UNIVERSITY OF IOANNINA
SCHOOL OF HEALTH SCIENCES
FACULTY OF MEDICINE
SECTOR OF INTERNAL MEDICINE
2nd DEPARTMENT OF INTERNAL MEDICINE

DOCTORATE THESIS

PREVALENCE AND INCIDENCE OF DIABETIC PERIPHERAL NEUROPATHY IN PREDIABETIC PATIENTS IN GREECE AND ASSOCIATION WITH POTENTIAL RISK FACTORS

Georgia Anastasiou
Internal Medicine Specialist

IOANNINA
2026

ΠΑΝΕΠΙΣΤΗΜΙΟ ΙΩΑΝΝΙΝΩΝ
ΣΧΟΛΗ ΕΠΙΣΤΗΜΩΝ ΥΓΕΙΑΣ
ΤΜΗΜΑ ΙΑΤΡΙΚΗΣ
ΠΑΘΟΛΟΓΙΚΟΣ ΤΟΜΕΑΣ
Β΄ ΠΑΘΟΛΟΓΙΚΗ ΚΛΙΝΙΚΗ

ΔΙΔΑΚΤΟΡΙΚΗ ΔΙΑΤΡΙΒΗ

ΕΠΙΠΟΛΑΣΜΟΣ ΚΑΙ ΕΠΙΠΤΩΣΗ ΤΗΣ ΠΕΡΙΦΕΡΙΚΗΣ ΔΙΑΒΗΤΙΚΗΣ ΝΕΥΡΟΠΑΘΕΙΑΣ ΣΕ ΠΡΟΔΙΑΒΗΤΙΚΟΥΣ ΑΣΘΕΝΕΙΣ ΣΤΗΝ ΕΛΛΑΔΑ ΚΑΙ ΣΥΣΧΕΤΙΣΗ ΜΕ ΠΙΘΑΝΟΥΣ ΠΑΡΑΓΟΝΤΕΣ ΚΙΝΔΥΝΟΥ

Γεωργία Αναστασίου
Ειδικός Παθολόγος

ΙΩΑΝΝΙΝΑ
2026

«Η έγκριση της διδακτορικής διατριβής από την Ιατρική Σχολή του Πανεπιστημίου Ιωαννίνων δεν υποδηλώνει αποδοχή των γνωμών του συγγραφέα» Ν.5343/32 άρθρο 202, παρ.2 (νομική κατοχύρωση του Ιατρικού τμήματος)».

Ημερομηνία αίτησης της κ. Αναστασίου Γεωργίας: 03-10-2018

Ημερομηνία ορισμού Τριμελούς Συμβουλευτικής Επιτροπής: Γ.Σ. αριθμ. 876α/18-12-2018

Μέλη Τριμελούς Συμβουλευτικής Επιτροπής:

Επιβλέπων:

Λυμπερόπουλος Ευάγγελος, Αναπληρωτής Καθηγητής Παθολογίας του Τμήματος Ιατρικής του Πανεπιστημίου Ιωαννίνων

Μέλη:

Λιάμης Γεώργιος, Αναπληρωτής Καθηγητής Παθολογίας του Τμήματος Ιατρικής του Πανεπιστημίου Ιωαννίνων

Παπάνας Νικόλαος, Καθηγητής Παθολογίας -Σακχαρώδη Διαβήτη του Τμήματος Ιατρικής του ΔΠΘ

Ημερομηνία ορισμού θέματος: 31-01-2019

«Επιπολασμός και επίπτωση της περιφερικής διαβητικής νευροπάθειας σε προδιαβητικούς ασθενείς στην Ελλάδα και συσχέτιση με πιθανούς παράγοντες κινδύνου»

"Prevalence and incidence of diabetic peripheral neuropathy in prediabetic patients in Greece and association with potential risk factors"

ΟΡΙΣΜΟΣ ΕΠΤΑΜΕΛΟΥΣ ΕΞΕΤΑΣΤΙΚΗΣ ΕΠΙΤΡΟΠΗΣ: 1222α/14-05-2026

1. Λυμπερόπουλος Ευάγγελος, Καθηγητής Παθολογίας-Μεταβολικών Νοσημάτων του Τμήματος Ιατρικής του ΕΚΠΑ
2. Λιάμης Γεώργιος, Καθηγητής Παθολογίας του Τμήματος Ιατρικής του Πανεπιστημίου Ιωαννίνων
3. Παπάνας Νικόλαος, Καθηγητής Παθολογίας-Σακχαρώδη Διαβήτη του Τμήματος Ιατρικής του Δημοκρίτειου Πανεπιστημίου Θράκης
4. Τεντολούρης Νικόλαος, Καθηγητής Παθολογίας του Τμήματος Ιατρικής του ΕΚΠΑ
5. Μηλιώνης Χαράλαμπος, Καθηγητής Παθολογίας του Τμήματος Ιατρικής του Πανεπιστημίου Ιωαννίνων
6. Ρίζος Χρήστος, Επίκουρος Καθηγητής Παθολογίας του Τμήματος Ιατρικής του Πανεπιστημίου Ιωαννίνων
7. Μπάρκας Φώτιος, Επίκουρος Καθηγητής Παθολογίας του Τμήματος Ιατρικής του Πανεπιστημίου Ιωαννίνων

Έγκριση Διδακτορικής Διατριβής με βαθμό **«ΑΡΙΣΤΑ»** στις **29-05-2026**

Ιωάννινα 12-06-2026

ΠΡΟΕΔΡΟΣ ΤΟΥ ΤΜΗΜΑΤΟΣ ΙΑΤΡΙΚΗΣ

Σπυρίδων Κονιτσιώτης

Καθηγητής Νευρολογίας

Η Γραμματέας του Τμήματος

Αικατερίνη Λαγού

Στους γονείς μου, Αγγελική και Ευάγγελο

Στο σύζυγό μου, Θάνο

Στην αδερφή μου, Παναγιώτα

Στον Καθηγητή μου,

Ευάγγελο Λυμπερόπουλο

ΠΡΟΛΟΓΟΣ

Η παρούσα διδακτορική διατριβή εκπονήθηκε στο Ιατρείο Μελέτης των Διαταραχών του Μεταβολισμού και των Λιπιδίων σε συνεργασία με το Ιατρείο Παχυσαρκίας του Πανεπιστημιακού Γενικού Νοσοκομείου Ιωαννίνων. Μέσα από αυτή την πορεία, η γνώση, η επιμονή και η συνεχής αναζήτηση αποτέλεσαν τη δύναμη που οδήγησε στην ολοκλήρωσή της. Ωστόσο, καμία διαδρομή δεν ολοκληρώνεται χωρίς συνοδοιπόρους. Νιώθω, λοιπόν, βαθιά ευγνωμοσύνη προς όλους εκείνους που συνόδευσαν και στήριξαν αυτή την προσπάθεια σε κάθε της στάδιο της με εμπιστοσύνη, καθοδήγηση και έμπρακτη στήριξη.

