Modulation of Leptin Levels by Oxidized Linoleic Acid: A Connection to Atherosclerosis?

Meera Penumetcha,¹ Nadya Merchant,² and Sampath Parthasarathy³

¹Division of Nutrition, School of Health Professions, Georgia State University, Atlanta; ²In Vasc Therapeutics, Tucker, Georgia; and ³The Ohio State University, Columbus, Ohio, USA

ABSTRACT The objective of the study was to determine the effects of oxidized linoleic acid (Ox-LA) on plasma leptin and to determine the relationship between plasma leptin levels and atherosclerosis in animals treated with Ox-LA. Low-density lipoprotein (LDL) receptor knockout (*LDL r^{-/-}*) mice were fed a high fat diet with or without Ox-LA for 11 weeks. Plasma leptin levels in the high fat group consuming Ox-LA were significantly higher ($14,052 \pm 601$ pg/mL vs. $10,950 \pm 541$ pg/mL; $P < .01$) compared to the group receiving the high fat diet alone. There was a highly significant correlation between the plasma leptin levels and aortic atherosclerotic lesions. From this we conclude that chronic exposure to dietary Ox-LA increases the plasma levels of leptin in *LDL r^{-/-}* mice on a high fat diet. Considering our previous finding that dietary Ox-LA increased atherosclerosis, the current findings emphasize the need to reduce dietary intake of oxidized fat.

KEY WORDS: • dietary oxidized fat • food efficiency • obesity

THE PREVALENCE OF OBESITY in the United States is on the rise. Data from the Behavioral Risk Factor Surveillance System (1985–2008) of the Centers for Disease Control and Prevention report that there has been a dramatic increase in obesity in most U.S. states. Several studies have shown that plasma leptin levels are well correlated with body fat.¹ Leptin, a hormone secreted primarily by adipocytes, has been shown to regulate food intake by acting as a satiety signal.^{2,3} However, this leptin-based regulation of food intake appears to have gone awry in obese individuals. Overweight individuals seem to have higher plasma leptin levels^{1,4} compared to their lean counterparts and are believed to be in a “leptin-resistant” state.^{5,6} Because obesity is known to be a risk factor for atherosclerosis, several investigators have tried to understand the relationship between plasma leptin levels and risk of cardiovascular disease. In general, the results are mixed with studies showing modest correlations between elevated plasma leptin levels and risk factors for cardiovascular disease,^{7–13} whereas others fail to show any association.^{14,15}

Oxidative stress has been implicated as a factor in the development of atherosclerosis.^{16,17} Previously, we reported that feeding oxidized linoleic acid (Ox-LA), a dietary component of fried foods, increases oxidative stress and promotes atherosclerosis in low-density lipoprotein (LDL) receptor knockout mice (*LDL r^{-/-}*) when they are simul-

taneously fed a high fat, high cholesterol diet.¹⁸ In the current study, we determined the plasma leptin levels and their association with aortic atherosclerotic lesions in *LDL r^{-/-}* mice fed a high fat, high cholesterol diet in the presence or absence of Ox-LA. To our knowledge this is the first study that looks at the regulation of plasma leptin levels by a common dietary component found in fried foods. Understanding this relationship is important because it could possibly explain the role of fried foods in the promotion of cardiovascular disease in obese individuals.

A 50 μ M solution of linoleic acid (LA) was made in phosphate-buffered saline, and the solution was oxidized with soybean lipoxidase (60–90 units/100 nmol, 2 hours at 37°C) to produce Ox-LA (hydroperoxyoctadecadienoic acid [HPODE] and hydroxyoctadecadienoic acid [HODE]). The formation of HPODE was determined by leukomethylene blue.¹⁹ In brief, 40 μ L of Ox-LA is added to 100 μ L of leukomethylene blue reagent in a microtiter plate and incubated at room temperature for 10 minutes. The sample is then read in an Anthos htII microtiter reader (Labtech International, Ringmer, United Kingdom). The amount of peroxide generated is quantitated against a standard curve generated by authentic 13-HPODE. Ox-LA and unoxidized LA were then extracted with ether, dried under nitrogen, and resuspended in ethanol. This process of extraction results in the conversion of 13-HPODE to 13-HODE. For studies with *LDL r^{-/-}* mice, the Ox-LA was frozen in aliquots at -80°C , after drying under nitrogen, and thawed just before feeding the animal.

Male *LDL r^{-/-}* mice ($n = 8$), 4–6 weeks old, were fed an atherogenic diet containing 21.2% fat and 0.15% cholesterol

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Address correspondence to: Sampath Parthasarathy, Ph.D., M.B.A., N-850 Doan Hall, The Ohio State University, 410 W 10th Avenue, Columbus, OH 43210, USA. E-mail: parthasarathy.6@osu.edu

(TD 88137; Harlan Teklad, Indianapolis, IN, USA). Another group ($n = 10$) was fed the atherogenic diet along with Ox-LA (8 mg/day). The mice were fed Ox-LA five times a week for a total of 11 weeks. At the end of 11 weeks the mice were euthanized by CO₂ asphyxia, and blood was drawn in a heparinized tube from the inferior vena cava. Red blood cells and plasma were separated by centrifugation and then frozen at -80°C .

