Elevated Triglycerides Correlate with Progression of Diabetic Neuropathy

Short running title: *Elevated triglycerides and DN progression*

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Objective: To evaluate mechanisms underlying diabetic neuropathy (DN) progression using indices of sural nerve morphometry obtained from two identical randomized placebo-controlled clinical trials.

Research Design and Methods: Sural nerve myelinated fiber density (MFD), nerve conduction velocities, vibration perception thresholds, clinical symptom scores and a visual analogue scale for pain were analyzed in participants with DN. A loss of ≥ 500 fibers/mm^2 in sural nerve MFD over 52 weeks was defined as progressing DN and a MFD loss of ≤ 100 fibers/mm^2 during the same time interval as non-progressing DN. The progressing and non-progressing cohorts were matched for baseline characteristics using an O'Brien rank sum and baseline MFD.

Results: At 52 weeks, the progressing cohort demonstrated a 25% decrease ($p < 0.0001$) from baseline in MFD while the non-progressing cohort remained unchanged. MFD was not affected by active drug treatment ($p=0.87$), diabetes duration ($p=0.48$), age ($p=0.11$) or body mass index (BMI) ($p=0.30$). Among all variables tested, elevated triglycerides and decreased peroneal motor NCV at baseline significantly correlated with loss of MFD at 52 weeks ($p=0.04$).

Conclusions: In this cohort of participants with mild/moderate DN, elevated triglycerides correlated with MFD loss independent of disease duration, age, diabetes control or other variables. These data support the evolving concept that hyperlipidemia is instrumental in the progression of DN.

Twenty-three million Americans have diabetes and the incidence is increasing by 5% per year. The most common complication of diabetes is peripheral neuropathy (DN) occurring in approximately 60% of all diabetic patients (1-3). In the United States, DN is the leading cause of diabetes-related hospital admissions and nontraumatic amputations (1-3). Current methods used to confirm DN and measure its progression include presence of symptoms, clinical signs, deficits in nerve conduction studies (NCV) and quantitative sensory measures (1-3). Changes in these parameters correlate with anatomical evidence of decreased large and small myelinated fiber densities in the sural nerve (myelinated fiber density, MFD) (4; 5) and the epidermis (intraepidermal nerve fiber density, IENF) (6). Although several risk factors for DN are identified in prior randomized or observational clinical trials (7; 8), a comprehensive understanding of their relationship and relevance for risk assessment is still lacking.

DN is positively correlated with the most common marker of hyperglycemia, HbA1c (9). Recent clinical evidence suggests that dyslipidemia is also associated with DN. The EURODIAB study established a significant association between cholesterol and fasting triglycerides and the development of DN (10) and cardiac autonomic neuropathy (11). A review by Steinmetz summarizes data from the United Kingdom Prospective Diabetes Study Group (UKPDS) and the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study indicate that lipid lowering therapy reduces the incidence of macrovascular complications and microvascular complications including retinopathy, nephropathy and autonomic neuropathy (12).

We are in possession of a unique repository of samples and data including human sural nerve biopsies with matched blood chemistries, electrophysiology and nerve function tests from participants in a large randomized placebo-controlled clinical DN trial testing acetyl-L-carnitine (ALC). Based on this material, we assessed predictors of DN by correlating the change in sural nerve MFD, assessed at study onset and again at study completion one year later, with baseline participant characteristics. Our results indicate that those participants with progressing DN exhibited significantly elevated triglyceride levels and deficits in peroneal motor NCV at baseline. These results indicate a role for dyslipidemia in the progression of DN and demonstrate that changes in motor NCV may precede sensory nerve fiber loss.

RESEARCH DESIGN AND METHODS

Design and Participants: We analyzed de-identified data obtained from two identical double-blind, placebo controlled, multi-center, 52-week clinical DN trials of two ALC doses (1.5 and 3.0 grams/day), conducted and supported by Sigma-Tau Research Inc. (USA) (13). Both placebo and drug treated participants were considered in the analyses. Design of these trials is described elsewhere (13). Eligibility criteria included: HbA1c >5.9%, age between 18 and 70 years, diabetes duration of greater than one year and DN as defined by the San Antonio conference (13; 14). Because the data analyzed in this report were de-identified, the University of Michigan Institutional Review Board concluded that no human subjects were involved in this project.

Blood Chemistry, Clinical Symptoms & Electrophysiology—Blood samples were collected at baseline and HbA1c, triglycerides, cholesterol, albumin and the hematocrit were recorded Clinical symptoms including pain, numbness, paresthesia, muscle weakness, postural dizziness, problems with

sweating, gastrointestinal problems and sexual dysfunction were recorded at baseline and at 52 weeks and scored on a scale of 0 (no symptoms) to 3 (incapacitating symptoms). In addition, the participants' own assessment of their most troublesome symptom at baseline was recorded.

Vibration perception thresholds of the index finger and great toe were assessed bilaterally in triplicate (15) at baseline and at 52 weeks using a Vibratron (Physitemp Instruments, Clifton, NJ) (16). These measures were completed during a 4-week run-in period prior to randomization (13).