Ο κύριος εμπνευστής και καθοδηγητής μου κατά τη διάρκεια της εκπόνησης της παρούσας διδακτορικής διατριβής ήταν ο δάσκαλός μου και επιβλέπων κ. Ευάγγελος Λυμπερόπουλος, Καθηγητής Παθολογίας. Υπήρξε ο άνθρωπος που κράτησε σταθερή την πυξίδα αυτής της πορείας, με ουσιαστική καθοδήγηση, υποστήριξη σε κάθε βήμα από την αρχή του εγχειρήματος αυτού, με εύστοχες υποδείξεις, εμπιστοσύνη και με λόγο που φώτιζε τον δρόμο ακόμη και στις πιο απαιτητικές στιγμές. Ταυτόχρονα, υπήρξε για μένα ο δάσκαλός μου στην κριτική προσέγγιση και τη συγγραφή των επιστημονικών εργασιών. Η πραγματοποίηση της παρούσας μελέτης δεν θα ήταν δυνατή χωρίς την αδιάκοπη καθοδήγησή του. Η συνεργασία μας είναι εξαιρετική τιμή για μένα, του έχω βαθιά ευγνωμοσύνη και θεωρώ βαθύτατη υποχρέωση να τον ευχαριστήσω από την καρδιά μου.

Θερμές ευχαριστίες από την καρδιά μου οφείλω επίσης και στα μέλη της τριμελούς συμβουλευτικής επιτροπής. Ιδιαίτερα θα ήθελα να εκφράσω την ευγνωμοσύνη μου στον Καθηγητή Παθολογίας κ. Γεώργιο Λιάμη, για την ανθρώπινη και διαρκή παρουσία του, τις ουσιαστικές παρατηρήσεις του, καθώς και το αδιάκοπο ενδιαφέρον με το οποίο στήριξε ενεργά την πρόοδο αυτής της διατριβής σε κάθε της στάδιο από την αρχή, φρόντιζε διαρκώς για τη

στρατολόγηση των ασθενών, την ομαλή εξέλιξη και την αποτελεσματική διεκπεραίωση της μελέτης. Για όλα αυτά, τον ευχαριστώ θερμά.

Ιδιαίτερα, θα ήθελα να ευχαριστήσω θερμά και τον Καθηγητή Παθολογίας κ. Νικόλαο Παπάνα, για την εμπιστοσύνη που έδειξε στο πρόσωπό μου, τη φωτεινή καθοδήγησή μου ήδη από τα πρώτα μου βήματα, τη διαρκή στήριξη και τις εύστοχες και σημαντικές παρατηρήσεις του. Η βαθιά εμπειρία του και η κρίση του σημάδεψαν καθοριστικά τη διαμόρφωση της μελέτης αυτής συμβάλλοντας ουσιαστικά στη βελτίωσή της. Για όλα αυτά, του είμαι βαθιά ευγνώμων.

Θα ήθελα επίσης να εκφράσω τις θερμές μου ευχαριστίες στον Καθηγητή Παθολογίας, κ. Νικόλαο Τεντολούρη για την πολύ σημαντική συμβολή του στη δημιουργία του πρωτοκόλλου της παρούσας διδακτορικής διατριβής. Οι καίριες παρατηρήσεις, η βαθιά γνώση και εμπειρία του, καθώς και η καθοδήγησή του αποτέλεσαν σταθερό σημείο αναφοράς σε κάθε στάδιο της μελέτης. Για τη στήριξη και την εμπιστοσύνη του, εκφράζω την ειλικρινή μου ευγνωμοσύνη.

Θερμές ευχαριστίες απευθύνω και στα μέλη της επταμελούς εξεταστικής επιτροπής, τον Καθηγητή Παθολογίας κ. Χαράλαμπο Μηλιώνη, τον Επίκουρο Καθηγητή Παθολογίας κ. Χρήστο Ρίζο και τον Επίκουρο Καθηγητή Παθολογίας κ. Φώτιο Μπάρκα, για το ενδιαφέρον και τη βοήθειά τους, καθώς και τις πολύτιμες γνώσεις τους που μοιράστηκαν μαζί μου για τη στατιστική επεξεργασία και συγγραφή των αποτελεσμάτων της παρούσας διδακτορικής διατριβής.

Ιδιαίτερες ευχαριστίες εκφράζω προς τον Καθηγητή Παθολογίας-Ενδοκρινολογίας κ. Χρήστο Μαντζώρο, που μου έδωσε την ευκαιρία να πραγματοποιήσω Research Fellowship στο ερευνητικό του εργαστήριο στο Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA. Μέσα από αυτήν την εμπειρία ζωής είχα την ευκαιρία να εκπαιδευτώ περαιτέρω στη μεθοδολογία της κλινικής και μεταφραστικής έρευνας. Η καθοδήγησή του, η ανθρώπινη παρουσία του, η εμπιστοσύνη και η συνεχής, πολύτιμη

υποστήριξή του αποτέλεσαν σημαντικό αρωγό στην κριτική προσέγγιση, επεξεργασία και ανάλυση των δεδομένων της παρούσας διατριβής. Για όλα αυτά, του είμαι βαθιά ευγνώμων.

Ευχαριστώ θερμά επίσης την Επίκουρη Καθηγήτρια Παθολογίας κα Ματίλντα Φλωρεντίν για τη συμβολή της στη στρατολόγηση ασθενών, τον κ. Ρήγα Καλαϊτζίδη και τον κ. Αριστείδη Μπεχλιούλη για την πραγματοποίηση των μετρήσεων της αρτηριακής σκληρίας, τη συνεργασία και τη βοήθειά τους, και την κα Ντόντη Ρέα, νοσηλεύτρια του Ιατρείου Μελέτης των Διαταραχών του Μεταβολισμού των Λιπιδίων του Πανεπιστημιακού Νοσοκομείου Ιωαννίνων για την πολύτιμη βοήθειά της.

Θα ήθελα να εκφράσω τη βαθύτερη ευγνωμοσύνη μου προς τη μητέρα μου, Αγγελική, τον πατέρα μου, Ευάγγελο, το σύζυγό μου, Αθανάσιο και την αδερφή μου, Παναγιώτα, και για τη διαρκή στήριξη, την αμέριστη αγάπη και την πίστη τους σε εμένα και στα όνειρά μου. Χωρίς τη δική τους παρουσία και την πολύτιμη, ηθική και πρακτική υποστήριξη σε κάθε βήμα μου, η ολοκλήρωση της παρούσας διατριβής δεν θα ήταν δυνατή. Αφανείς ήρωες, με ταπεινό κι αθόρυβο καθημερινό αγώνα για τη στήριξη της οικογένειας. Το λιγότερο που μπορώ να προσφέρω ως αντίδωρο αυτών είναι να αφιερώσω την παρούσα εργασία στη μητέρα μου, τον πατέρα μου, το σύζυγό μου, την αδερφή μου, και τον δάσκαλό μου.

