

SHORT REPORT

LOX-1-mediated injury in sensory neurons in type 2 diabetes

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Abstract

Our laboratory recently identified several differentially expressed genes (DEG) through microarray analysis of the sciatic nerves of 24-week-old BKS db/db (diabetic) and db/+ (nondiabetic) mice. We noted prominent increases in lipid metabolism genes, consistent with another model, the high fat-fed C57BL6/J mouse, where we postulated that oxidized low-density lipoproteins (oxLDL) activate a receptor-mediated inflammatory pathway of neuronal injury. Our findings support clinical observations that plasma lipids associate with neuropathy, and suggest that glucose and lipids together will be responsible for the most aggressive development of diabetic neuropathy. A role for oxLDL-induced injury remains to be proved *in vivo*, and this is the goal of the present study. First, we performed biostatistical network analysis to identify molecular interactions of our LOX-1 gene of interest, *Olr1*. Next, nondiabetic BKSdb/+ and diabetic BKSdb/db littermates received intraperitoneal injections of LOX-1 neutralizing antibody or control non-immune IgG every 48 h from 6-12 wk of age. At 12 wk of age, blood and tissues were harvested for metabolic and neuropathy phenotyping. We confirmed our network analysis by demonstrating that NFkappaB, a gene closely linked to *Olr1* in our network, was decreased in anti-LOX-1 treated mice. Most metabolic parameters (blood glucose, glycated hemoglobin, plasma triglycerides and cholesterol) and also the sciatic motor nerve conduction velocity deficit were unchanged in the presence of anti-LOX-1 treatment compared with control IgG. However, there was a striking preservation of the sural nerve conduction velocity in anti-LOX-1-treated mice, demonstrating that this receptor is involved in sural nerve injury in type 2 diabetes.

Keywords: Lipid metabolism genes, LOX-1, Sensory neurons, type 2 diabetes

Short Report

A network of DEG related to LOX-1 was generated by searching Pubmed abstracts for same sentence citations with the LOX-1 gene *Olr1*. This co-citation analysis, annotated using our previous microarray data¹ in the db/db and db/+ mice, reveals few direct interactions of *Olr1* with other genes (Fig. 1). The largest hub of related genes is linked to LOX-1 via NFkappaB. Two other hubs link through TNF α and Bcl-2. The data strongly suggest that LOX-1 may mediate lipid-induced injury via activation of inflammation or programmed cell death. Therefore, we set out to determine whether we can prevent peripheral neuronal injury in dyslipidemic, diabetic mice via inhibition of LOX-1.

We used BKS db/db mice and non-diabetic db/+ littermates (Jackson Laboratories, stock number 000642, Bar Harbor ME). Mice were maintained in the University of Michigan, pathogen-free environment following the University of Michigan Committee on the Care and Use of Animals guidelines. Mice had continuous access to food (Purina 5001, Purina Mills LZLC, St. Louis, MO) and water. At 6

wk of age, mice were randomly assigned to receive either anti-LOX-1 antibody (R&D Systems, cat. no. AF 1564 affinity purified goat IgG) or control non-immune goat IgG with 6 mice in each group. Injections (I.P. 100 μ L of 1.6 μ g/mL IgG in PBS) were performed every 48 h for 6 wk. At 12 wk of age, metabolic and neuropathy phenotyping was performed per our standard protocols¹⁻³ and then mice were euthanized for collection of plasma and dorsal root ganglia (DRG).

We confirmed the network association of *Olr1* with NFkappaB expression by Western blotting for NFkappaB in the DRG using BD Transduction Laboratories cat. no. 610869, followed by HRP-conjugated secondary antibody and confirming even loading by probing for actin. NFkappaB p65 protein was significantly downregulated in both db/+ and db/db mice that received the anti-LOX-1 treatments (Fig. 1 inset). The protein also was significantly increased in db/db compared with db/+ mice.

Db/db mice have significantly increased body weight, plasma glucose, GHb, plasma cholesterol, and plasma triglycerides compared with db/+ littermates (Fig. 2). There was no significant difference between mice receiving non-immune IgG and those receiving anti-LOX-1. By 12 weeks of age, the db/db mice treated with control non-immune IgG (or no injection, not shown) had a significantly reduced response to heat in the hind paw, with an increase in latency from 5.29 \pm 0.19s to 6.71 \pm 0.57 s (Fig. 3A). Blocking LOX-1 did not prevent this symptom of neuropathy.

