Journal of the Peripheral Nervous System 14:257–267 (2009)

2009 PNS PLENARY LECTURE AND REVIEW

Hyperlipidemia: a new therapeutic target for diabetic neuropathy

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Abstract Emerging data establish dyslipidemia as a significant contributor to the development of diabetic neuropathy. In this review, we discuss how separate metabolic imbalances, including hyperglycemia and hyperlipidemia, converge on mechanisms leading to oxidative stress in dorsal root ganglia (DRG) sensory neurons. We conclude with suggestions for novel therapeutic strategies to prevent or reverse diabetes-induced nerve degeneration.

Key words: low-density lipoprotein, mitochondria, NAD(P)H oxidase, oxLDL, triglycerides

Significance of the Problem

Diabetes mellitus affects over 20 million people in the United States and a number of diabetic patients are increasing by 5% per year (www.diabetes.org). The most common complication of diabetes is diabetic neuropathy. Depending on the diagnostic criteria used, at least 50% up to 90% of individuals with diabetes will develop diabetic neuropathy. The most common form of diabetic neuropathy is diabetic polyneuropathy, a symmetric loss of nerve function beginning in the toes and progressing in a distal to proximal fashion, yielding what is commonly called a stocking/glove pattern of sensory loss *(Edwards et al., 2008)*. While all sensory modalities are eventually affected, recent studies show that initially small unmyelinated and thinly myelinated fibers are injured and, especially early in the disease, patients can present with pain. Twenty-five years after the diagnosis of diabetes, the cumulative risk of a lower extremity amputation is 22% and, in the general population, 60% of all lower extremity amputations are secondary to diabetic neuropathy. This represents a cost of over $22 billion per year and a significant loss of quality of life for diabetic patients *(Barrett et al., 2007)*. Although therapies are available to alleviate the symptoms of diabetic neuropathy, these rarely impact upon the root causes of the disease *(Feldman et al., 2002)*. The immense physical, psychological, and economic cost of diabetic neuropathy underscores the need for causally targeted therapies *(Kles and Vinik, 2006)*.

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Oxidative Stress in Diabetes

There is a growing consensus, driven by both clinical and basic studies, that oxidative stress underlies the development of the microvascular complications of diabetes, including diabetic neuropathy *(Low et al., 1997; Ziegler et al., 2004; Russell et al., 2008; Vincent et al., 2008)*. In type 1 diabetic patients, the severity of microvascular complications parallels the degree of systemic oxidative stress *(Sullivan and Feldman, 2005; Giugliano et al., 2008)*. With disease and diabetic neuropathy progression, antioxidant potential decreases while lipid peroxidation products increase. Type 2 diabetic patients have a similar oxidative stress profile which directly relates the onset and progression of microvascular complications *(Greene et al., 1999; Vincent et al., 2004b)*.

In the late 1990s, our group introduced the idea that glucose-mediated oxidative stress injures the

peripheral nervous system, leading to eventual death and loss of neurons and supporting Schwann cells *(Feldman et al., 1997; Russell et al., 1998; 1999; 2001)*. Investigation of the basic mechanisms underlying this process in DRG neurons identified multiple mechanisms by which hyperglycemia mediates DRG neuron injury. Mitochondrial overload is the principal site of reactive oxygen species (ROS) generation in hyperglycemia *(Vincent et al., 2005a)*. DRG neurons may be preserved *in vitro* in the face of hyperglycemic insult by uncoupling agents that relieve the mitochondrial overload *(Vincent et al., 2004a)* and by lipophilic antioxidants that protect the mitochondria against ROS injury *(Vincent et al., 2005a; 2005b)*.

Additional cellular mechanisms are activated by hyperglycemia to produce ROS. Hyperglycemia leads to the formation of advanced glycation end products (AGE). DRG neurons express the receptor for AGE and exposure to AGE leads to oxidative stress and injury in DRG neurons that is partially mediated through activation of the nicotinamide adenine dinucleotide phosphate [NAD(P)H] oxidase complex *(Vincent et al., 2007a)*. In addition, direct exposure to hyperglycemia leads to the activation of NAD(P)H oxidase *(Vincent et al., 2005a)*. This complex is formed through recruitment of a combination of a p22-phox subunit and five NOX subunits *(Baumer et al., 2008)*. Within neurons, NOX2 (or gp91-phox) and p22-phox are expressed within the cell membrane; p47-phox and p67-phox are recruited from the cytoplasm to the membrane when the neuron is exposed to an NAD(P)H oxidase activating stimulus *(Murdoch et al., 2006)*. NAD(P)H oxidase activity generates superoxide ($O_2^{\cdot -}$) that promotes mitochondrial dysfunction and apoptosis in the setting of inflammation, neurodegeneration and in atherosclerosis *(Stamler, 1996; Silver et al., 2007)*. Over time, these mechanisms act in concert with accumulating ROS-induced damage that impairs nerve function and results in the signs and symptoms of diabetic neuropathy *(Feldman et al., 1997; 2002; Greene et al., 1999; Vincent and Feldman, 2004; Vincent et al., 2004b; 2007b)*.

Pathophysiology of Diabetic Neuropathy: More Than Just Glucose

Until recently, we *(Feldman et al., 1997; 2002; Greene et al., 1999; Vincent et al., 2004b)* and other investigators *(Low et al., 1997; Brownlee, 2005; Osawa and Kato, 2005; Tomlinson and Gardiner, 2008)* in the field contended that hyperglycemia was the driving force underlying the development of diabetic neuropathy. Our opinions were based originally on results from The Diabetes Control and Complications Trial (DCCT). In the DCCT, type 1 diabetic subjects receiving intensive therapy with an average glycosylated hemoglobin (HbA1c) of 7.2% had a reduced 60% cumulative incidence of diabetic neuropathy when compared to patients receiving conventional treatment (average HbA1c of 9.0%) *(The Diabetes Control and Complications Trial Research Group, 1993)*. However, the continuing longitudinal study of the DCCT, the Epidemiology of Diabetes Complications and Interventions Cohort (EDIC) yielded unanticipated results 20 years later *(Genuth, 2006; Martin et al., 2006; Pop-Busui et al., 2009)*. Within 1 year of discontinuing the DCCT and beginning EDIC, the glycemic control in the two treatment groups equalized to an average HbA1c of 8% *(Genuth, 2006)*. All 1,300 patients were examined annually for diabetic neuropathy; one decade later, patients from the intensive-DCCT cohort had a lower incidence of diabetic neuropathy compared to patients from the conventional-DCCT cohort, despite 10 years of convergent glycemic control *(Martin et al., 2006)*. The underlying mechanism(s) of this result is not determined, but one interesting difference is the lipid profiles of the two groups: a subset of the intensive-DCCT cohort has less dyslipidemia than the conventional cohort *(Diabetes Control and Complications Trial Cohort, 1999; Lyons et al., 2004)*. This interesting, unanticipated finding is further supported by the Eurodiab Trial, a longitudinal study of over 3,000 individuals with type 1 diabetes *(Tesfaye et al., 2005; Tesfaye, 2007)*. Of the 1,200 subjects who did not have diabetic neuropathy at baseline, hypertension, serum lipids and body mass index were each independently associated with the risk of developing diabetic neuropathy during a 7-year follow-up period. Of these risk factors, dyslipidemia was closely linked with the onset and progression of diabetic neuropathy [reviewed in *(Leiter, 2005)*]. In support of these findings, we recently evaluated the mechanisms underlying diabetic neuropathy progression using indexes of sural nerve morphometry obtained from two identical randomized, placebo-controlled clinical trials *(Wiggin et al., 2009)*. Sural nerve myelinated fiber density, nerve conduction velocities, vibration perception thresholds, clinical symptom scores, and a visual analog scale for pain were analyzed in participants with mild-to-moderate diabetic neuropathy. A loss of $\geq$500 fibers/mm^2 in sural nerve myelinated fiber density over 52 weeks was defined as progressing diabetic neuropathy, and a myelinated fiber density loss of $\leq$100 fibers/mm^2 during the same time interval as nonprogressing diabetic neuropathy. In this cohort of participants, elevated triglycerides were the only clinical parameter