Γεωργία Αναστασίου

Ιωάννινα, Μάιος 2026

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

1hPG: 1-hour Plasma Glucose

2hPG: 2-hour Plasma Glucose

25(OH)D: 25-Hydroxyvitamin D

ABI: Ankle-Brachial Index

ACE: Angiotensin-Converting Enzyme

ACEs: Angiotensin-Converting Enzyme Inhibitors

ACCORD: Action to Control Cardiovascular Risk in Diabetes

ADA: American Diabetes Association

AGE: Advanced Glycation End-products

Akt: Protein Kinase B

ANDA: Australian National Diabetes Audit

A-P: Amitriptyline-Pregabalin

apoA1: Apolipoprotein A1

apoB: Apolipoprotein B

AR: Aldose Reductase

ARBs: Angiotensin II Receptor Blockers

B-blockers: Beta Blockers

BENDIP: Benfotiamine in Diabetic Polyneuropathy

BIA: Bioelectrical Impedance Analysis

BMI: Body-Mass Index

CCB: Calcium Channel Blocker

CCBs: Calcium Channel Blockers

cfPWV: Carotid-Femoral Pulse Wave Velocity

CGRP: Calcitonin Gene-Related Peptide

CGM: Continuous Glucose Monitoring

CI: Confidence Interval

CMAP: Compound Motor Action Potential

CNS: Central Nervous System

COMBO-DN: Combination vs Monotherapy of Duloxetine and Pregabalin in Diabetic Neuropathy

CVD: Cardiovascular Disease

D-P: Duloxetine-Pregabalin

DBP: Diastolic Blood Pressure

DCCT: Diabetes Control and Complications Trial

DPP: Diabetes Prevention Program

DPPOS: Diabetes Prevention Program Outcomes Study

DRG: Dorsal Root Ganglion

DSPN: Distal Symmetric Sensorimotor Polyneuropathy

eGFR: Estimated Glomerular Filtration Rate

EMA: European Medicines Agency

EMG: Electromyography

eNOS: Endothelial Nitric Oxide Synthase

ER: Endoplasmic Reticulum

ESC: European Society of Cardiology

ESH: European Society of Hypertension

ET-1: Endothelin-1

F-6-P: Fructose-6-Phosphate

FDA: Food and Drug Administration

FIELD: Fenofibrate Intervention and Event Lowering in Diabetes

FPG: Fasting Plasma Glucose

GAP43: Growth-Associated Protein 43

GLU: Glucose

GLUT1: Glucose Transporter 1

GSH: Glutathione

GV: Glucose Variability

HbA1c: Hemoglobin A1c

HDL: High-Density Lipoprotein

HDL-C: High-Density Lipoprotein Cholesterol

HIV: Human Immunodeficiency Virus

HK: Hexokinase

HOMA-IR: Homeostatic Model Assessment for Insulin Resistance

HR: Hazard Ratio

HRV: Heart Rate Variability

HSP27: Heat Shock Protein 27

ICAM-1: Intercellular Adhesion Molecule 1

IDF: International Diabetes Federation

IEC: International Expert Committee

IENFD: Intraepidermal Nerve Fiber Density

IFG: Impaired Fasting Glucose

IGT: Impaired Glucose Tolerance

IQR: Interquartile Range

IR: Insulin Resistance

IU: International Unit

LDL-C: Low-Density Lipoprotein Cholesterol

LOOK AHEAD: Action for Health in Diabetes

Lp(a): Lipoprotein(a)

MAPK: Mitogen-Activated Protein Kinase

MCP-1: Monocyte Chemoattractant Protein-1

MedDiet: Mediterranean Diet

MetS: Metabolic Syndrome

miRNA: MicroRNA

MNSI: Michigan Neuropathy Screening Instrument

mRNA: Messenger Ribonucleic Acid

MRAs: Mineralocorticoid Receptor Antagonists

NADPH: Nicotinamide Adenine Dinucleotide Phosphate

Na+/K+-ATPase: Sodium–Potassium Adenosine Triphosphatase

NATHAN 1: Neurological Assessment of Thioctic Acid in Diabetic Neuropathy 1

NCV: Nerve Conduction Velocity

NCS: Nerve Conduction Studies

NDS: Neuropathy Disability Score

NF-κB: Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells

NGT: Normal Glucose Tolerance

NIDDM: Non-Insulin-Dependent Diabetes Mellitus

NO: Nitric Oxide

NRS: Numerical Rating Scale

NSS: Neuropathy Symptom Score

OGTT: Oral Glucose Tolerance Test

OPTION-DM: Optimal Pathway for Treating Neuropathic Pain in Diabetes Mellitus

OR: Odds Ratio

OXPHOS: Oxidative Phosphorylation

P-A: Pregabalin-Amitriptyline

PAI-1: Plasminogen Activator Inhibitor-1

PARP: Poly(ADP-Ribose) Polymerase

PCSK9i: Proprotein Convertase Subtilisin/Kexin Type 9 Inhibitor

PI3K: Phosphoinositide 3-Kinase

PKC: Protein Kinase C

PNS: Peripheral Nervous System

PROMINENT: Pemafibrate to Reduce Cardiovascular Outcomes by Reducing Triglycerides in Patients with Diabetes

PROMISE: Prospective Metabolism and Islet Cell Evaluation

PWV: Pulse Wave Velocity

RAGE: Receptor for Advanced Glycation End-products

QST: Quantitative Sensory Testing

RAGs: Regeneration-Associated Genes

RCTs: Randomized Controlled Trials

RNA: Ribonucleic Acid

ROS: Reactive Oxygen Species

RR: Relative Risk

SARM1: Sterile Alpha and TIR Motif Containing 1

SBP: Systolic Blood Pressure

SNRIs: Serotonin-Norepinephrine Reuptake Inhibitors

SP: Substance P

SPNSQ: Subjective Peripheral Neuropathy Screen Questionnaire

T1D: Type 1 Diabetes

T2D: Type 2 Diabetes

TC: Total Cholesterol

TCA: Tricarboxylic Acid

TCAs: Tricyclic Antidepressants

TCMAP: Tibial Compound Motor Action Potential

TGF-β: Transforming Growth Factor-Beta

TF: Transcription Factor

TGs: Triglycerides

TIND: Treatment-Induced Neuropathy in Diabetes

TIR: Time in Range

TMNCV: Tibial Motor Nerve Conduction Velocity

TriNetX: TriNetX Research Network

TSH: Thyroid-Stimulating Hormone

UACR: Urinary Albumin-to-Creatinine Ratio

UK: United Kingdom

UKPDS: United Kingdom Prospective Diabetes Study

USA: United States of America

VACSDM: Veterans Affairs Cooperative Study on Glycemic Control and Complication in NIDDM