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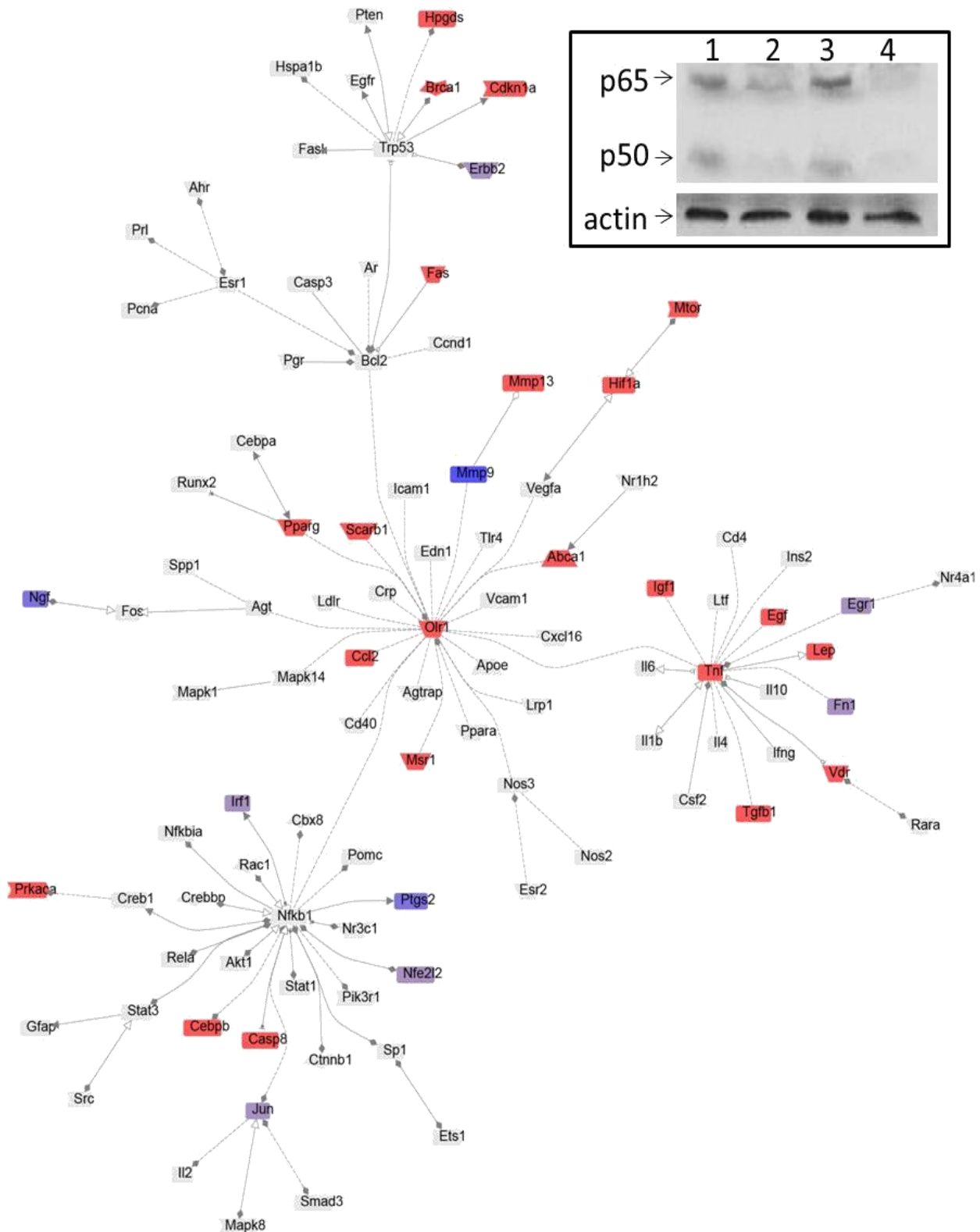