Electrophysiological measurements included bilateral sural nerve amplitude and conduction velocity (NCV), peroneal amplitude and NCV on the dominant side, and median motor and sensory amplitudes and NCVs on the nondominant side. Sural nerve amplitude ≥ 1 µV was required for inclusion.

Sural Nerve Biopsies—Sural nerve biopsies were collected from the majority of the study participants. A biopsy was taken from one ankle at the beginning of the study, and a second biopsy was taken from the opposite ankle after 52 weeks. Morphometric parameters measured included total myelinated fiber number, fascicular area, mean fiber size, MFD, fiber occupancy and axon-to-myelin ratio (17). MFD (fibers/mm^2) in the largest fascicle was determined in semi-thin para-phenylene diamine stained sections. Details of counting methods have been previously described (4; 5).

Primary Outcome Measure—The primary outcome measure of the present study was the difference between the initial and 52 week sural nerve MFD. Participants without both a primary and secondary sural nerve biopsy or blood chemistry data were excluded from our current data analyses. Of the 748 participants in the study, 321 were excluded due to missing data. 427 participants were included in the primary data analysis of the present study (Figure 1).

Data Analysis—The analysis of these data was divided into two stages. In the primary data analysis, variables were tested for a simple correlation with the rate of MFD loss. However, a simple correlation assumes a consistent, linear progression of DN, which may not be the case. In the secondary analysis, we balanced groups based on initial DN status and tested the significant variables correlated with divergent outcomes.

Primary Data Analysis

In the cohort of 427 participants, 99.5% of clinical symptoms, vibration perception and electrophysiological measures were available. The small number of missing values were imputed by the k-nearest neighbor technique (18). The O'Brien rank sum (19) of each patient was calculated at baseline using the values for NCV, amplitude, vibration perception and the clinical symptom score. Continuous variables (e.g. HbA1c) from the initial time point were correlated with change in sural nerve MFD using the Spearman non-parametric method, and a significance value of the correlation was calculated. Categorical variables (treatment, gender, diabetes type, most bothersome symptom at baseline, insulin treatment) were tested for significant differences in sural nerve MFD by a Mann-Whitney test (20) (two categories) or Kruskal-Wallis test (21) (more than two categories).

Participant Selection for the Secondary Data Analysis: In order to identify factors driving DN progression, two groups of participants with a similar sural nerve MFD and DN at baseline but differing degrees of sural nerve MFD at 52 weeks were defined. A Perl program evaluated the change in sural nerve MFD and identified participants with an absolute loss of 500 fibers/mm^2 over 52 weeks as having rapidly progressing DN. Participants with a loss of 100 fibers/mm^2 or less over 52 weeks were identified as having non-progressing DN. Participants with a 52 week sural nerve MFD >1,000/mm^2 greater

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than baseline were excluded. DN was also estimated in the participants using the O'Brien rank score, a non-parametric combination of neuropathy measures. The O'Brien sum is composed of a linear combination of the variables listed in Table 2, excluding the "Most Bothersome Symptom at Baseline," demographic data and drug treatment information. These 16 variables describe the nerve conduction velocity in three nerves, along with the corresponding nerve amplitudes, vibration perception thresholds in the fingers and toes, and the Total Clinical Symptom Score. The program matched each participant with rapidly progressing DN with a non-progressing DN participant with similar sural nerve MFD and O'Brien score at baseline. The maximum difference in MFD was set as 1,000 fibers/mm2 and the maximum difference in the O'Brien was set as 1,000. This O'Brien threshold required that at least 3 of the 16 measures of neuropathy differ by a large degree between the participants at baseline. When multiple participants were under the similarity thresholds defined, the samples with the most similar initial fiber densities were matched.

Based on these unbiased criteria, type I diabetic and insulin treated participants were over-represented in the non-progressing group. This enrichment was not statistically significant (diabetes type p=0.19, insulin treatment p=0.11). However, because insulin is known to be neuroprotective (22; 23) the two groups were then explicitly balanced for diabetes type and insulin treatment to prevent a potential confounding effect. After balancing, 104 rapidly progressing and 104 non-progressing participants were identified for further analysis, for a total *n* = 208 (Figure 1).

Secondary Data Analysis—In the secondary data analysis, rapidly progressing participants were compared to non-progressing participants. Variables that were significantly correlated or associated with decreased sural nerve MFD in the primary analysis were advanced to the secondary analysis. The variables were tested for significant differences between the rapidly progressing and non-progressing groups using the Mann-Whitney non-parametric test (20).