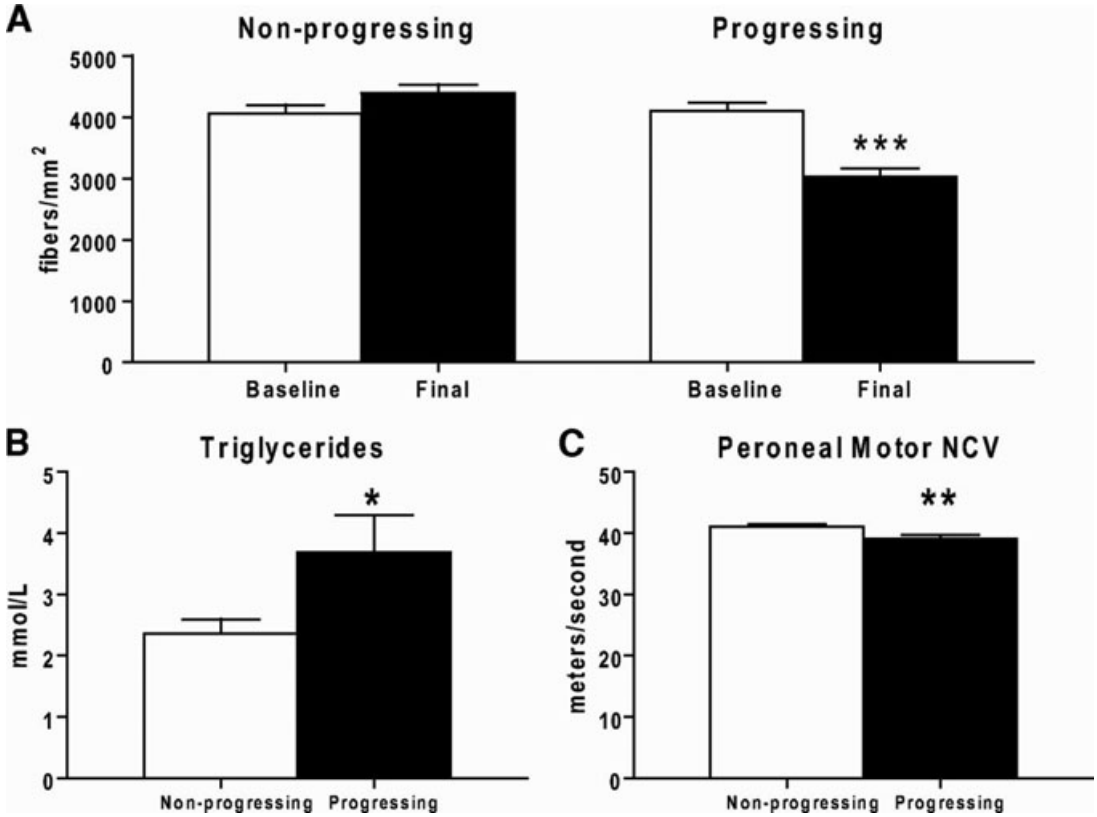


Figure 1. Myelinated fiber density (MFD) of the rapidly progressing and nonprogressing diabetic patients. (A) The nonprogressing dataset shows no change in MFD (fibers/mm^2) over 52 weeks, while the progressing dataset shows a highly significant decrease in MFD. Baseline measurements of triglyceride levels (B) and peroneal motor nerve conduction velocity (C) are significantly different between the progressing and nonprogressing participants. *$p < 0.05$; **$p < 0.01$; ***$p < 0.0001$ (reproduced from *Wiggin et al., 2009*).

that correlated with a loss of myelinated fiber density, independent of disease duration, age, diabetes control, or other variables *(Wiggin et al., 2009)*. The nerve fiber densities, triglycerides, and motor nerve conduction velocities for the two groups are presented in Fig. 1.

Dyslipidemia and Neuropathy: An Expanding Problem

The emerging idea that dyslipidemia contributes to the development of diabetic neuropathy may explain the earlier incidence of diabetic neuropathy in individuals with type 2 compared to type 1 diabetes. Lipid profiles are commonly abnormal early in the course of type 2 diabetes in a temporal pattern that correlates with the presence of diabetic neuropathy *[Diabetes Atorvastin Lipid Intervention (DALI) Study Group, 2001; Clemens et al., 2004]*. In contrast, lipid profiles are nearly always normal in type 1 patients at the time of diabetes diagnosis *(Leiter, 2005)*. Dyslipidemia develops later in the course of type 1 diabetes, and these abnormal lipid profiles coincide with the delayed onset and progression of diabetic neuropathy *(Young et al., 1993; Kempler et al., 2002)*. Accumulating data from several large scale trials of patients with type 2 diabetes also point to early dyslipidemia as a major independent risk factor for the development of diabetic neuropathy [reviewed in *(Cameron et al., 2003; Leiter, 2005; Gordon and Robinson, 2006)*]. In the United Kingdom Prospective Diabetes Study (UKPDS), 3,867 newly diagnosed type 2 patients were randomized into either intensive treatment with an oral hypoglycemic agent or insulin or conventional treatment with diet. After 10 years, intensive treatment resulted in approximately 1% lower HbA1c vs. conventional treatment but there was no difference in the development of diabetic neuropathy between the two groups, which had similar lipid and blood pressure profiles *[UK Prospective Diabetes Study (UKPDS) Group, 1998]*. This finding, at first unexpected in light of the earlier DCCT data, was supported by the VA Cooperative Study, which demonstrated no difference in the prevalence of diabetic neuropathy in type 2 diabetic patients over a 2-year period comparing standard and intensive

glycemic control *(Azad et al., 1999)*. These results suggested that independent factors other than glycemic control are critical to the development of diabetic neuropathy *(Leiter, 2005)*.

As with EDIC and Eurodiab, analysis of the UKPDS and VA cooperative data points to dyslipidemia as a critical independent factor for the development of diabetic neuropathy *(Leiter, 2005)*. Type 2 diabetes clusters with risk factors for coronary heart disease including obesity, hypertension, and dyslipidemia; individuals with two or more of these factors are diagnosed with the metabolic syndrome *(Fonseca, 2005; Grundy, 2005; Bonora, 2006; Zimmet and Alberti, 2008)*. In a cross-sectional study of 548 type 2 diabetic subjects, those with metabolic syndrome were twice as likely to have diabetic neuropathy *(Isomaa et al., 2001)*, and the driving factor was dyslipidemia. In a European study of 85 type 2 diabetic patients with at least two additional metabolic syndrome parameters, the prevalence of microvascular complications, including diabetic neuropathy, increased with each additional parameter present *(Isomaa et al., 2001)*; abnormalities in (low-density lipoprotein) LDL profiles were more closely related to diabetic neuropathy than hyperglycemia. Finally, prospective studies of patients with idiopathic neuropathy, including our own recently published work, confirm a higher prevalence of hyperlipidemia than impaired glucose tolerance or hypertension, suggesting that dyslipidemia is an essential factor underlying nerve injury *(Gordon and Robinson, 2006; Wiggin et al., 2009)*. Collectively, this evolving and exciting literature links dyslipidemia to the development and progression of diabetic neuropathy. Fig. 2 outlines our current understanding of the factors that contribute to the development of diabetic neuropathy.