Figure 1: GePS Literature Co-citation Network of *Olr1*. The network depicts literature co-citation relationships for *Olr1*. Nodes represent genes; edges represent literature co-citation of genes. Node color represents fold change of gene regulation in the 24 week old db/db mouse sciatic nerve: red: up-regulation, blue: down-regulation, grey: no regulation. [Inset] DRG were harvested from the 12 week old mice and 5 DRG per condition were pooled and processed for Western blotting. Lane 1 contains db/+ treated with IgG; lane 2 contains db/+ treated with anti-LOX-1; lane 3 contains db/db treated with IgG; lane 4 contains db/db treated with anti-LOX-1. Membranes were stripped and re-probed with antibody to actin in order to compare loading.

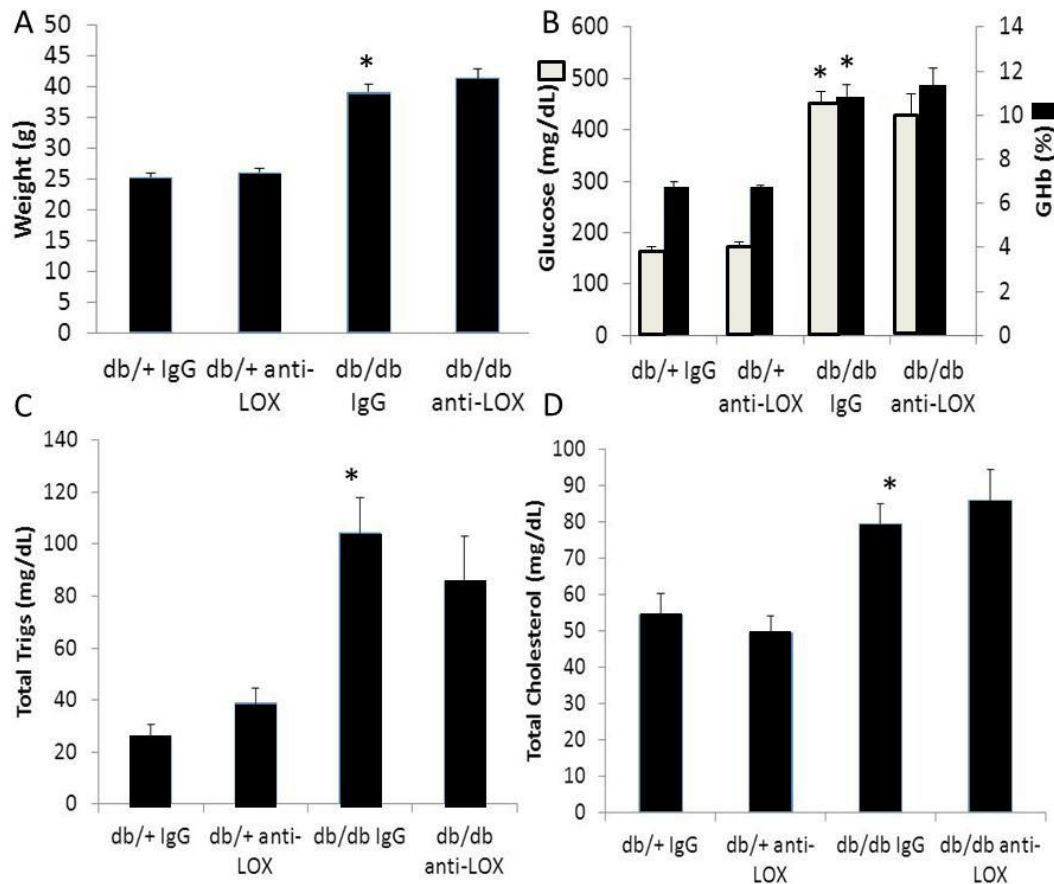


Figure 2: Metabolic Profiles of the db/+ and db/db Mice. After 12 weeks of treatment with control non-immune IgG or anti-LOX-1, six mice per group were assessed for the degree of diabetes. Mean and standard errors are plotted of (A) body weight, (B) fasting blood glucose (left Y-axis) and glycated hemoglobin (right Y-axis), (C) plasma total triglycerides, and (D) plasma total cholesterol. (*)All these parameters were significantly increased in db/db mice compared with db/+ mice, $p < 0.05$, and there was no difference between IgG or anti-LOX-1 treatments in either db/+ or db/db genotype.