VCAM-1: Vascular Cell Adhesion Molecule 1

VEGF: Vascular Endothelial Growth Factor

VIF: Variance Inflation Factor

VPT: Vibration Perception Threshold

WC: Waist Circumference

WHO: World Health Organization

INTRODUCTION

1. PREDIABETES

1.1 Definition and diagnostic criteria

At present, distinct definitions of prediabetes have been established by professional organizations, including the American Diabetes Association (ADA), the World Health Organization (WHO), and the International Expert Committee (IEC) [4-6]. WHO refers to the term "intermediate hyperglycemia" and not "prediabetes" [6]. The definitions are differentiated based on levels of fasting plasma glucose (FPG), 2-hour plasma glucose (2hPG), and hemoglobin A1c (HbA1c) (***Table 1***). There are different degrees of insulin resistance (IR) and defects in beta-cell functions in different glycemic states. Notably, there is significant IR and an approximate 80% impairment in β-cell function in people with impaired glucose tolerance (IGT) [7]. As HbA1c reflects average glycemic exposure over the preceding 2-3 months, phenotypes defined by elevated HbA1c typically capture individuals with combined abnormalities in both fasting and postprandial glucose regulation. According to ADA Standards of Care in Diabetes-2026 [4] and the European Society of Cardiology (ESC) Guidelines on Diabetes, Prediabetes, and Cardiovascular Diseases-2023 [8, 9], prediabetes represents an intermediate metabolic state characterized by glucose levels that are higher than normal but below the diagnostic threshold for type 2 diabetes (T2D). Guidelines issued by both organizations define prediabetes with the same glycemic parameters: a FPG level of 100-125 mg/dL (5.6-6.9 mmol/L), a 2hPG level of 140-199 mg/dL (7.8-11.0 mmol/L) following a 75 g oral glucose tolerance test (OGTT), or HbA1c level of 5.7-6.4% (39-47 mmol/mol) (**Table 1**) [4, 8].

Table 1 Prediabetes diagnostic criteria according to international guidelines

Organization	FPG	2hPG	HbA1c
ADA	100-125 mg/dL (5.6-6.9 mmol/L)	140-199 mg/dL (7.8-11.0 mmol/L)	5.7-6.4% (39-47 mmol/mol)
ESC	100-125 mg/dL (5.6-6.9 mmol/L)	140-199 mg/dL (7.8-11.0 mmol/L)	5.7-6.4% (39-47 mmol/mol)
WHO	110-125 mg/dL (6.1-6.9 mmol/L)	140-199 mg/dL (7.8-11.0 mmol/L)	*N/A*
IEC	110-125 mg/dL (6.1-6.9 mmol/L)	140-199 mg/dL (7.8-11.0 mmol/L)	6.0-6.4% (42-47 mmol/mol)
DCCPG	110-125 mg/dL (6.1-6.9 mmol/L)	140-199 mg/dL (7.8-11.0 mmol/L)	6.06.4% (42-47 mmol/mol)
NICE (UK)	110-125 mg/dL (6.1-6.9 mmol/L)	140-199 mg/dL (7.8-11.0 mmol/L)	6.0-6.4% (42-47 mmol/mol)

Abbreviations: FPG, Fasting Plasma Glucose; 2hPG, 2-hour plasma glucose following a 75 g OGTT; HbA1c, Hemoglobin A1c; ADA, American Diabetes Association; ESC, European Society of Cardiology; WHO, World Health Organization; IEC, International Expert Committee; DCCPG, Diabetes Canada Clinical Practice Guidelines [4-6, 10]

Within this spectrum, impaired fasting glucose (IFG) and IGT are two distinct categories of prediabetes that reflect different underlying pathophysiological mechanisms. IFG is defined as a FPG level between 100 and 125 mg/dL (5.6-6.9 mmol/L) after at least 8 fasting hours and is primarily associated with hepatic IR and decreased basal insulin secretion (**Table 2**, **Figure 1**) [4]. In contrast, IGT is defined by a 2hPG level of 140-199 mg/dL (7.8-11.0 mmol/L) following a 75 g OGTT (**Table 2**) and is characterized predominantly by peripheral (skeletal muscle) IR and inadequate compensatory insulin secretory response, particularly in the late phase,

resulting in sustained post-load hyperglycemia (**Figure 1**) [4]. In isolated IFG, FPG levels are elevated (100-125 mg/dL [5.6-6.9 mmol/L]), but plasma glucose following a 75 g OGTT declines to levels near to baseline by 2h (**Table 2**, **Figure *2***) [4]. In isolated IGT, FPG levels are comparable to normoglycemic, but post 75 g OGTT plasma glucose increases at all time points and remains elevated (140-199 mg/dL [7.8-11.0 mmol/L]) at 2h (**Table 2**, **Figure *2***) [4]. In isolated IFG, FPG levels are elevated (100-125 mg/dL [5.6-6.9 mmol/L]), while the 1-hour post-load plasma glucose (1hPG) is typically normal or only modestly elevated, and plasma glucose declines toward baseline by 2h [6]. In isolated IGT, FPG levels are comparable to normoglycemia, but following a 75 g OGTT, plasma glucose rises markedly at 1h and remains elevated (140-199 mg/dL [7.8-11.0 mmol/L]) at 2h. Current ADA diagnostic criteria do not incorporate the 1h PG for the classification of prediabetes [11]. In contrast, the IDF recognizes the 1h PG as a sensitive marker of early dysglycemia, with a threshold ≥155 mg/dL (8.6 mmol/L) identifying individuals at high risk for progression to T2D and cardiometabolic complications [12]. Physiologically, the 1h PG represents an integrated marker of early-phase insulin secretion and peripheral (predominantly skeletal muscle) insulin sensitivity, with elevated values reflecting an early defect in β-cell function with impaired insulin-mediated glucose disposal [12].