Leptin levels in the plasma of *LDL r^{-/-}* mice were determined by an enzyme immunometric assay kit (Assay Designs Inc, Ann Arbor, MI, USA). The assay was performed according to the manufacturer's instructions. Plasma from the *LDL r^{-/-}* mice was diluted 1:50, and a 100- μL aliquot was used in the assay.

Food efficiency was calculated by dividing the weight gain (in g) by the total amount of food (in g) eaten during the experimental period.

Results are expressed as mean $\pm$ SE values. For the mouse study the difference between the groups treated with Ox-LA versus unoxidized LA was determined by Student's *t* test, and the significance was set at $P \leq .05$. The relationship between plasma leptin and aortic atherosclerotic lesions was determined by Pearson's correlation.

Feeding Ox-LA (8 mg/day for 11 weeks) along with a high fat, high cholesterol diet caused a significant increase in plasma leptin levels in *LDL r^{-/-}* mice (Table 1). Furthermore, the plasma leptin levels were strongly and significantly associated with aortic atherosclerotic lesion area (Fig. 1). There was no significant difference in food efficiency between the control and Ox-LA-fed groups (Table 1). The amount of Ox-LA given to the mice can be extrapolated to equal an intake of 2.3 g of fat/day in a 70-kg adult male or $\sim 1\%$ of the total calories of a 2,200 Kcal/day diet.

Recent reports have demonstrated a positive association between hyperleptinemia and cardiovascular disease.^{20–22} Because leptin is an important regulator of hunger and satiety, we wanted to see if Ox-LA, a component of fried foods, can modulated leptin levels and if this modulation has any association with the development of atherosclerotic lesions. Our laboratory has previously shown that Ox-LA promotes atherosclerosis in *LDL r^{-/-}* mice fed a high fat, high cholesterol diet. We analyzed the plasma samples of these animals and determined leptin levels. This is the first study to demonstrate that a component of fried food, Ox-LA, increases leptin levels in *LDL r^{-/-}* mice fed a high fat diet and that this increase is modestly associated with atherosclerotic lesions. Regulation

FIG. 1. Correlation plot of plasma leptin levels and aortic atherosclerotic lesions in low-density lipoprotein receptor knockout mice fed a high fat diet with or without oxidized linoleic acid.

of leptin secretion by dietary factors and in particular by dietary fat has been investigated.^{23–28} Those studies suggested that the mechanism of regulation include pre- and posttranscriptional modulation of leptin by the direct action of fatty acids as well as their indirect action on certain hormones or cytokines. We think that either Ox-LA or its metabolites could increase plasma leptin. Because we have previously demonstrated²⁹ that Ox-LA is absorbed by the intestine and based on evidence, by others, that oxidized lipids are ligands of peroxisome proliferative-activated receptor γ , it is entirely possible that Ox-LA itself is mediating this effect. Adipose tissue, the primary site of leptin production, is under the influence of peroxisome proliferative-activated receptor γ for its differentiation. Studies have shown that oxidized fatty acids are strong ligands of peroxisome proliferative-activated receptor γ compared to their unoxidized counterparts.³⁰ We hypothesize that Ox-LA acting through peroxisome proliferative-activated receptor γ could influence adipose tissue metabolism and thus increase leptin secretion. Future studies should test this hypothesis in a more appropriate mouse model. Measuring adipose tissue mass and adipocyte-derived cytokines in *LDL r^{-/-}* mice could yield results that are difficult to interpret. Instead, using a diet-induced model of obesity in C57BL/6J mice would be more appropriate. Finally, an increase in plasma leptin should result in reduced food intake in normal-weight animals. In fact, we previously reported that the mice in the Ox-LA group ate about 20% less food (in g). However, there was no difference in food efficiency between the two groups, suggesting that Ox-LA did not influence weight gained per gram of food consumed.

Our results suggest that consumption of fried foods along with a high fat diet could elevate plasma leptin levels and in the presence of hyperlipidemia could promote the development of atherosclerotic lesions in individuals with mild or moderate obesity.

TABLE 1. PLASMA LEPTIN LEVELS AND FOOD EFFICIENCY IN LOW-DENSITY LIPOPROTEIN RECEPTOR KNOCKOUT MICE FED HIGH FAT DIET AND LINOLEIC ACID

Linoleic acid	Leptin (pg/mL)	Food efficiency (g/g [weight gain/food intake])
Unoxidized	10,950 $\pm$ 495	0.074 $\pm$ 0.003
Oxidized	14,053 $\pm$ 567 ^a	0.08 $\pm$ 0.005 ^b

^a $P \leq .05$ by Student's *t* test.

^bDifference not significant.

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AUTHOR DISCLOSURE STATEMENT

N.M. is an employee of In Vasc Therapeutics (Tucker, GA, USA). M.P. and S.P. have no competing financial interests to disclose.

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