Lipid Modification in Diabetes

We have now employed cell culture and mouse models of diabetic neuropathy and suggest that oxLDLs are one notable 'lipid factor' responsible for nervous system injury *(Vincent et al., 2009)*. LDL is the primary carrier of cholesterol *(Hammer et al., 1995)* and vitamin E *(Heinecke, 1987)* within the plasma. Systemic oxidative stress results in the modification of these lipoproteins, which is well characterized in atherosclerosis *(Willems et al., 1998; Tsuzura et al., 2004)*. LDLs spontaneously oxidize in the presence of ROS such as superoxide ($O_2^{\cdot -}$) to form oxLDLs *(Hammer et al., 1995)*. Cholesterol carrying LDLs is more prone to oxidation than smaller, high-density particles *(Krentz, 2003)*.

oxLDLs are critically involved in endothelial cell dysfunction, evident from the large body of literature implicating oxLDLs in atherosclerotic lesion formation *(Li and Mehta, 2005; Ceriello, 2006; Genuth, 2006)*. oxLDL is strongly cytotoxic, which may explain the areas of necrosis detected within atherosclerotic lesions *(Tsuzura et al., 2004; Li and Mehta, 2005; Pennathur and Heinecke, 2007; Thum and Borlak, 2008)*. In man, oxLDL is a highly analytic marker for macrovascular disease, including stroke and myocardial infarction *(Tsimikas et al., 2005)*. In patients with types 1 and 2 diabetes, serum levels of oxLDL in proportion to total LDL particles are associated with diabetic neuropathy *(Willems et al., 1998; Tsuzura et al., 2004)*. Fig. 3 illustrates our findings that oxLDLs increase significantly in mice on a high-fat diet. These oxLDLs can be found in the DRG and the mice develop early signs and symptoms of diabetic neuropathy *(Vincent et al., 2009)*.

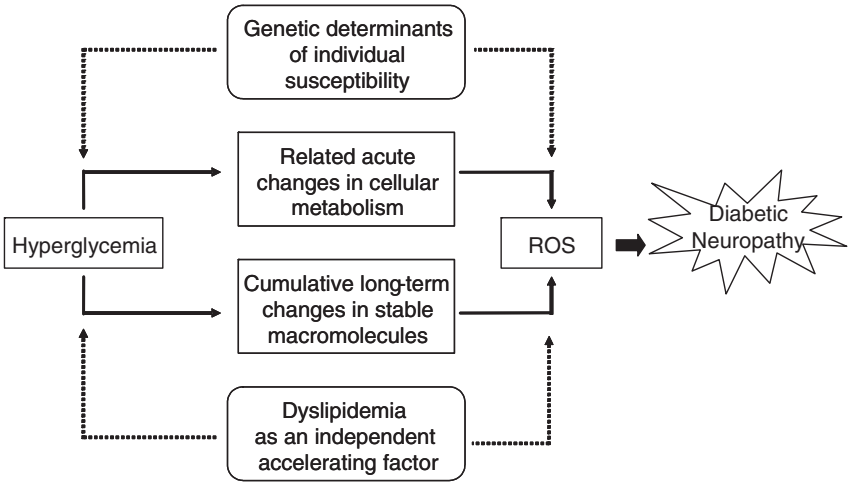
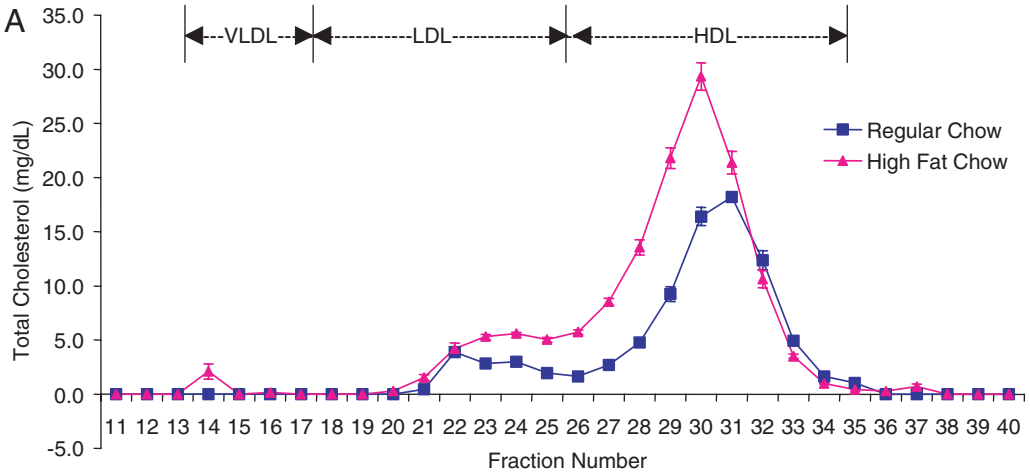


Figure 2. Hyperglycemia and hyperlipidemia contribute to the pathogenesis of diabetic neuropathy. Adapted from *Brownlee, 2005*.



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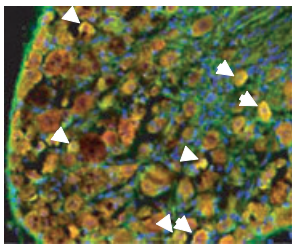
LDL Oxidation	Control	High Fat
HODE (pmol/mg protein)	116±14	193±10
Dityrosine (μmol/mol tyrosine)	23±2	546±91
Nitrotyrosine	90±11	144±57



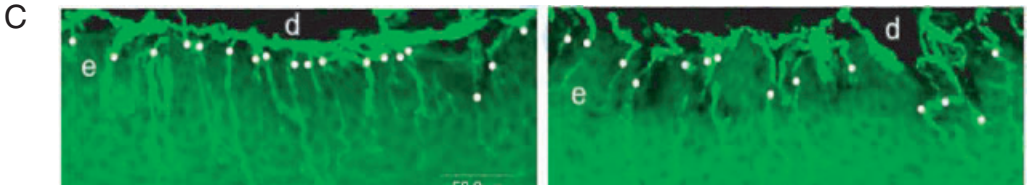


Figure 3. Mice on a high-fat diet increase oxLDL and develop neuropathy. (A) Pooled plasma samples (2 pools/group, each pool analyzed three times) were subjected to fractionation by FPLC, then cholesterol (A) was measured in each fraction. The graph shows the mean and standard error of the mean for n = 2 pools/group. (B) Oxidative stress measures in the low-density lipoprotein (LDL) fraction by reverse phase high performance liquid chromatography. HODE, dityrosine, and nitrotyrosine were all significantly increased ($p < 0.05$). (C) Representative intraepidermal nerve fiber density images from one control and one high-fat fed mouse footpad. Bar = 50 μm; d = dermis, e = epidermis. White dots indicate nerve fibers counted. (D) In a different mouse study using obese db/db mice, we immunostained the dorsal root ganglia for ApoB and MDA-oxidized LDL. We observed colocalization of MDA-LDL in green and ApoB in red (yielding a yellow signal) around the neurons (arrows) (A-C reproduced from *Vincent et al., 2009*).

oxLDLs Mediate Cellular Injury via the Scavenger Receptor, LOX-1

oxLDLs cause apoptotic injury and death in both endothelial cells *(Dimmeler et al., 1997)* and neurons *(Papassotiropoulos et al., 1996; Draczynska-Lusiak et al., 1998a; 1998b; Keller et al., 1999; 2000; Schroeter et al., 2000)*. In endothelial cells, oxLDLs induce multiple events associated with apoptotic injury, including Bid degradation, cytochrome *c* release, and caspase-3 activation *(Vindis et al., 2005)*. oxLDLs are associated with increased proapoptotic Bax and decreased levels of the anti-apoptotic type I IGF receptor (IGF-IR) in smooth muscle cells *(Higashi et al., 2005)*. Both death receptor and mitochondrial pathways are involved in atherosclerotic-plaque associated apoptosis induced by oxLDLs *(Napoli, 2003)*. In neurons, oxLDLs induce DNA fragmentation characteristic of apoptosis in DRG *(Papassotiropoulos et al., 1996; Vincent et al., 2009)*, striatal neurons *(Draczynska-Lusiak et al., 1998b; Schroeter et al., 2000)*, and PC-12 cells *(Draczynska-Lusiak et al., 1998a)*. In motor neurons, oxLDLs increase ROS and activate a caspase-3-dependent death mechanism *(Keller et al., 2000)*. Specific effects of oxLDL on mitochondria and mitochondrial-mediated apoptotic events in neurons remain unknown.