Table 2 Diagnostic Criteria for IFG and IGT

Glycemic state	**FPG (mg/dL)**	**2hPG (mg/dL, OGTT 75 g)**
Normal glucose	<100	<140
Isolated IFG	100-125	<140
Isolated IGT	<100	140-199
IFG	100-125	<200
IGT	(-)	140-199
Combined IFG/IGT	100-125	140-199

Abbreviations: IFG, Impaired Fasting Glucose; IGT, Impaired Glucose Tolerance; FPG, Fasting Plasma Glucose; 2h-PG, 2-hour Plasma Glucose; OGTT, Oral Glucose Tolerance Test

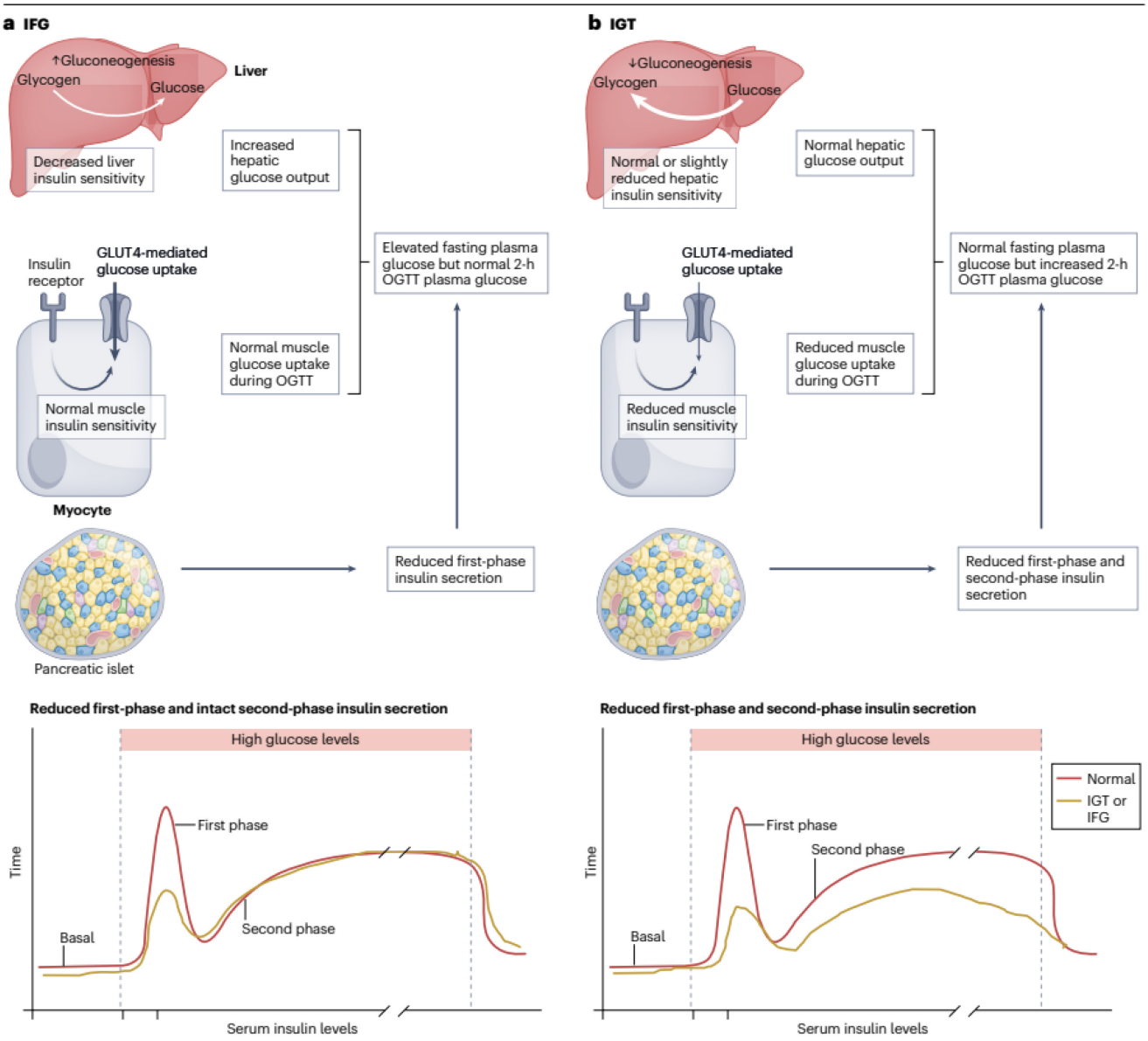


Figure 1 Comparison of the pathophysiology of IFG and IGT

Impaired fasting glucose (IFG) is primarily characterized by hepatic insulin resistance with relatively preserved skeletal muscle insulin sensitivity, resulting in elevated fasting plasma glucose (FPG) due to increased hepatic glucose production, while postprandial glucose levels remain near normal. Early-phase insulin secretion is reduced, whereas late-phase insulin secretion is generally preserved. In contrast, impaired glucose tolerance (IGT) is mainly associated with peripheral (skeletal muscle) insulin resistance, leading to impaired glucose disposal and elevated post-load glucose levels despite relatively normal FPG levels. Both early- and late-phase insulin secretion are impaired in IGT. Adapted from Unnikrishnan R et al [13].

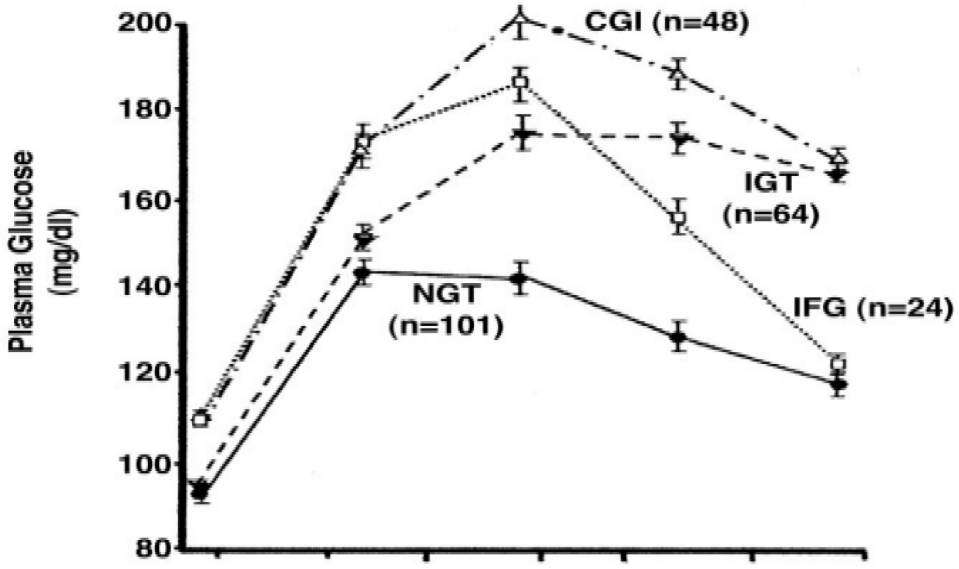


Figure 2 Plasma glucose levels during Oral Glucose Tolerance Test (OGTT) in individuals with impaired fasting glucose (IFG), impaired glucose tolerance (IGT), normal glucose tolerance (NGT), or a combination of IFG/IGT (CGI) [14]