Machine Learning Analysis—Machine learning analysis was performed according to the ADA Consensus Statement on Computer Modeling of Diabetes (24). The rapidly progressing and non-progressing groups (Figure 1) were used as a "training" set for seven machine learning techniques (Naive Bayes, K-Nearest Neighbor, Support Vector Machine, Linear Regression, Random Forest, Classification Tree and CN2 Rule Based) (25). The accuracy, sensitivity and specificity of each model was estimated using leave-one-out cross validation (26). In order to ensure that over-fitting did not take place, the highest performing and most sensitive models were then tested on an independent dataset from the same population. The dataset was taken from 56 participants who fit the criteria of rapidly progressing (28 participants) or non-progressing (28 participants), but who were not included in the secondary analysis cohort. A classification confidence threshold was chosen using this independent set, creating a third category of "unclassified" participants that the model lacked confidence to classify. Classification accuracy was reported based on this independent set.

All analyses were performed by one investigator (T.D.W.) using GraphPad Prism 5.01 for Windows (San Diego, CA) and Orange (Ljubljana, Slovenia) (25)

RESULTS

The dataset included 748 participants in the ALC clinical trials, but blood chemistries, initial sural nerve MFD and 52 week sural nerve MFD were only available from 427 participants (Figure 1). There were

no significant demographic, treatment or metabolic differences between the excluded cohort (321 participants) and those with the necessary data for the primary analysis (427 participants). ALC treatment did not affect sural nerve MFD loss (p = 0.87); therefore data from ALC treated participants were pooled with placebo treated participants in tests related to outcome. The participants included in the primary analysis were primarily male (67%), the majority had type 2 diabetes (78%), and the majority (59%) were treated with insulin (Table 1).

In the primary analysis, the baseline values of the patient symptoms, functional neurological exams, blood chemistries and demographics were tested for association with change of sural nerve MFD between the initial and 52 week sural nerve biopsies (Table 2). Five baseline variables were significantly correlated with a loss of sural nerve MFD over the 52 weeks of the study. They were: dominant peroneal motor NCV (R=0.13, p=0.005), non-dominant median motor NCV (R=0.11, p=0.02), sural sensory NCV (R=0.10, p=0.05), HbA1C (R=-0.12, p=-0.02), and triglyceride level (R=-0.11, p=0.02). The primary analysis was potentially confounded by the effect of initial sural nerve MFD on MFD change. There was a positive correlation between initial sural nerve MFD and the size of the decrease of sural nerve MFD over 52 weeks (R=0.14). In order to account for this confounding factor, the variables with a nominal p-value < 0.05 were tested in a subset of this dataset controlled for initial MFD.

Using the methodology in *Participant Selection for the Secondary Data Analysis*, two groups of participants were selected (*n=208*). The groups did not differ significantly in mean initial sural nerve MFD (p=0.87), O'Brien rank sum for neuropathy (p=0.09), duration of diabetes (p=0.48), age (p=0.11) or body mass index (BMI) (p=0.30). At 52 weeks, the rapidly progressing group had significantly decreased mean sural nerve MFD i.e. sural nerve MFD had decreased by more than 25% over the course of the study (p<0.0001) (Figure 2A). In contrast, the non-progressing participants had no significant change in sural nerve MFD over the study period. The divergence of DN progression between these two groups over the 52-week study period served as the basis for the secondary data analysis.

When comparing the rapidly progressing and non-progressing participants, baseline triglycerides were significantly higher in the progressing group (p = 0.04) (Figure 2B). Baseline peroneal motor NCV was significantly lower in the rapidly progressing participants (p = 0.008) (Figure 2C), despite the similar sural nerve MFD of the groups at baseline. There was no significant effect of median motor NCV, sural sensory NCV or HbA1c in the secondary analysis.

A machine learning paradigm was used to test the hypothesis that multiple factors may combine to predict the outcome of participants with DN. Using the same diverging groups of participants defined for the secondary analysis, blood chemistry, demographic data and clinical symptom score were assigned significance values with regard to decreasing or stable sural nerve MFD. This process was repeated seven times using different machine learning techniques (see *Methods*). The Naïve Bayes classifier achieved the highest sensitivity in detecting rapidly progressing participants (57%) based on their baseline characteristics (Figure 3A). The three most influential measures in this model for predicting patient outcome were triglycerides, cholesterol, and the clinical symptom score. A refinement of this classifier creates a third category of "unclassified," where the model lacks confidence to classify the participant as more likely progressing or non-progressing. If only those participants with a high classification

confidence (>56%) were assigned a prediction, overall accuracy increased to 63% (Figure 3B), which was greater than any other model tested.

DISCUSSION

Of the 748 initially recruited participants in this randomized placebo-controlled ALC trial, we were able to analyze data from 427 participants using change in sural nerve MFD over the one-year study period as the primary outcome measure. The primary analysis showed a correlation between the outcome measure and baseline motor and sensory NCVs, HbA1c and triglycerides. To identify factors specific to the rate of DN progression as measured by MFD, participants with the same degree of DN at baseline were divided into two groups with either no disease progression or rapid progression over the one-year study. Triglyceride levels and peroneal motor NCV were the only factors that significantly differed between the non-progressing and rapidly progressing participants. A model for predicting the progression of DN based on these data performed with 63% overall classification accuracy.