oxLDLs exert effects on cells through two primary cell surface receptors, lectin-like oxidized LDL receptor-1 (LOX-1) on endothelial cells *(Chen et al., 2006)* and CD36 on macrophages *(Yamashita et al., 2007)*. Upon receptor-mediated uptake of oxLDL into

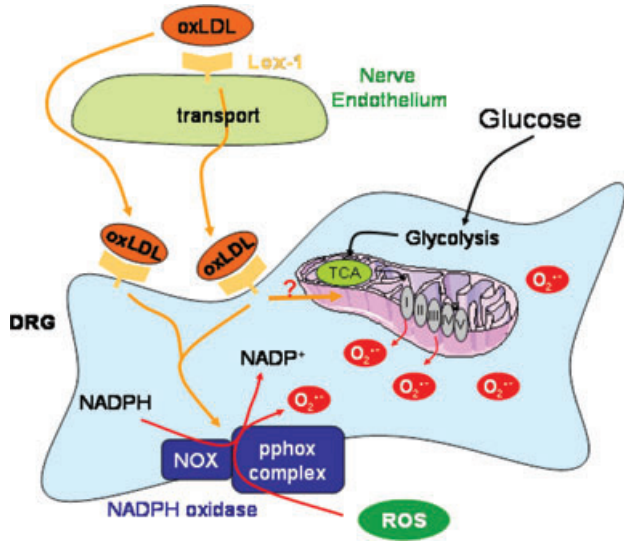


Figure 4. Schematic of effects of oxLDL binding to LOX-1. LOX-1 on both vascular endothelial cells and dorsal root ganglia neurons will bind oxLDL. Subsequently, the oxLDL may be endocytosed or transcytosed. Receptor binding initiates a signaling pathway leading to the activation of NAD(P)H oxidase and may also alter mitochondrial generation of reactive oxygen species. Glucose independently affects these same cellular targets.

endothelial cells, oxLDL is transported through the endothelial cell and extruded into the subendothelial region within tissues *(Li and Mehta, 2005)*. A schematic showing the expression of LOX-1 and the potential effects of oxLDL binding is shown in Fig. 4. LOX-1 expression is upregulated by oxLDL, which in turn increases intracellular $O_2^{\cdot -}$ production *(Hu et al., 2003)*. Shear stress, tumor necrosis factor-α, and free radicals all increase LOX-1 expression levels *(Hu et al., 2003)*, while lipid lowering drugs decrease expression *(Draude et al., 1999)*. Hyperglycemia, AGE, and C-reactive protein also increase LOX-1 expression *(Iwashima et al., 2000; Schalkwijk and Stehouwer, 2005; Rudijanto, 2007)*. Indeed, glucose stimulates LOX-1 expression in both endothelial cells and macrophages, prevented by antioxidants and inhibitors of MAPK and NF-κB *(Li et al., 2003; 2004; Dandapat et al., 2007)*. LOX-1 is upregulated in renal tubules in obese, diabetic rats and its activation leads to inflammation and nephropathy *(Ueno et al., 2003; Dominguez et al., 2008; Kelly et al., 2008)*. Finally, LOX-1 is also expressed on neurons, and polymorphisms in the LOX-1 gene are associated with neurodegenerative disease in humans *(Papassotiropoulos et al., 2005)*. We examined LOX-1 expression in DRG neurons, and found basal expression that was further increased by exposure to oxLDL *(Vincent et al., 2009)*. Selected data are presented in Fig. 5. Subsequent to LOX-1 activation, the DRG neurons rapidly activated NAD(P)H oxidase, increased superoxide generation, and activated a programmed cell death mechanism. DRG neuron injury in the presence of oxLDL was prevented by a LOX-1 blocking antibody, the NAD(P)H oxidase inhibitor apocyanin, or the antioxidant a-lipoic acid *(Vincent et al., 2009)*.

These data suggest that, in diabetes, neurons are exposed to both glucose and oxLDL which independently increase ROS, and glucose may sensitize neurons to oxLDL-mediated damage via upregulation of LOX-1. Interestingly, oxLDL decreases native LDL-receptor expression in a LOX-1-dependent manner *(Hu et al., 2003)*. Given the critical role for native LDL receptors in neuronal functioning, synapse maintenance, and myelination following injury *(Herz and Bock, 2002)*, oxLDL could also predispose neurons to glucose-mediated injury by decreasing native LDL receptor. These ideas await further exploration.

Lipids and Diabetic Neuropathy

If the idea that dyslipidemia contributes to the development of diabetic neuropathy is true *(McManis et al., 1994)*, lipid lowering drugs may be beneficial in the treatment of diabetic neuropathy. Fenofibrate is a PPARa agonist that lowers plasma lipids by improving their removal by the liver and improving fatty acid metabolism *(Harano et al., 2006; Aasum et al., 2008)*. In genetic dyslipidemia in mice, including ApoE knockout, leptin deficient, and LDL receptor knockout mice, fenofibrate improves the lipid profile and increases high-density lipoprotein *(Lie et al., 2005; Kooistra et al., 2006; Srivastava et al., 2006)*. These lipid improvements correlate with prevention of insulin resistance and atherosclerosis *(Calkin et al., 2007; Xie et al., 2007; Aasum et al., 2008)*. Interest in this drug treatment has expanded with the demonstration that fenofibrate dramatically improves hyperglycemia, insulin resistance, albuminuria, and glomerular lesions in db/db mice *(Park et al., 2006)*. The FIELD trial demonstrated that fenofibrate improves signs and progression of retinopathy and nephropathy *(FIELD Study, 2007; Keech et al., 2007; Simo and Hernandez, 2007; Davis et al., 2008; Firth, 2008)*. The Fremantle Diabetes Study was an observational investigation of 1,237 patients with type 2 diabetes. The data suggest that therapy with a statin or fibrate protects against diabetic peripheral sensory neuropathy, but calls for confirmatory evidence via a randomized clinical trial *(Davis et al., 2008)*. Fenofibrate may also provide neuroprotection against stroke *(Deplanque et al., 2003)*. We recently demonstrated the potent ability of fenofibrate to prevent hyperglycemia-induced DRG neuron injury *in vitro* by decreasing mitochondrial

