; F_2-IsoP, F_2-Isoprostanes; HDoHE, Hydroxydocosahexaenoic acid; HEPE, Hydroxyeicosapentaenoic acid; HETE, Hydroxyeicosatetraenoic acid; HETrE, Hydroxyeicosatrienoic acid; HODE, Hydroxyoctadecadienoic acid; HOMA-IR, Homeostasis model assessment of insulin resistance; HOTE, Hydroxyoctadecatrienoic acid; HPODE, Hydroperoxyoctadecadienoic acid; KODE, Ketooctadecadienoic acid; LA, Linoleic acid; LOX, Lipoxygenase; MUFA, Monounsaturated fatty acids; OGTT, Oral glucose tolerance test; PUFA, Polyunsaturated fatty acid; sEH, Soluble epoxide hydrolase; ROS, Reactive oxygen species; TriHOME, Trihydroxyoctadecaenoic acid

* Corresponding author at: USDA-ARS Grand Forks Human Nutrition Research Center 2420 2nd Avenue North Grand Forks, ND 58203-9034, USA. Fax: + 1 701 795 8240,
E-mail address: matthew.picklo@ars.usda.gov (M.J. Picklo Sr.).

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among known oxylipins varies with structure and includes pro- and anti-inflammatory actions, vasodilatory/relaxant properties, and mediation of cell growth and proliferation [9]. These lipid mediators include products derived from cyclooxygenases (COX), lipoxygenases (LOX), and cytochrome P450 (CYP)-mediated oxygenation, as well as products derived from free-radical-catalyzed PUFA oxidation [9].

Vitamin E (as α-tocopherol) and vitamin C are two of the most widely used antioxidant supplements, often used in combination [10,11]. These compounds reduce indices of lipid peroxidation in vivo and interact with enzymes involved in oxylipin metabolism. For instance, vitamin E has been reported to inhibit lipoxygenases, induce CYP2C fatty acid epoxygenases, and act as CYP4F ω-hydroxylase substrates, enzymes also responsible for the ω-hydroxylation of leukotrienes and ArA [12–15]. Vitamin C has been found to induce COX and LOX product formation in endothelial cell cultures [16]. Yet it is not known whether supplementation with these antioxidants modifies oxylipin disposition. Such data will provide an improved understanding of dietary antioxidant impacts on hepatic oxylipins in obesity and provide unique insight into the mechanistic relationship between obesity, lipid metabolism, and oxidative stress.

The purpose of this work is to determine the extent to which supplementation with α-tocopherol and vitamin C modifies obesity-induced changes in hepatic oxylipins derived from enzymatic and free-radical-catalyzed auto-oxidative processes. We used obesity-prone Sprague–Dawley rats fed a high-fat, hypercaloric diet as the model. Our data indicate that the obese phenotype increased the content of CYP-generated fatty acid epoxides while reducing the content of the corresponding soluble epoxide hydrolase (sEH)-derived diol products in the liver. Antioxidant supplementation reduced indices of free-radical-catalyzed lipid peroxidation in addition to reducing levels of multiple putatively LOX-derived products. Furthermore, our data indicate that induction of an obese, insulin-resistant phenotype was not accompanied by elevations in hepatic oxidative stress.

Table 1
Diet compositions.

Ingredient	Lean	Obese	Obese+CE
Energy, kcal/g diet	4.2 ± 0.1[a]	5.1 ± 0.2	5.0 ± 0.1
% Energy			
Carbohydrate	70	35	35
Protein	20	20	20
Fat	10	45	45
Vitamin C (mg/kg)[b]	N.D.	N.D.	500
Vitamin E (IU/kg)[c]	72	89	633

[a] Data are mean ± the S.D; $n=3$. Values were determined by bomb calorimetry.
[b] Vitamin C added as *L*-ascorbic acid. Vitamin C was not included in the AIN93 vitamin mix used to prepare the diets. Vitamin C was not detectable (N.D.) in the lean and obese diets.
[c] Vitamin E as *d/l*-α-tocpherol for the Lean and Obese diets and as added *d*-α-tocopherol acetate in the Obese+CE diet