Our primary outcome measure, MFD, is a quantitative, highly reproducible measure of nerve health (13; 27-29). MFD correlates strongly with both motor and sensory NCV in DN and other neuropathies (13; 27-29), but unlike motor NCV, MFD does not vary with acute metabolic disturbances (30-32).

Our primary data analysis on the 427 participants revealed correlations between decreased sural nerve MFD over the one year study period and peroneal motor, median motor and sural sensory NCV, HbA1c and levels of triglycerides. While statistically significant, these correlations were not robust. This may be partially due to the fact that these data were confounded by the effect of initial sural nerve MFD . Variation in initial sural nerve MFD may be due to type and duration

of diabetes, age and gender, the range of HbA1c values at baseline and other metabolic variables. Similar variability in baseline DN has been reported in other clinical, observational and treatment trials (4; 5). Our goal was to examine parameters associated specifically with DN progression; therefore the study cohort was separated into two distinct populations with similar baseline DN and the presence or absence of a significant loss of sural nerve MFD over the 52 weeks of the study. By defining our groups as having a similar degree of DN at baseline as defined by the O'Brien score, we may underestimate the true statistical significance of NCV differences at baseline. However, this underestimation is necessary to ensure that we have truly diverging groups. Comparisons of these two groups indicate that participants with rapidly progressing DN (MFD loss greater than $500/mm^2$) exhibited elevated triglycerides and greater deficits in peroneal NCV at baseline as compared to the non-progressing participants.

The Diabetes Control and Complications Trial (DCCT) and its continuation, the Epidemiology of Diabetes and its Complications (EDIC) (7; 8), established hyperglycemia as the primary cause of diabetic complications. Consistent with these studies, we initially found that elevated HbA1c correlated with loss of sural nerve MFD. However, when directly comparing participants with a similar degree of baseline DN, i.e. similar sural nerve MFD, HbA1c did not differ between rapidly progressing and non-progressing participants; it was not a specific marker for DN progression in this study. This suggests that other factors may underlie variation in the progression of DN. In the last decade, abnormalities in insulin signaling, caused by insulin-deficiency as in type 1 diabetes or insulin-resistance as in type 2 diabetes, have been invoked as additional pathogenetic components in DN. This is underscored by

the data from the longitudinal Rochester study in which type 1 diabetes was found to be a major risk factor for severity of DN (33). Experimental studies also suggest that insulin-deficiency is a major contributor to DN, because of the prominent neurotrophic effects of insulin (22; 23). For this reason, the number of participants with type 1 diabetes and those treated with insulin was balanced when defining the progressing and non-progressing groups.

In contrast to HbA1C, baseline serum triglycerides were significantly elevated in the rapidly progressing compared to the non-progressing groups. Triglycerides are components of HDL, LDL and VLDL lipid transporters. When measured in serum, free triglycerides are a surrogate marker of endogenous lipid transport pathway activity. Free triglycerides are released from VLDL, leading to their conversion to LDL (34).

Our findings support the emerging idea that dyslipidemia contributes to the development of DN. This hypothesis may explain the earlier incidence of DN in individuals with type 2 compared to type 1 diabetes. Dyslipidemia develops later in the course of type 1 diabetes, and the delayed development of an abnormal lipid profile coincides with the delayed onset and progression of DN (35; 36). In this study, triglycerides were significantly elevated in those participants exhibiting DN progression independent of diabetes type or insulin treatment. These data confirm reports from several large scale trials of participants with type 2 diabetes that also point to early dyslipidemia as a major independent risk factor for the progression of DN [reviewed in (37; 38)]. Correction of dyslipidemia with statins has an ameliorative affect on the development and progression of DN (39; 40).

Peroneal motor NCV also differed between the progressing and non-progressing groups at baseline. This finding is more indicative of concordant damage to peroneal and sural nerve function than of a specific mechanism for that damage. Multiple studies agree with our findings and report a correlation between MFD and NCV (13; 27-29). However, in the current study decreased peroneal motor NCV was detectable prior to the loss of a significant amount of sural nerve sensory fibers, as assessed by sural nerve MFD. This most likely reflects metabolic nerve dysfunction in the peroneal nerve rather than earlier nerve fiber loss and is consistent with experimental models of DN (31). While NCV and fiber density are closely related, factors other than fiber density, such as acute metabolic disruption (41; 42) affect NCV without resulting in nerve fiber loss.

Modeling done on this dataset was motivated by the desire to identify non-invasive predictors of the loss of MFD. The ADA has issued guidelines (24) for the use of modeling and machine learning that specify that validation of a model should be done in three ways: the model should first be validated on the initial data set, then the data should be validated on an independent set from the same experiment, and finally an independent set from a different experiment from which the same parameters were collected. In this study, only the first two parts of the recommended validation could be completed due to the lack of additional published datasets with serial sural nerve biopsies. We found that a model for predicting the progression of DN using the ADA guidelines for modeling and machine learning performed with 63% overall classification accuracy. The three most influential measures in this model for predicting patient outcome were triglycerides, cholesterol, and the clinical symptom score. Interestingly, despite being significantly different between progressing and non-progressing patients, NCV was not a major contributor to this predictive model. This may be because the difference between the two groups, while significant, was

approximately 5%. The Baysean model used may not be sensitive enough to include this subtle change. A specialized learning algorithm or a measure with greater dynamic range may allow us to include this important predictor in future modeling. Future informatics studies on DN hold promise and are being proposed on the DCCT/EDIC cohort.