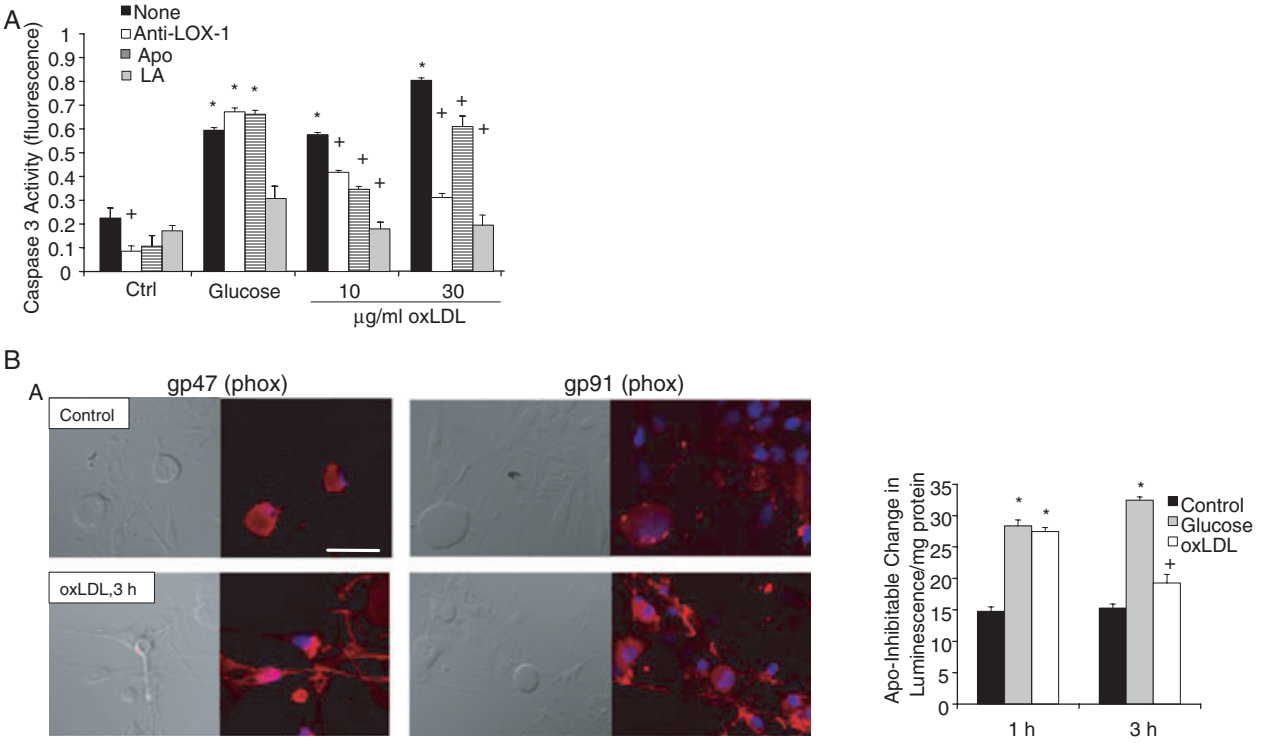


Figure 5. High glucose and oxLDL cause cell death in dorsal root ganglia (DRG) neurons via NAD(P)H oxidase. Adult DRG neurons were exposed to high glucose (25.7 mM) or increasing concentrations of oxLDL and then cell death was quantitated by caspase 3 activation after 5 h. DRG neurons were additionally pretreated with LOX-1 neutralizing antibody (Anti-LOX-1, 100 mg/ml), apocyanin (Apo, 1 mM), or a-lipoic acid (LA, 100 mM). n = 9, *p < 0.01 compared to untreated control, +p < 0.01 compared to no pretreatment (None). (B) Adult DRG neurons were exposed to 30 mg/ml oxLDL and then immunolabeled for NAD(P)H oxidase subunits p47 or gp91. In (B), adult DRG neurons were exposed to high glucose (25.7 mM) or oxLDL (30 mg/ml) for 1 h or 3 h, then lysed for biochemical assays of NAD(P)H oxidase. *p < 0.01 compared to untreated control, +p < 0.05 compared to untreated control (reproduced from *Vincent et al., 2009*).

$O_2^{\cdot -}$ generation *(Vincent and Feldman, 2008)*. We are following this study with an intervention in type 1 diabetic mice and have demonstrated that 0.1% w/w fenofibrate chow significantly decreases total cholesterol and LDL triglycerides (unpublished data).

Future Directions

We maintain our stand that hyperglycemia is also a key mediator of DRG neuron injury particularly in poorly controlled diabetes. Therefore, compounds that improve glycemic control will assist in the prevention of complications. Metformin, a biguanide compound, improves insulin resistance by reducing gluconeogenesis and enhancing peripheral glucose uptake, promoting reduction of the plasma glucose level *(Yoon et al., 2007)*. Metformin remains as one of the most used glucose regulating drugs in type 2 diabetes *(Saenz et al., 2005)* and is used in preclinical trials in mice *(Yoon et al., 2007; Algire et al., 2008)*. Interestingly, dyslipidemia increases in adolescent type 1 diabetic patients with poor glycemic control, again highlighting the complex interplay between glycemia and dyslipidemia *(Shamir et al., 2008)*. Taken together, the data indicate that strategic use of NAD(P)H oxidase inhibition, antioxidants, anti-LOX-1 therapy, anti-hyperglycemia, and lipid lowering therapies will prevent diabetic neuropathy. Each of these components has been tested in rodents with positive results *(Cotter and Cameron, 2003; Park et al., 2006; Dominguez et al., 2008)*. Individually, these strategies have not produced significant results in clinical trials, with the exception of a-lipoic acid *(Ziegler et al., 2006)*. Current investigations are focusing on metabolic deficits in the axon, particularly at the mitochondria *(Figueroa-Romero et al., 2008; Wiggin et al., 2008; Edwards et al., 2009)*. Further drug refinement and subcellular targeting may be the key to improved efficacy against neuronal injury.

Acknowledgements

The Feldman Laboratory is supported by the Juvenile Diabetes Research Foundation (A.M.V.,

E.L.F., R.P.B.), the American Diabetes Association (A.M.V., E.L.F., R.P.B.), the Animal Models of Diabetes Complications Consortium (NIH UO1 DK076160, E.L.F.), the Program for Neurology Research and Discovery, and the A. Alfred Taubman Medical Institute.

References

Aasum E, Khalid AM, Gudbrandsen OA, How OJ, Berge RK, Larsen TS (2008). Fenofibrate modulates cardiac and hepatic metabolism and increases ischemic tolerance in diet-induced obese mice. J Mol Cell Cardiol 44:201–209.

Algire C, Zakikhani M, Blouin MJ, Shuai JH, Pollak M (2008). Metformin attenuates the stimulatory effect of a high-energy diet on in vivo LLC1 carcinoma growth. Endocr Relat Cancer 15:833–839.

Azad N, Emanuele NV, Abraira C, Henderson WG, Colwell J, Levin SR, Nuttall FQ, Comstock JP, Sawin CT, Silbert C, Rubino FA (1999). The effects of intensive glycemic control on neuropathy in the VA cooperative study on type II diabetes mellitus (VA CSDM). J Diabetes Complicat 13:307–313.

Barrett AM, Lucero MA, Le T, Robinson RL, Dworkin RH, Chappell AS (2007). Epidemiology, public health burden, and treatment of diabetic peripheral neuropathic pain: a review. Pain Med 8:S50–S62.

Baumer AT, Ten Freyhaus H, Sauer H, Wartenberg M, Kappert K, Schnabel P, Konkol C, Hescheler J, Vantler M, Rosenkranz S (2008). Phosphatidylinositol 3-kinase-dependent membrane recruitment of Rac-1 and p47phox is critical for alpha-platelet-derived growth factor receptor-induced production of reactive oxygen species. J Biol Chem 283:7864–7876.

Bonora E (2006). The metabolic syndrome and cardiovascular disease. Ann Med 38:64–80.

Brownlee M (2005). The pathobiology of diabetic complications: a unifying mechanism. Diabetes 54:1615–1625.

Calkin AC, Jandeleit-Dahm KA, Sebekova E, Allen TJ, Mizrahi J, Cooper ME, Tikellis C (2007). PPARs and diabetes-associated atherosclerosis. Curr Pharm Des 13:2736–2741.

Cameron N, Cotter M, Inkster M, Nangle M (2003). Looking to the future: diabetic neuropathy and effects of rosuvastatin on neurovascular function in diabetes models. Diabetes Res Clin Pract 61:S35–S39.