1.2 Epidemiology

Recent global analyses present updated estimates of impaired glucose regulation and projections through 2050 [15]. A large comprehensive systematic review was conducted including more than 4800 studies published between 2020 and 2024, data from 215 countries and data from the 11th edition of the IDF Diabetes Atlas [15]. The analysis applied WHO diagnostic criteria to define impaired glucose regulation, with IGT specified as a 2hPG of 140-199 mg/dL (7.8-11.1 mmol/L) and IFG as a FPG level of 110-125 mg/dL (6.1-6.9 mmol/L). Multivariate logistic regression models were used to estimate global and regional prevalence and the age distribution of each respective country. In 2024, an estimated 634.8 million adults (12.0%) were living with IGT, while 487.7 million (9.2%) had IFG [15]. By 2050, these are projected to rise to 846.5 million (12.9%) and 647.5 million (9.8%), respectively [15]. The

greatest relative increases are expected in low- and middle-income regions, highlighting global disparities in metabolic health. Regionally, the highest prevalence of IGT was observed in Southeast Asia, whereas IFG was most prevalent in North America and the Caribbean; both parameters were lowest in Europe. The study shows a remarkable rise in the prevalence of prediabetes compared to the estimates in 2021; IGT from 9.1% to 12.0% and IFG from 5.8% to 9.2% [15]. Despite increasing data availability, data on IGT or IFG are derived from only approximately 40% of countries, suggesting that the real global burden of prediabetes remains substantially underestimated [15].

Recent national evidence highlights a considerable burden of prediabetes in Greece. According to findings from the Hellenic National Health Examination Survey (EMENO), the prevalence of prediabetes among Greek adults was 12.4% (95% Confidence Interval [CI]: 11.4 -13.6) [16]. According to the IDF, the prevalence of T2D among individuals aged 20-79 years in Greece was 9.6% in 2021, similar to the European regional average [17]. These findings emphasize the growing metabolic burden in Greece and reinforce the need for targeted and effective preventive strategies addressing both prediabetes and established T2D.

In the 2025 Lancet Global Health pooled analysis which included 76,092 adults from 19 international cohorts (median prospective follow-up: 9.8 years), individuals with prediabetes had a 12.5% probability of progression to T2D and a 36.1% probability of reverting to normoglycemia [18]. However, probability ratios varied based on baseline FPG levels and demographic characteristics. Specifically, in the highest quartile of FPG levels (110-125 mg/dL [6.2-6.9 mmol/L]), the probability of progression was 16.1% and reversion 13.4% [18]. Progression rates were highest among males, adults aged ≥55 years, and Latinx populations [18]. In contrast, individuals from Japan demonstrated the lowest rate ratios [18].

1.3 Natural History of IFG and IGT

IFG and IGT are intermediate states of dysglycemia linked to a markedly increased risk of developing T2D (**Figure 3**). IFG is primarily associated with hepatic IR and defects in early-phase insulin secretion, while IGT is more linked to peripheral IR and impaired late-phase insulin response [19]. Although both IFG and IGT can progress to T2D, the risk of progression is substantially higher when they are combined [20].

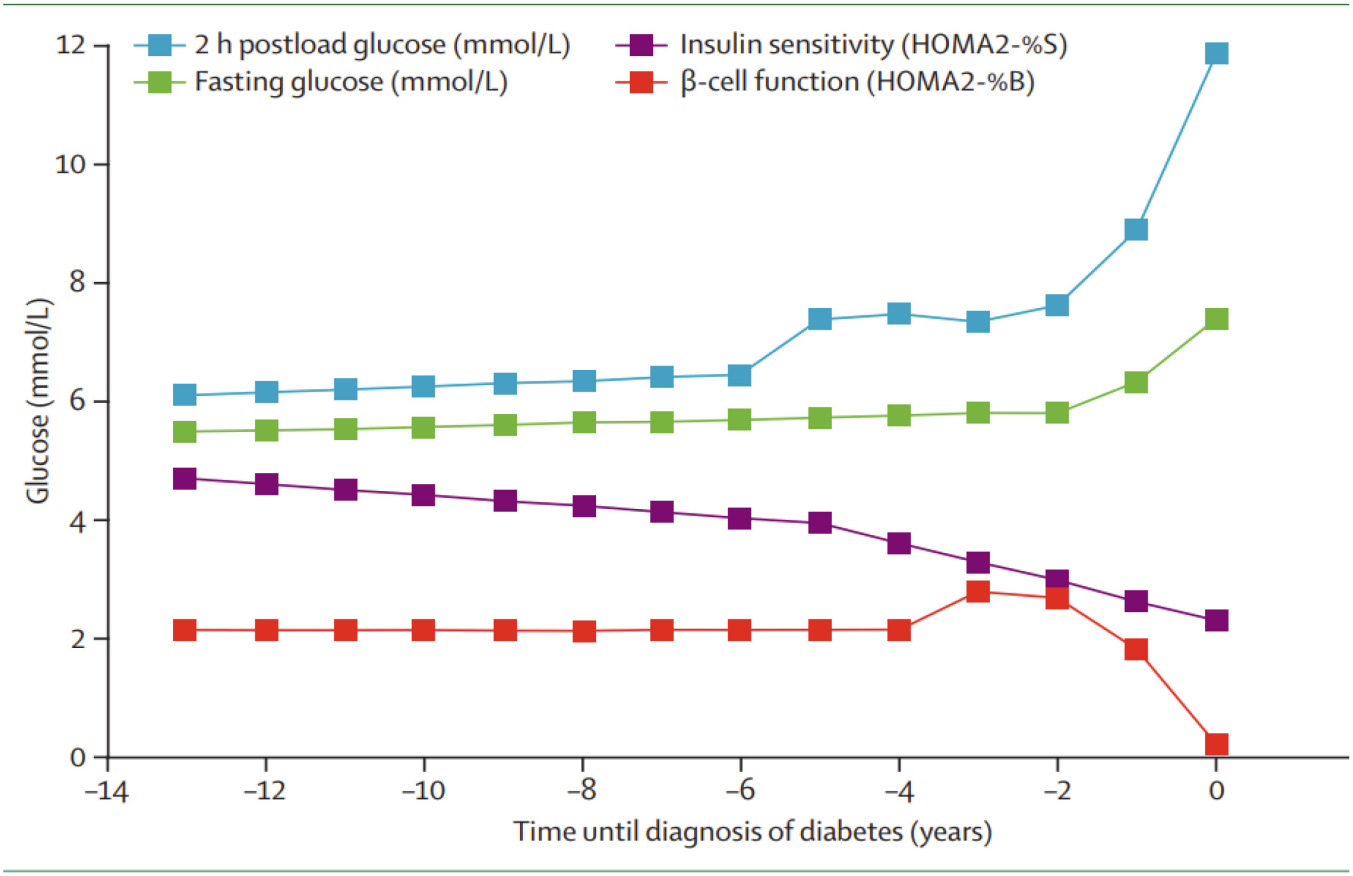


Figure 3 Fasting and 2hPG, insulin sensitivity, and β-cell function trajectories before the diagnosis of diabetes