Both the sural sensory (by around 5 m/s) and sciatic motor (by around 20 m/s) NCV were decreased at 12 wk in db/db mice (Fig. 3B-C). In the group that received the anti-LOX-1 antibody sural but not sciatic NCV was preserved.

Comment

Biostatistical analyses of diabetes models are an exciting new tool for discovery science that may identify disease mechanisms. Our microarray studies in db/db mice confirmed the importance of both inflammatory and lipid metabolism pathways in diabetic neuropathy.¹ We previously suggested a role for the oxLDL receptor LOX-1 in neuropathy, based on our examination of high fat fed mice.³ In these mice, hyperphagia produced dyslipidemia and insulin resistance, leading to diabetes.¹ Hyperglycemia produces tissue and systemic oxidative stress that accelerates oxidation of LDL to cytotoxic oxLDL.⁴ Our co-citation network suggested that LOX-1 is functionally related to genes primarily involved in inflammation and cell survival such as *Pparg*, *Scarb1*, *Mmp9*, *Tnf*, *Nfkb*, and *Bcl2*. In order to determine the *in vivo* role of LOX-1 in diabetic neuropathy, we elected to inhibit its activity in db/db mice. A previous study demonstrated the utility of the anti-LOX-1 antibody *in vivo*,⁵ so we employed the same technique. Anti-LOX-1 did not significantly alter the course of the

development of obesity, hyperglycemia, and hyperlipidemia, so any protective effects were downstream of these metabolic stressors.

Hind paw latency and nerve conduction velocity measures demonstrate that 12 wk db/db mice have slower responses than age-matched db/+ littermates, evidence of neuropathy.^{1,3} Treatment of the mice with anti-LOX-1 during the period when neuropathy develops in these mice prevented the loss of sural NCV but did not alter the loss of sciatic NCV or hind paw sensitivity to heat. Since the sural measurement primarily measures sensory neuron function, while the sciatic measurement focuses on motor neuron function, LOX-1 inhibition seems to preferentially protect the sensory neurons. Thus, the motor neurons likely sustain an injury in the presence of LOX-1 inhibition via other mechanisms. Mechanisms for differential protection of sural but not sciatic NCV could be proposed. Our previous work showed that LOX-1 is expressed by DRG neurons at the plasma membrane of both the cell body and the neuritis.³

However, LOX-1 is widely reported to be expressed on macrovascular endothelium⁶ and we have unpublished data demonstrating robust expression on microvascular endothelium. Since our db/db array demonstrated