Ceriello A (2006). Oxidative stress and diabetes-associated complications. Endocr Pract 12(Suppl 1):60–62.

Chen J, Liu Y, Liu H, Hermonat PL, Mehta JL (2006). Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) transcriptional regulation by Oct-1 in human endothelial cells: implications for atherosclerosis. Biochem J 393:255–265.

Clemens A, Siegel E, Gallwitz B (2004). Global risk management in type 2 diabetes: blood glucose, blood pressure, and lipids – update on the background of the current guidelines. Exp Clin Endocrinol Diabetes 112:493–503.

Cotter MA, Cameron NE (2003). Effect of the NAD(P)H oxidase inhibitor, apocynin, on peripheral nerve perfusion and function in diabetic rats. Life Sci 73:1813–1824.

Dandapat A, Hu C, Sun L, Mehta JL (2007). Small concentrations of oxLDL induce capillary tube formation from endothelial cells via LOX-1-dependent redox-sensitive pathway. Arterioscler Thromb Vasc Biol 27:2435–2442.

Davis TM, Yeap BB, Davis WA, Bruce DG (2008). Lipid-lowering therapy and peripheral sensory neuropathy in type 2 diabetes: the Fremantle Diabetes Study. Diabetologia 51:562–566.

Deplanque D, Gele P, Petrault O, Six I, Furman C, Bouly M, Nion S, Dupuis B, Leys D, Fruchart JC, Cecchelli R, Staels B, Duriez P, Bordet R (2003). Peroxisome proliferator-activated receptor-alpha activation as a mechanism of preventive neuroprotection induced by chronic fenofibrate treatment. J Neurosci 23:6264–6271.

Diabetes Atorvastin Lipid Intervention (DALI) Study Group (2001). The effect of aggressive versus standard lipid lowering by atorvastatin on diabetic dyslipidemia: the DALI study: a double-blind, randomized, placebo-controlled trial in patients with type 2 diabetes and diabetic dyslipidemia. Diabetes Care 24:1335–1341.

Diabetes Control and Complications Trial Cohort (1999). Epidemiology of Diabetes Interventions and Complications (EDIC). Design, implementation, and preliminary results of a long-term follow-up of the Diabetes Control and Complications Trial cohort. Diabetes Care 22:99–111.

Dimmeler S, Haendeler J, Galle J, Zeiher AM (1997). Oxidized low-density lipoprotein induces apoptosis of human endothelial cells by activation of CPP32-like proteases. A mechanistic clue to the response to injury hypothesis. Circulation 95:1760–1763.

Dominguez JH, Mehta JL, Li D, Wu P, Kelly KJ, Packer CS, Temm C, Goss E, Cheng L, Zhang S, Patterson CE, Hawes JW, Peterson R (2008). Anti-LOX-1 therapy in rats with diabetes and dyslipidemia: ablation of renal vascular and epithelial manifestations. Am J Physiol Renal Physiol 294:F110–F119.

Draczynska-Lusiak B, Chen YM, Sun AY (1998a). Oxidized lipoproteins activate NF-kappaB binding activity and apoptosis in PC12 cells. Neuroreport 9:527–532.

Draczynska-Lusiak B, Doung A, Sun AY (1998b). Oxidized lipoproteins may play a role in neuronal cell death in Alzheimer disease. Mol Chem Neuropathol 33:139–148.

Draude G, Hrboticky N, Lorenz RL (1999). The expression of the lectin-like oxidized low-density lipoprotein receptor (LOX-1) on human vascular smooth muscle cells and monocytes and its down-regulation by lovastatin. Biochem Pharmacol 57:383–386.

Edwards JL, Quattrini A, Lentz SI, Figueroa-Romero C, Cerri F, Backus C, Hong Y, Feldman EL (2009). Diabetes regulates mitochondrial biogenesis and fission in mouse neurons. Diabetologia Oct 22 [epub ahead of print].

Edwards JL, Vincent AM, Cheng HT, Feldman EL (2008). Diabetic neuropathy: mechanisms to management. Pharmacol Ther 120:1–34.

Feldman EL, Stevens MJ, Greene DA (1997). Pathogenesis of diabetic neuropathy. Clin Neurosci 4:365–370.

Feldman EL, Stevens MJ, Russell JW (2002). Diabetic peripheral and autonomic neuropathy. In: Contemporary Endocrinology: Type 1 Diabetes: Etiology and Treatment, Sperling MA, ed., Humana Press, Totowa, NJ pp. 437–461.

FIELD Study (2007). Reducing ocular damage in type 2 diabetes: the FIELD study shows fenofibrate benefits. Cardiovasc J S Afr 18:400.

Figueroa-Romero C, Sadidi M, Feldman EL (2008). Mechanisms of disease: the oxidative stress theory of diabetic neuropathy. Rev Endocr Metab Disord 9:301–314.

Firth J (2008). Fenofibrate and diabetic retinopathy. Lancet 371:722; author reply 722.

Fonseca VA (2005). The metabolic syndrome, hyperlipidemia, and insulin resistance. Clin Cornerstone 7:61–72.

Genuth S (2006). Insights from the diabetes control and complications trial/epidemiology of diabetes interventions and complications study on the use of intensive glycemic treatment to reduce the risk of complications of type 1 diabetes. Endocr Pract 12(Suppl 1):34–41.

Giugliano D, Ceriello A, Esposito K (2008). Glucose metabolism and hyperglycemia. Am J Clin Nutr 87:217S–222S.

Gordon SA, Robinson SJ (2006). Idiopathic neuropathy, prediabetes and the metabolic syndrome. J Neurol Sci 242:9–14.

Greene DA, Stevens MJ, Obrosova I, Feldman EL (1999). Glucose-induced oxidative stress and programmed cell death in diabetic neuropathy. Eur J Pharmacol 375:217–223.

Grundy SM (2005). A constellation of complications: the metabolic syndrome. Clin Cornerstone 7:36–45.

Hammer A, Kager G, Dohr G, Rabl H, Ghassempur I, Jurgens G (1995). Generation, characterization, and histochemical application of monoclonal antibodies selectively recognizing oxidatively modified apoB-containing serum lipoproteins. Arterioscler Thromb Vasc Biol 15:704–713.

Harano Y, Yasui K, Toyama T, Nakajima T, Mitsuyoshi H, Mimani M, Hirasawa T, Itoh Y, Okanoue T (2006). Fenofibrate, a peroxisome proliferator-activated receptor alpha agonist, reduces hepatic steatosis and lipid peroxidation in fatty liver Shionogi mice with hereditary fatty liver. Liver Int 26:613–620.

Heinecke JW (1987). Free radical modification of low-density lipoprotein: mechanisms and biological consequences. Free Radic Biol Med 3:65–73.

Herz J, Bock HH (2002). Lipoprotein receptors in the nervous system. Annu Rev Biochem 71:405–434.

Higashi Y, Peng T, Du J, Sukhanov S, Li Y, Itabe H, Parthasarathy S, Delafontaine P (2005). A redox-sensitive pathway mediates oxidized LDL-induced downregulation of insulin-like growth factor-1 receptor. J Lipid Res 46:1266–1277.

Hu B, Li D, Sawamura T, Mehta JL (2003). Oxidized LDL through LOX-1 modulates LDL-receptor expression in human coronary artery endothelial cells. Biochem Biophys Res Commun 307:1008–1012.

Isomaa B, Henricsson M, Almgren P, Tuomi T, Taskinen MR, Groop L (2001). The metabolic syndrome influences the risk of chronic complications in patients with type II diabetes. Diabetologia 44:1148–1154.

Iwashima Y, Eto M, Hata A, Kaku K, Horiuchi S, Ushikubi F, Sano H (2000). Advanced glycation end products-induced gene expression of scavenger receptors in cultured human monocyte-derived macrophages. Biochem Biophys Res Commun 277:368–380.