Longitudinal changes in fasting glucose, 2 hour plasma glucose (2hPG) following oral glucose tolerance test (OGTT), insulin sensitivity, and β-cell function during progression toward type 2 diabetes (T2D). Declining insulin sensitivity and β-cell dysfunction precede the onset of overt hyperglycemia. Time 0 is diagnosis of diabetes. The updated homoeostasis model assessment (HOMA2) was used to calculate insulin sensitivity (HOMA2-%S) and β-cell function (HOMA2-%B) trajectories. Adapted from Baranowska-Jurkun A, et al.[3]

The prevalence of IFG and IGT varies significantly across different ethnic groups, age categories, and between genders [21-29]. Both IGF and IGT increase with age and are higher prevalent in females [24, 30, 31]. The natural history of IFG and IGT is heterogeneous, with outcomes including progression to T2D, sustained dysglycemia, or reversion to normoglycemia. Over 3-5 years of follow-up, approximately 25% of individuals with prediabetes progress to T2D, 50% remain with prediabetes, and 25% revert to normoglycemia [32-34]. The risk of progression increases with age, obesity, and the presence of metabolic syndrome (MetS) [35, 36]. Notably, individuals with combined IFG and IGT have the highest incidence of T2D compared to those with isolated IFG or IGT [37]. Moreover, those at increased risk of developing T2D typically demonstrate both impaired insulin secretion and increased IR [36, 38].

Multiple epidemiological studies have demonstrated that both IFG and IGT are associated with a 1.1- to 1.4-fold increased risk of cardiovascular disease (CVD) compared to normoglycemia, with IGT identified as a slightly stronger predictor [26, 39-44]. However, additional cardiovascular risk factors such as hypertension, low high-density lipoprotein cholesterol (HDL-C) levels, and elevated triglycerides (TGs) are prevalent in individuals with IFG and/or IGT [45-47]. Notably, after adjusting these risk factors, both IFG and IGT have been identified as independent predictors of CVD in several studies [26, 39-44, 48].

1.4 Pathophysiology of IFG and IGT

Epidemiological and clinical evidence demonstrate that IFG and IGT are characterized by distinct pathophysiological mechanisms, which reflect discrete impairments in glucose homeostasis [14, 49-54]. In isolated IGT, FPG levels are typically within the normoglycemic range [14, 49-54]. However, during a standard OGTT (75 g of anhydrous glucose), post-load

plasma glucose levels rise abnormally and remain elevated at 120 min, consistent with the IGT diagnostic threshold (140-199 mg/dL; 7.8-11.0 mmol/L) (**Figure 2**). In isolated IFG, FPG levels are elevated (100-125 mg/dL; 5.6–6.9 mmol/L) compared to those with normoglycemia and those with isolated IGT. Following a 75 g OGTT, plasma glucose typically declines to near-fasting levels by 120 min. These 2 OGTT glycemic curves observed in IFG and IGT reflect distinct underlying pathophysiological defects in glucose homeostasis (**Figure 2**). In those with combined IFG and IGT, the plasma glucose curve demonstrates a dual-pattern phenotype, characterized by both hepatic and peripheral IR with impairments in both 1st- and 2^{nd} -phase insulin secretion [14, 49-54].

Although both isolated IFG and isolated IGT are characterized by IR, differences are observed in the primary tissues affected [19, 50, 55, 56]. In isolated IFG, IR is predominantly hepatic, while insulin sensitivity in skeletal muscle is generally preserved (**Figure 1**). Conversely, in isolated IGT, hepatic insulin sensitivity is typically normal or mildly reduced, whereas IR is more pronounced in skeletal muscle (**Figure 1**). In individuals with both IFG and IGT, IR is observed in both hepatic and skeletal muscle tissues [19, 50, 55, 56].

The pattern of insulin secretion differs between individuals with IFG and those with IGT. In isolated IFG, there is a marked reduction in 1^{st} -phase insulin secretion (0-10 min) following intravenous glucose administration, as well as impaired early-phase insulin secretion (within the first 30 min) in response to oral glucose [52, 57]. However, the late-phase insulin response (60-120 min) during an OGTT is typically preserved in these individuals [52, 57]. In contrast, isolated IGT is characterized by impaired early-phase insulin secretion following oral glucose, and a more pronounced reduction in late-phase insulin secretion, resulting in sustained postprandial hyperglycemia (**Figure 1**) [52, 57].

In isolated IFG, the combination of hepatic IR and reduced insulin secretion leads to excessive hepatic gluconeogenesis and thus fasting hyperglycemia. Impaired early-phase insulin

secretion along with hepatic IR, contributes to a further rise in plasma glucose during the 1st h of the OGTT [50, 57]. However, glucose levels typically return to baseline by 120 min due to preserved late-phase insulin secretion and intact skeletal muscle insulin sensitivity in isolated IFG state [50, 57]. In contrast, isolated IGT is characterized by deficient late-phase insulin secretion combined with both hepatic and peripheral (skeletal muscle) IR, resulting in sustained postprandial hyperglycemia following oral glucose administration [50, 57].

2. DIABETIC NEUROPATHY

2.1 Definition and classification

Diabetic distal symmetric polyneuropathy (DSPN) is a devastating complication of diabetes, leading to diabetic foot ulcers, neuropathic pain, decreased quality of life, and increased morbidity [58-61]. It is the commonest neuropathy in developed countries and one of the most frequent complications of diabetes with a median prevalence of 28-34% in community-based studies [62-64]. Classification of diabetic neuropathy is shown in **Table 3** [58]**.**

Table 3 Classification of diabetic neuropathy

	Subclassification
Diffuse neuropathy (DSPN)	Primarily small-fiber neuropathy
	Primarily large-fiber neuropathy
	Mixed small- and large-fiber neuropathy (most common)

Autonomic	**Cardiovascular**
	Reduced heart rate variability
	Resting tachycardia
	Orthostatic hypotension
	Sudden death (malignant arrhythmia)
	Gastrointestinal
	Diabetic gastroparesis (gastropathy)
	Diabetic enteropathy (diarrhea)
	Colonic hypomotility (constipation)
	Urogenital
	Diabetic cystopathy (neurogenic bladder)
	Erectile dysfunction
	Female sexual dysfunction
	Sudomotor dysfunction
	Distal hypohydrosis/anhidrosis
	Gustatory sweating
	Hypoglycemia unawareness
	Abnormal pupillary function
Mononeuropathy	Isolated cranial or peripheral nerve (e.g., cranial nerve III, ulnar, median, femoral, peroneal)
	Mononeuritis multiplex

Radiculopathy/ Polyradiculopathy	Radiculoplexus neuropathy
	Thoracic radiculopathy
Other neuropathies common in diabetes	Pressure palsies
	Chronic inflammatory demyelinating polyneuropathy
	Radiculoplexus neuropathy
	Acute painful small-fiber neuropathies (treatment-induced)

Abbreviations: DSPN, Diabetic Sensory Peripheral Neuropathy

2.2 Epidemiology

In a community-based study of 15,692 subjects with diabetes from Northwest England, the prevalence of diabetic DSPN, defined by the loss of pinprick, vibration, and temperature sensation, was 49%, while the prevalence of painful neuropathic symptoms was 34% [65]. In a cross-sectional multi-centre study of 6487 patients with diabetes in the United Kingdom, the overall prevalence of diabetic DSPN was 28.5% [66]. Subjects with T2D had a higher overall prevalence of DSPN than those with type 1 diabetes (T1D): 32.1% (30.6-33.6%) vs 22.7% (21.0-24.4%), respectively ($p<0.001$), regardless of diabetes duration[66]. In the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial, DSPN was present in 42% of adults with T2D at baseline [67].