Table 2
Percent energy of individual fatty acids

Major fatty acids	10% fat en[a]	45% fat en
C4:0–C14:0	0.7	1.3
C16:0	1.0	9.6
C18:0	0.5	7.2
C18:1n9	2.1	15.0
C18:2n6	4.0	8.4
C18:3n3	0.3	0.5
Total n3 PUFA	0.3	0.7
Total n6 PUFA	4.5	9.0
Total MUFA	2.8	16.9
Total saturated	2.5	18.5
Total % en	10.0	45.0

[a] Data are the means of three samples assayed independently with relative standard deviations <5%. PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids.

2. Methods and materials

2.1. Animal experimentation

All experiments were performed in accordance with the NIH guidelines for the use of live animals and were approved by the Institutional Animal Care and Use Committee of the USDA/Agricultural Research Service, Grand Forks Human Nutrition Research Center. Forty-two (42) male obesity-prone Sprague–Dawley rats (Crl:OP(CD) strain code: 463) four weeks of age were ordered from Charles River Laboratories International, Inc. (Wilmington, MA). Animals were placed on a modified AIN-93G diet with 10% of the energy (% en) derived from fat for the first two weeks of the study.

Starting in week 2, the animals were regrouped by lean body mass ($n=14$/group) measured by an EchoMRI whole body composition analyzer (Echo Medical Systems, Houston, TX) and placed on one of three dietary treatments with 10% en fat (Group 1, control; lean), 45% en fat (Group 2, obese), or 45% en fat with additional vitamin E and vitamin C (Group 3, obese+CE; Table 1). One rat in the lean group died during the study. Diet components for the low- and high-fat diets are essentially the same as previously published [17]. Lean rats received a diet composed of protein (20% en), fat (10% en), and carbohydrate (70% en). For the lean group, the 10% en fat was supplied as lard (Dyets Inc; Bethlehem, PA) with added linoleic acid (18:2n6) and α-linolenic acid (18:3n3) to meet nutritional requirements (both from MP Biomedicals, Solon, OH). The fatty acid composition of the diets is provided in Table 2. Obese rats received a diet composed of protein (20% en), fat (45% en), and carbohydrate (35% en), with fat energy solely from lard. Obese+CE rats were the same as the obese group except that the diet contained 500 mg/kg of added *L*-ascorbic acid (Acros Organics, Geel, Belgium) and 400 mg/kg *d*-α-tocopheryl acetate (Sigma-Aldrich, St. Louis, MO) as a source of vitamin E. All diets contained AIN93 mineral mix (Dyets Inc.; Bethlehem, PA) and AIN93G vitamin mix (Harlan/Teklad; Madison, WI). In the AIN93 vitamin mix, vitamin E was provided as *d/l* α-tocopheryl acetate.

At week 14, approximately 1.5 mL of whole blood was drawn via tail artery into a 3 mL syringe containing 75 μL of 4.5% EDTA–disodium salt with animals under ether anesthetic. Whole blood was then centrifuged at 2000*g* for 15 min at 4 °C. The remaining 200 μL of plasma was immediately added to a 1.5 mL microcentrifuge tube containing 800 μL of a diethylenetriaminepentaacetic acid (DTPA) solution [90% methanol:10% ultrapure water:25 μM DTPA] to stabilize the ascorbic acid. The solution was vortexed and incubated on ice for 10 min and then centrifuged at 12,000*g* for 10 min at 4 °C. The supernatant was removed from the pellet and stored at −80 °C until it was analyzed for ascorbic acid.

Following oral glucose tolerance testing at 16 weeks (see below), animals were placed back on their respective diets for two weeks prior to euthanasia. Animals were euthanized under fasting conditions by administration of a 1.37:1 mixture of ketamine (100 mg/mL):xylazine (100 mg/mLl) at 1 mL/kg body weight i.p. and exsanguinated by descending vena cava blood draw. Tissues were quickly removed, frozen in liquid nitrogen, and stored at −80 °C until use.