In summary, both elevated triglycerides and reduced peroneal motor NCV are predictive of a dramatic decrease in sural nerve MFD over a one year period. The correlation between triglycerides and DN progression suggests that hyperglycemia and aberrant glucose metabolism are not the only factors contributing to nerve damage. The exact mechanism underlying triglyceride mediated injury has yet to be elucidated but may dysregulated lipid metabolism within motor and/or sensory neurons. These same factors, along with acute metabolic flux, may explain the correlation between reduced peroneal motor NCV and rapidly progressing DN. We have also demonstrated that given an adequate data set, predictive models of DN progression may be trained using standard machine learning techniques.

REFERENCES
1. Edwards JL, Vincent AM, Cheng HT, Feldman EL: Diabetic neuropathy: Mechanisms to management. *Pharmacol Ther* 120:1-34, 2008
2. Feldman EL, Stevens MJ, Russell JW, Peltier A, Inzucchi S, Porte JD, Sherwin RS, Baron A: Somatosensory neuropathy. In *The Diabetes Mellitus Manual*, McGraw-Hill, 2005, p. 366-384
3. Feldman EL: Diabetic neuropathy. *Curr Drug Targets* 9:1-2, 2008
4. Sima A, Cherian V, Albers JW, Greene DA, The Tolrestat Study G: Nerve fiber loss in diabetic neuropathy correlates with impaired evoked potential amplitudes and nerve conduction velocity. *Diabetologia* 35:606, 1992
5. Greene DA, Arezzo JC, Brown MB: Effect of aldose reductase inhibition on nerve conduction and morphometry in diabetic neuropathy. Zenarestat Study Group. *Neurology* 53:580-591, 1999
6. Lauria G, Lombardi R: Skin biopsy: a new tool for diagnosing peripheral neuropathy. *Bmj* 334:1159-1162, 2007
7. Factors in development of diabetic neuropathy. Baseline analysis of neuropathy in feasibility phase of Diabetes Control and Complications Trial (DCCT). The DCCT Research Group. *Diabetes* 37:476-481, 1988
8. 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, 1999
9. Chisholm DJ: The Diabetes Control and Complications Trial (DCCT). A milestone in diabetes management. *Medical Journal of Australia* 159:721-723, 1993
10. Tesfaye S, Chaturvedi N, Eaton SE, Ward JD, Manes C, Ionescu-Tirgoviste C, Witte DR, Fuller JH: Vascular risk factors and diabetic neuropathy. *N Engl J Med* 352:341-350, 2005
11. Kempler P, Tesfaye S, Chaturvedi N, Stevens LK, Webb DJ, Eaton S, Kerenyi Z, Tamas G, Ward JD, Fuller JH: Autonomic neuropathy is associated with increased cardiovascular risk factors: the EURODIAB IDDM Complications Study. *Diabet.Med* 19:900-909, 2002
12. Steinmetz A: Lipid-lowering therapy in patients with type 2 diabetes: the case for early intervention. *Diabetes Metab Res Rev* 24:286-293, 2008
13. Sima AA, Calvani M, Mehra M, Amato A: Acetyl-L-carnitine improves pain, nerve regeneration, and vibratory perception in patients with chronic diabetic neuropathy: an analysis of two randomized placebo-controlled trials. *Diabetes Care* 28:89-94, 2005
14. American Diabetes Association: clinical practice recommendations 1995. *Diabetes Care* 18 Suppl 1:1-96, 1995
15. Sima AA, Laudadio C: Design of controlled clinical trials for diabetic polyneuropathy. *Semin Neurol* 16:187-191, 1996
16. Arezzo JC: New developments in the diagnosis of diabetic neuropathy. *Am J Med* 107:9S-16S, 1999
17. Sima AAF, Blaivas M: Peripheral Neuropathies. In *Neuropathology: The Diagnostic Approach* Garcia J, McKeevar P, Sima AAF, Eds. Philadelphia, Mosby, 1997, p. 758-809
18. Troyanskaya O, Cantor M, Sherlock G, Brown P, Hastie T, Tibshirani R, Botstein D, Altman RB: Missing value estimation methods for DNA microarrays. *Bioinformatics* 17:520-525, 2001
19. Huang P, Woolson RF, O'Brien PC: A rank-based sample size method for multiple outcomes in clinical trials. *Stat Med* 27:3084-3104, 2008
20. Mann HB, Whitney DR: On a Test of Whether one of Two Random Variables is Sochastically Larger than the Other *Annals of Mathematical Statistics* 18:50-60, 1947