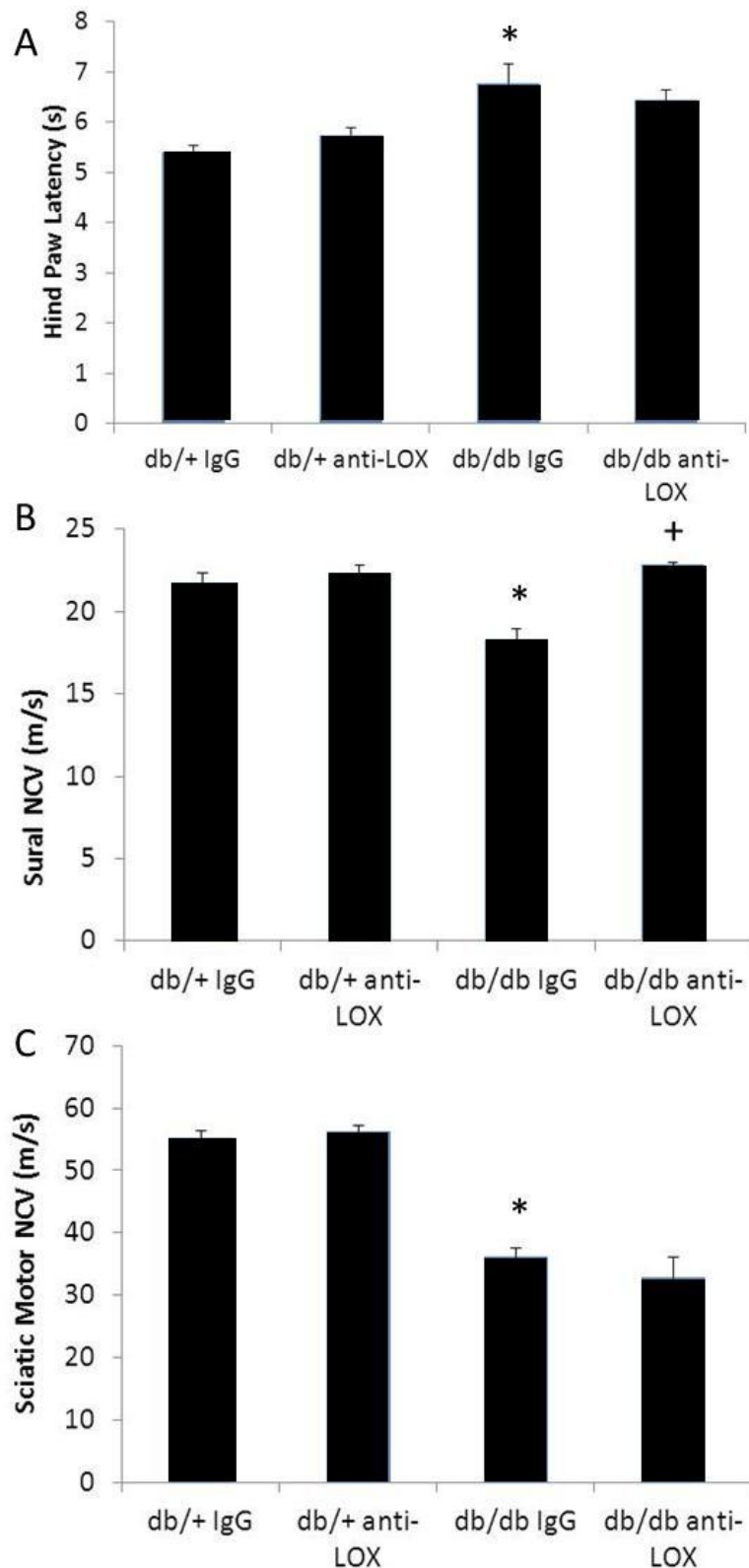


Figure 3: Neuropathy Phenotype of the db/+ and db/db Mice. After 12 weeks of treatment with control non-immune IgG or anti-LOX-1, six mice per group were assessed for the development of diabetic neuropathy. Mean and standard errors are plotted of (A) hind paw latency to a heat stimulus, (B) sural nerve conduction velocity, (C) sciatic motor nerve conduction velocity. (*)All these parameters were significantly altered in db/db compared with db/+ mice, $p < 0.05$. (+)In sural NCV only, treatment with anti-LOX-1 prevented the loss of function in the db/db mice (compare db/db IgG with db/db anti-LOX).

significant *Olr1* mRNA in sciatic nerve,¹ we postulate that LOX-1 also may be present in Schwann cells, thus the site of action of the antibody may be dependent upon the cellular site of LOX-1 activity. Sural nerve injury may be predominantly generated via altered blood flow so that oxLDL-mediated vascular injury is the critical mediator of sural nerve dysfunction in db/db early neuropathic deficits.⁷ Thermal sensory neuropathy tends to relate to other clinically evident neuropathic symptoms, so the inability of LOX-1 inhibition to prevent the decay in hind paw responses suggest that neuropathy is progressing via mechanisms other than oxLDL-mediated LOX-1 signaling. This is not surprising, given the clear role of hyperglycemia in the development of neuropathy⁸ and other potential mechanisms such as insulin resistance.^{4,9}

The striking decrease in p65 NFkappaB protein in the DRG of LOX-1-treated db/+ and db/db mice is interesting because this protein is redox-sensitive.¹⁰ This finding strongly links oxLDL with cellular inflammation in the peripheral nervous system. While blocking the consequences of LOX-1 activation is not a complete therapy for diabetic neuropathy, it underscores the necessity of targeting more than hyperglycemia alone in order to prevent or reverse this disease in patients.

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