Keech AC, Mitchell P, Summanen PA, O'Day J, Davis TM, Moffitt MS, Taskinen MR, Simes RJ, Tse D, Williamson E, Merrifield A, Laatikainen LT, d'Emden MC, Crimet DC, O'Connell RL, Colman PG (2007). Effect of fenofibrate on the need for laser treatment for diabetic retinopathy (FIELD study): a randomised controlled trial. Lancet 370:1687–1697.

Keller JN, Hanni KB, Kindy MS (2000). Oxidized high-density lipoprotein induces neuron death. Exp Neurol 161:621–630.

Keller JN, Hanni KB, Pedersen WA, Cashman NR, Mattson MP, Gabbita SP, Friebe V, Markesbery WR (1999). Opposing actions of native and oxidized lipoprotein on motor neuron-like cells. Exp Neurol 157:202–210.

Kelly KJ, Wu P, Patterson CE, Temm C, Dominguez JH (2008). LOX-1 and inflammation: a new mechanism for renal injury in obesity and diabetes. Am J Physiol Renal Physiol 294:F1136–F1145.

Kempler P, Tesfaye S, Chaturvedi N, Stevens LK, Webb DJ, Eaton S, Kerenyi Z, Tamas G, Ward JD, Fuller JH (2002). Autonomic neuropathy is associated with increased cardiovascular risk factors: the EURODIAB IDDM complications study. Diabet Med 19:900–909.

Kles KA, Vinik AI (2006). Pathophysiology and treatment of diabetic peripheral neuropathy: the case for diabetic neurovascular function as an essential component. Curr Diab Rev 2:131–145.

Kooistra T, Verschuren L, de Vries-van der Weij J, Koenig W, Toet K, Princen HM, Kleemann R (2006). Fenofibrate reduces atherogenesis in ApoE*3Leiden mice: evidence for multiple antiatherogenic effects besides lowering plasma cholesterol. Arterioscler Thromb Vasc Biol 26:2322–2330.

Krentz AJ (2003). Lipoprotein abnormalities and their consequences for patients with type 2 diabetes. Diabetes Obes Metab 5(Suppl 1):S19–S27.

Leiter LA (2005). The prevention of diabetic microvascular complications of diabetes: is there a role for lipid lowering? Diabetes Res Clin Pract 68(Suppl 2):S3–S14.

Li D, Mehta JL (2005). Oxidized LDL, a critical factor in atherogenesis. Cardiovasc Res 68:353–354.

Li L, Sawamura T, Renier G (2003). Glucose enhances endothelial LOX-1 expression: role for LOX-1 in glucose-induced human monocyte adhesion to endothelium. Diabetes 52:1843–1850.

Li L, Sawamura T, Renier G (2004). Glucose enhances human macrophage LOX-1 expression: role for LOX-1 in glucose-induced macrophage foam cell formation. Circ Res 94:892–901.

Lie J, Lankhuizen IM, Gross B, van Gent T, van Haperen R, Scheek L, Staels B, de Crom R, van Tol A (2005). Fenofibrate reverses the decline in HDL cholesterol in mice overexpressing human phospholipid transfer protein. Biochim Biophys Acta 1738:48–53.

Low PA, Nickander KK, Tritschler HJ (1997). The roles of oxidative stress and antioxidant treatment in experimental diabetic neuropathy. Diabetes 46(Suppl 2):S38–S42.

Lyons TJ, Jenkins AJ, Zheng D, Lackland DT, McGee D, Garvey WT, Klein RL (2004). Diabetic retinopathy and serum lipoprotein subclasses in the DCCT/EDIC cohort. Invest Ophthalmol Vis Sci 45:910–918.

Martin CL, Albers J, Herman WH, Cleary P, Waberski B, Greene DA, Stevens MJ, Feldman EL (2006). Neuropathy among the diabetes control and complications trial cohort 8 years after trial completion. Diabetes Care 29:340–344.

McManis PG, Windebank AJ, Kiziltan M (1994). Neuropathy associated with hyperlipidemia. Neurology 44:2185–2186.

Murdoch CE, Grieve DJ, Cave AC, Looi YH, Shah AM (2006). NADPH oxidase and heart failure. Curr Opin Pharmacol 6:148–153.

Napoli C (2003). Oxidation of LDL, atherogenesis, and apoptosis. Ann N Y Acad Sci 1010:698–709.

Osawa T, Kato Y (2005). Protective role of antioxidative food factors in oxidative stress caused by hyperglycemia. Ann N Y Acad Sci 1043:440–451.

Papassotiropoulos A, Ludwig M, Naib-Majani W, Rao GS (1996). Induction of apoptosis and secondary necrosis in rat dorsal root ganglion cell cultures by oxidized low density lipoprotein. Neurosci Lett 209:33–36.

Papassotiropoulos A, Wollmer MA, Tsolaki M, Brunner F, Molyva D, Lutjohann D, Nitsch RM, Hock C (2005). A cluster of cholesterol-related genes confers susceptibility for Alzheimer's disease. J Clin Psychiatry 66:940–947.

Park CW, Zhang Y, Zhang X, Wu J, Chen L, Cha DR, Su D, Hwang MT, Fan X, Davis L, Striker G, Zheng F, Breyer M, Guan Y (2006). PPARalpha agonist fenofibrate improves diabetic nephropathy in db/db mice. Kidney Int 69:1511–1517.

Pennathur S, Heinecke JW (2007). Oxidative stress and endothelial dysfunction in vascular disease. Curr Diab Rep 7:257–264.

Pop-Busui R, Low PA, Waberski BH, Martin CL, Albers JW, Feldman EL, Sommer C, Cleary PA, Lachin JM, Herman WH (2009). Effects of prior intensive insulin therapy on cardiac autonomic nervous system function in type 1 diabetes mellitus: the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications study (DCCT/EDIC). Circulation 119:2886–2893.

Rudijanto A (2007). The expression and down stream effect of lectin like-oxidized low density lipoprotein 1 (LOX-1) in hyperglycemic state. Acta Med Indones 39:36–43.

Russell JW, Berent-Spillson A, Vincent AM, Freimann CL, Sullivan KA, Feldman EL (2008). Oxidative injury and neuropathy in diabetes and impaired glucose tolerance. Neurobiol Dis 30:420–429.

Russell JW, Gong C, Vincent A, Berent AR, Mentzer LE, Brownlee M (2001). Nitric oxide (NO) and mitochondrial manganese superoxide dismutase (MnSOD) regulate glucose-induced oxidative stress and programmed cell death in neurons. Neurology 56:A394.

Russell JW, Sullivan KA, Windebank AJ, Herrmann DN, Feldman EL (1999). Neurons undergo apoptosis in animal and cell culture models of diabetes. Neurobiol Dis 6:347–363.

Russell JW, van Golen C, Feldman EL (1998). IGF-I prevents glucose mediated loss of mitochondrial membrane integrity and programmed cell death in models of diabetic neuropathy. Ann Neurol 44:439.

Saenz A, Fernandez-Esteban I, Mataix A, Ausejo M, Roque M, Moher D (2005). Metformin monotherapy for type 2 diabetes mellitus. Cochrane Database Syst Rev 3:CD002966.

Schalkwijk CG, Stehouwer CD (2005). Vascular complications in diabetes mellitus: the role of endothelial dysfunction. Clin Sci (Lond) 109:143–159.

Schroeter H, Williams RJ, Matin R, Iversen L, Rice-Evans CA (2000). Phenolic antioxidants attenuate neuronal cell death following uptake of oxidized low-density lipoprotein. Free Radic Biol Med 29:1222–1233.