21. Gaddis GM, Gaddis ML: Introduction to biostatistics: Part 5, Statistical inference techniques for hypothesis testing with nonparametric data. *Annals of Emergency Medicine* 19:1054-1059, 1990

22. Toth C, Brussee V, Zochodne DW: Remote neurotrophic support of epidermal nerve fibres in experimental diabetes. *Diabetologia* 49:1081-1088, 2006

23. Toth C, Brussee V, Martinez JA, McDonald D, Cunningham FA, Zochodne DW: Rescue and regeneration of injured peripheral nerve axons by intrathecal insulin. *Neuroscience* 139:429-449, 2006

24. Guidelines for computer modeling of diabetes and its complications. *Diabetes Care* 27:2262-2265, 2004

25. Demsar J, Zupan B, Leban G: Orange: From Experimental Machine Learning to Interactive Data Mining. 2004

26. Kearns M, Ron D: Algorithmic Stability and Sanity-Check Bounds for Leave-One -Out Cross-Validation. *Neural Computation* 11:1427-1453, 1999

27. Veves A, Malik RA, Lye RH, Masson EA, Sharma AK, Schady W, Boulton AJ: The Relationship Between Sural Nerve Morphometric Findings and Measures of Peripheral Nerve Function in Mild Diabetic Neuropathy. *Diabet Med* 8:917-921, 1991

28. Russell J, Karnes J, Dyck P: Sural nerve myelinated fiber density differences associated with meaningful changes in clinical and electrophysiological measurements. *Journal of the Neurological Sciences* 135:114-117, 1996

29. Malik RA, Veves A, Walker D, Siddique I, Lye RH, Schady W, Boulton AJ: Sural nerve fibre pathology in diabetic patients with mild neuropathy: relationship to pain, quantitative sensory testing and peripheral nerve electrophysiology. *Acta Neuropathol.(Berl)* 101:367-374, 2001

30. Saini AK, Arun KH, Kaul CL, Sharma SS: Acute hyperglycemia attenuates nerve conduction velocity and nerve blood flow in male Sprague-Dawley rats: reversal by adenosine. *Pharmacol Res* 50:593-599, 2004

31. Sima AA, Kamiya H: Diabetic neuropathy differs in type 1 and type 2 diabetes. *Ann N Y Acad Sci* 1084:235-249, 2006

32. Ward JD, Barnes CG, Fisher DJ, Jessop JD, Baker RW: Improvement in nerve conduction following treatment in newly diagnosed diabetics. *Lancet* 1:428-430, 1971

33. Dyck PJ, Davies JL, Litchy WJ, O'Brien PC: Longitudinal assessment of diabetic polyneuropathy using a composite score in the Rochester diabetic neuropathy study cohort. *Neurology* 49:229-239, 1997

34. Walker KH, Hall DW, Hurst WJ: *clinical Methods: The History, Physical and Laboratory Examinations*, Butterworths, 1990

35. Tomlin AM, Dovey SM, Tilyard MW: Risk factors for hospitalization due to diabetes complications. *Diabetes Res Clin Pract* 80:244-252, 2008

36. Chaturvedi N, Sjoelie AK, Porta M, Aldington SJ, Fuller JH, Songini M, Kohner EM: Markers of insulin resistance are strong risk factors for retinopathy incidence in type 1 diabetes. *Diabetes Care* 24:284-289, 2001

37. Pennathur S, Heinecke JW: Oxidative stress and endothelial dysfunction in vascular disease. *Curr Diab Rep* 7:257-264, 2007

38. Tsuzura S, Ikeda Y, Suehiro T, Ota K, Osaki F, Arii K, Kumon Y, Hashimoto K: Correlation of plasma oxidized low-density lipoprotein levels to vascular complications and human serum

paraoxonase in patients with type 2 diabetes. *Metabolism: Clinical and Experimental* 53:297-302, 2004

39. Fried LF, Forrest KY, Ellis D, Chang Y, Silvers N, Orchard TJ: Lipid modulation in insulin-dependent diabetes mellitus: effect on microvascular outcomes. *J Diabetes Complications* 15:113-119, 2001

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

41. Vinik AI, Bril V, Litchy WJ, Price KL, Bastyr EJ, 3rd: Sural sensory action potential identifies diabetic peripheral neuropathy responders to therapy. *Muscle Nerve* 32:619-625, 2005

42. Grewal J, Bril V, Lewis G, Perkins BA: Objective Evidence for the Reversibility of Nerve Injury in Diabetic Neuropathic Cachexia. *Diabetes Care* 29:473-474, 2006

Table 1 - Patient characteristics at baseline. Continuous variables are reported as mean (standard deviation).

		All Participants *n = 748*	Participants with Complete Data *n = 427*	Matched Rapidly Progressing & Non-Progressing Participants *n = 208*
Treatment				
	1000 Mg ALC	252	150	69
	500 mg ALC	252	143	71
	Placebo	244	134	68
Gender				
	Male	475	284	150
	Female	273	143	58
Diabetes Type				
	Type 1	168	95	40
	Type 2	580	332	168
Most Bothersome Symptom at Baseline				
	Burning	65	39	21
	Numbness	213	114	55
	Pain	213	126	58
	Paresthesia	32	12	5
	Tingling	88	59	29
	Weakness	9	7	5
	Other	128	126	36
Insulin Treatment				
	Yes	447	251	118
	No	301	176	90
Age	Years	53.0 (11)	53.1 (10)	53.4 (11)
Diabetes Duration	Years	12.3 (8)	12.3 (8)	11.6 (8)
Body Mass Index	kg/cm^2	29.7 (6)	29.7 (6)	29.7 (6)
Hemoglobin A1C	%	8.8 (1.8)	8.8 (1.7)	8.8 (1.7)

Table 2 – Significance of correlation or association between possible risk factors and MFD loss

Measure	Nominal p-value	Correlation
Most Bothersome Symptom	0.58	na
Total Clinical Symptom Score	0.44	-0.038
Toe Vibration Perception	0.40	-0.041
Left Finger Vibration perception	0.43	-0.038
Right Finger Vibration Perception	0.96	-0.003
Dominant Peroneal Motor NCV	0.005	0.136
Dominant Peroneal Motor Amplitude -Knee	0.71	0.018
Dominant Peroneal Motor Amplitude - Ankle	0.77	0.014
Sural Sensory NCV	0.05	0.095
Sural Sensory Nerve Amplitude	0.42	0.039
Nondominant Median Motor NCV	0.02	0.110
Nondominant Median Motor Amplitude - Elbow	0.90	0.006
Nondominant Median Motor Amplitude - Wrist	0.83	-0.011
Nondominant Median Sensory NCV - Elbow	0.08	0.086
Nondominant Median Sensory Amplitude - Elbow	0.05	0.094
Nondominant Median Sensory NCV - Wrist	0.36	0.045
Nondominant Median Sensory Amplitude - Wrist	0.27	0.053
HbA1c	0.012	-0.115
Triglycerides	0.02	-0.110
Body Mass Index	0.54	-0.029
Cholesterol	0.27	-0.053
Hematocrit	0.84	0.010
Serum Albumin	0.46	-0.036
Diabetes Duration	0.07	0.086
Age	0.13	-0.074
Insulin	0.46	na
Gender	0.10	na
Drug Treatment	0.87	na

Figure Legends

Figure 1 – Schematic outline of participant selection.
This flow diagram represents the decision process for including or excluding participants at each stage of the analysis.

Figure 2 – MFD of the rapidly progressing and non-progressing participants and significant changes between the groups.
The non-progressing dataset shows a no change in MFD (fibers/mm^2) over 52 weeks, while the progressing dataset shows a highly significant decrease in MFD (A). Baseline measurements of triglyceride levels (B) and peroneal motor NCV (C) are significantly different between the progressing and non-progressing participants. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$.