Shamir R, Kassis H, Kaplan M, Naveh T, Shehadeh N (2008). Glycemic control in adolescents with type 1 diabetes mellitus improves lipid serum levels and oxidative stress.Pediatr Diabetes 9:104–109.

Silver AE, Beske SD, Christou DD, Donato AJ, Moreau KL, Eskurza I, Gates PE, Seals DR (2007). Overweight and obese humans demonstrate increased vascular endothelial NAD(P)H oxidase-p47(phox) expression and evidence of endothelial oxidative stress. Circulation 115:627–637.

Simo R, Hernandez C (2007). Fenofibrate for diabetic retinopathy. Lancet 370:1667–1668.

Srivastava RA, Jahagirdar R, Azhar S, Sharma S, Bisgaier CL (2006). Peroxisome proliferator-activated receptor-alpha selective ligand reduces adiposity, improves insulin sensitivity and inhibits atherosclerosis in LDL receptor-deficient mice. Mol Cell Biochem 285:35–50.

Stamler JS (1996). Alzheimer's disease. A radical vascular connection. Nature 380:108–111.

Sullivan KA, Feldman EL (2005). New developments in diabetic neuropathy. Curr Opin Neurol 18:586–590.

Tesfaye S (2007). Advances in the management of painful diabetic neuropathy. Clin Med 7:113–114.

Tesfaye S, Chaturvedi N, Eaton SE, Ward JD, Manes C, Ionescu-Tirgoviste C, Witte DR, Fuller JH (2005). Vascular risk factors and diabetic neuropathy. N Engl J Med 352:341–350.

The Diabetes Control and Complications Trial Research Group (1993). The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 329:977–986.

Thum T, Borlak J (2008). Lox-1 receptor blockade abrogates oxLDL-induced oxidative DNA damage and prevents activation of the transcriptional repressor OCT-1 in human coronary arterial endothelium. J Biol Chem 283: 19456–19464.

Tomlinson DR, Gardiner NJ (2008). Glucose neurotoxicity. Nat Rev 9:36–45.

Tsimikas S, Brilakis ES, Miller ER, McConnell JP, Lennon RJ, Kornman KS, Witztum JL, Berger PB (2005). Oxidized phospholipids, Lp(a) lipoprotein, and coronary artery disease. N Engl J Med 353:46–57.

Tsuzura S, Ikeda Y, Suehiro T, Ota K, Osaki F, Arii K, Kumon Y, Hashimoto K (2004). Correlation of plasma oxidized low-density lipoprotein levels to vascular complications and human serum paraoxonase in patients with type 2 diabetes. Metabolism 53:297–302.

Ueno T, Kaname S, Takaichi K, Nagase M, Tojo A, Onozato ML, Fujita T (2003). LOX-1, an oxidized low-density lipoprotein receptor, was upregulated in the kidneys of chronic renal failure rats. Hypertens Res 26:117–122.

UK Prospective Diabetes Study (UKPDS) Group (1998). Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet 352:837–853.

Vincent AM, Edwards JL, Sadidi M, Feldman EL (2008). The antioxidant response as a drug target in diabetic neuropathy. Curr Drug Targets 9:94–100.

Vincent AM, Feldman EL (2004). New insights into the mechanisms of diabetic neuropathy. Rev Endocr Metab Disord 5:227–236.

Vincent AM, Feldman EL (2008). Can drug screening lead to candidate therapies for testing in diabetic neuropathy? Antioxid Redox Signal 10:387–393.

Vincent AM, Hayes JM, McLean LL, Vivekanandan-Giri A, Pennathur S, Feldman EL (2009). Dyslipidemia-induced neuropathy in mice: the role of oxLDL/LOX-1. Diabetes 58:2376–2385.

Vincent AM, McLean LL, Backus C, Feldman EL (2005a). Short-term hyperglycemia produces oxidative damage and apoptosis in neurons. FASEB J 19:638–640.

Vincent AM, Olzmann JA, Brownlee M, Sivitz WI, Russell JW (2004a). Uncoupling proteins prevent glucose-induced neuronal oxidative stress and programmed cell death. Diabetes 53:726–734.

Vincent AM, Perrone L, Sullivan KA, Backus C, Sastry AM, Lastoskie C, Feldman EL (2007a). Receptor for advanced

glycation end products activation injures primary sensory neurons via oxidative stress. Endocrinology 148: 548–558.

Vincent AM, Russell JW, Low P, Feldman EL (2004b). Oxidative stress in the pathogenesis of diabetic neuropathy. Endocr Rev 25:612–628.

Vincent AM, Russell JW, Sullivan KA, Backus C, Hayes JM, McLean LL, Feldman EL (2007b). SOD2 protects neurons from injury in cell culture and animal models of diabetic neuropathy. Exp Neurol 208:216–227.

Vincent AM, Stevens MJ, Backus C, McLean LL, Feldman EL (2005b). Cell culture modeling to test therapies against hyperglycemia-mediated oxidative stress and injury. Antioxid Redox Signal 7:1494–1506.

Vindis C, Elbaz M, Escargueil-Blanc I, Auge N, Heniquez A, Thiers JC, Negre-Salvayre A, Salvayre R (2005). Two distinct calcium-dependent mitochondrial pathways are involved in oxidized LDL-induced apoptosis. Arterioscler Thromb Vasc Biol 25:639–645.

Wiggin TD, Kretzler M, Pennathur S, Sullivan KA, Brosius FC, Feldman EL (2008). Rosiglitazone treatment reduces diabetic neuropathy in streptozotocin-treated DBA/2J mice. Endocrinology 149:4928–4937.

Wiggin TD, Sullivan KA, Pop-Busui R, Amato A, Sima AA, Feldman EL (2009). Elevated triglycerides correlate with progression of diabetic neuropathy. Diabetes 58: 1634–1640.

Willems D, Dorchy H, Dufrasne D (1998). Serum antioxidant status and oxidized LDL in well-controlled young type 1 diabetic patients with and without subclinical complications. Atherosclerosis 137:S61–S64.

Xie W, Nie Y, Du L, Zhang Y, Cai G (2007). Preventive effects of fenofibrate on insulin resistance, hyperglycaemia, visceral fat accumulation in NIH mice induced by small-dose streptozotocin and lard. Pharmacol Res 55:392–399.

Yamashita S, Hirano K, Kuwasako T, Janabi M, Toyama Y, Ishigami M, Sakai N (2007). Physiological and pathological roles of a multi-ligand receptor CD36 in atherogenesis; insights from CD36-deficient patients. Mol Cell Biochem 299:19–22.

Yoon SH, Han EJ, Sung JH, Chung SH (2007). Anti-diabetic effects of compound K versus metformin versus compound K-metformin combination therapy in diabetic db/db mice. Biol Pharm Bull 30:2196–2200.

Young MJ, Boulton AJM, Macleod AF, Williams DRR, Sonksen PH (1993). A multicentre study of the prevalence of diabetic peripheral neuropathy in the United Kingdom hospital clinic population. Diabetologia 36:150–154.

Ziegler D, Ametov A, Barinov A, Dyck PJ, Gurieva I, Low PA, Munzel U, Yakhno N, Raz I, Novosadova M, Maus J, Samigullin R (2006). Oral treatment with alpha-lipoic acid improves symptomatic diabetic polyneuropathy: the SYDNEY 2 trial. Diabetes Care 29:2365–2370.

Ziegler D, Sohr CG, Nourooz-Zadeh J (2004). Oxidative stress and antioxidant defense in relation to the severity of diabetic polyneuropathy and cardiovascular autonomic neuropathy. Diabetes Care 27:2178–2183.

Zimmet P, Alberti G (2008). The metabolic syndrome: progress towards one definition for an epidemic of our time. Nat Clin Pract Endocrinol Metab 4:239.