Figure 3 – Naïve Bayes classifier performance in an independent group of participants
A) Important variables in the model are triglycerides, cholesterol and clinical symptom score. B) The model assigns a probability of progressing to each participant. When the probability is greater than 56% or less than 44%, a specific outcome is predicted.
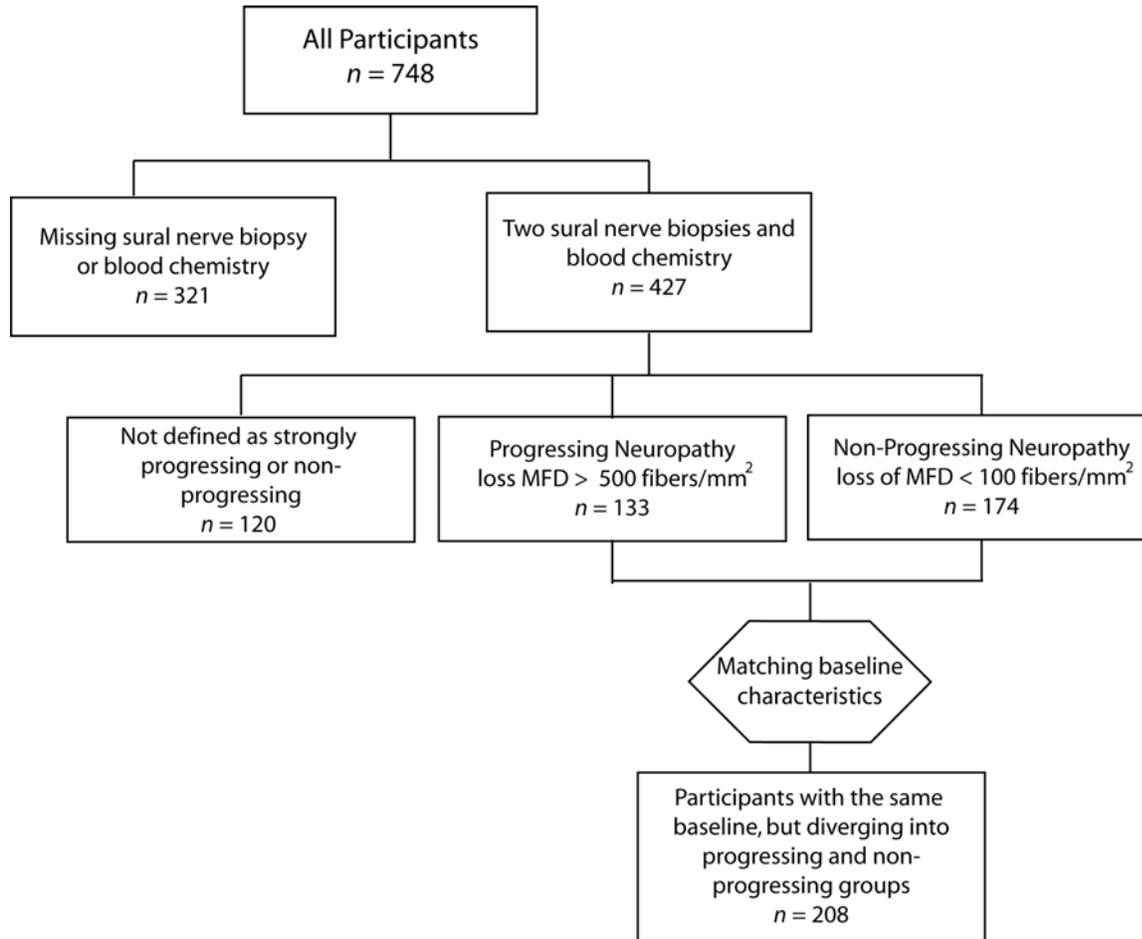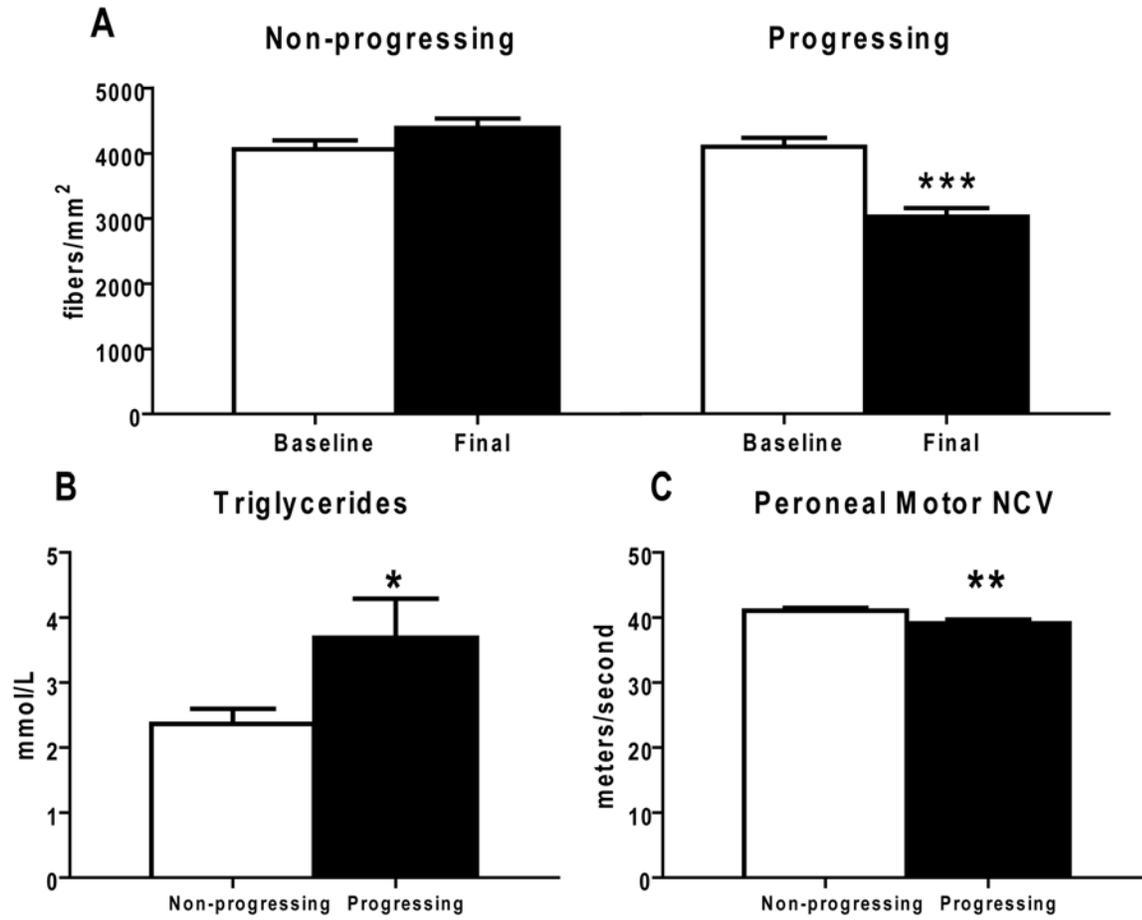
Figure 1

Figure 2

Figure 3