According to several studies, approximately 50% to 66% of patients with diabetes will eventually develop DSPN [68]. Diabetes duration and glucose control are major predictors of diabetic DSPN development and progression [62]. In the UK Prospective Diabetes Study (UKPDS) study (including subjects with newly diagnosed T2D), DSPN was defined by a vibration perception threshold (VPT)>25 V [69, 70]. DSPN developed in approximately 20% of subjects regardless of treatment, resulting in an annual incidence of approximately 2%

during the first 12 years [69]. In the Danish Addition study, the incidence of DSPN was 10% over the 13-year follow-up in patients with newly diagnosed T2D without DSPN at study entry [71].

2.3 Pathological findings in diabetic DSPN: sensory neurons, dorsal root ganglia, and distal axon degeneration

From a neuroscientific perspective, diabetic DSPN is driven by intricate and not well understood biological processes that underline the development of a distinct, sensory-predominant axonal degenerative disorder. Diabetic DSPN is a length-dependent, sensory-predominant axonopathy and its characteristic "stocking-glove" distribution reflects that the most distal sensory terminals that innervate the skin of the toes and fingers [72-74]. Core neurophysiological and pathological features include slowed conduction velocity, altered sensory behavior, and marked loss of dermal/epidermal cutaneous axons [75-77].

1) Distal sensory axon terminal dysfunction as the initiating lesion in DSPN

A central neuropathophysiological concept is that DSPN begins at distal sensory terminals in skin, where axons normally require ongoing structural plasticity to maintain epidermal innervation and continuous keratinocyte turnover [78]. In diabetes this adaptive plasticity is impaired: sensory axons shift phenotype, retract, and disappear, producing progressive distal denervation that clinically manifests as numbness, pain and impaired protective sensation [72, 77-79]. This distal-first pattern aligns with the "stocking-glove" phenotype and the prominence of intraepidermal nerve fiber loss in human and experimental disease (**Figure 4**) [17-20].

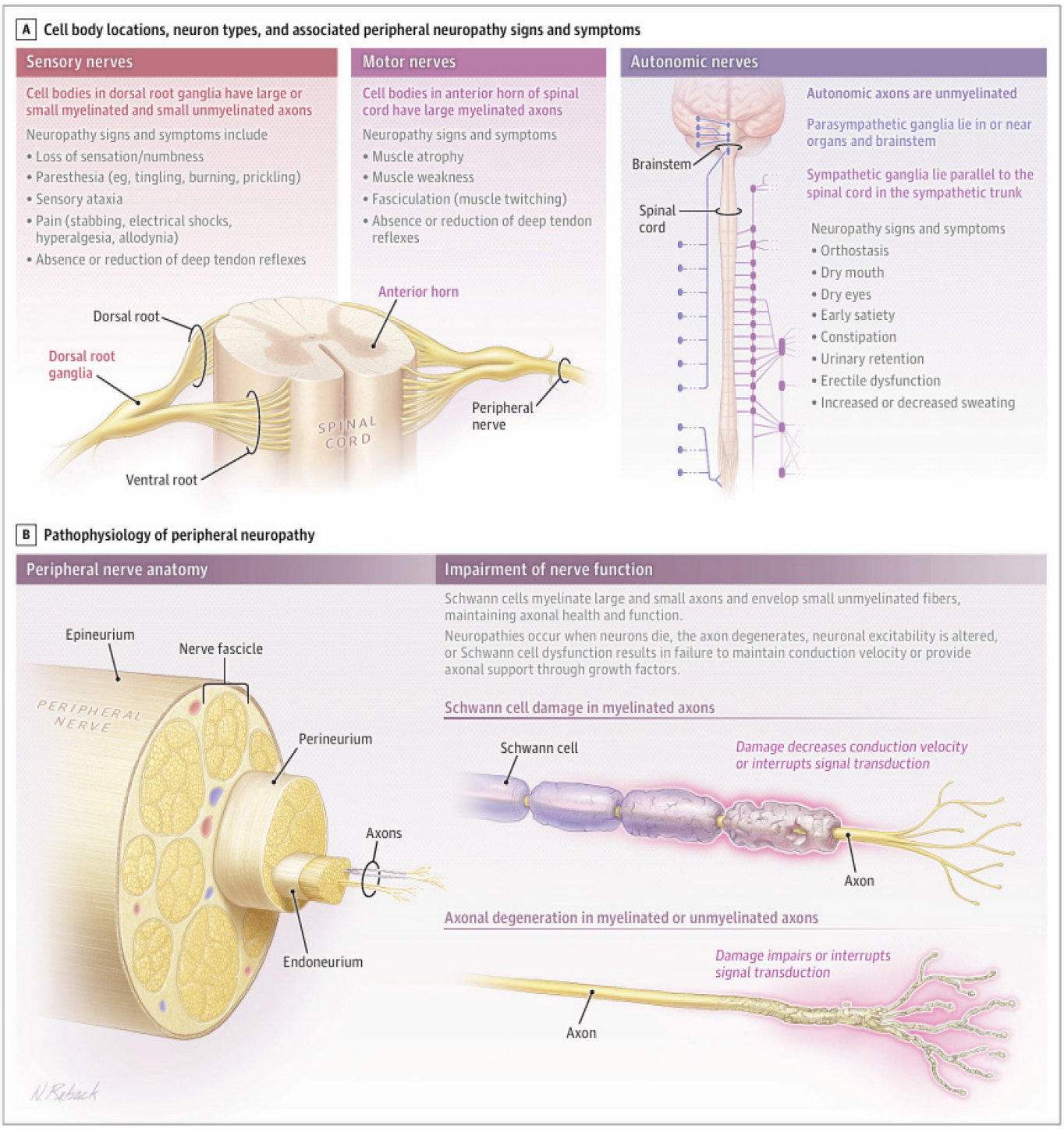


Figure 4 Anatomical basis of diabetic DSPN

Neuronal cell bodies are located in the anterior horn of the spinal cord (motor neurons), dorsal root ganglia (sensory neurons), and autonomic ganglia (autonomic neurons). DSPN may result from axonal degeneration and/or demyelination, leading to dysfunction of sensory, motor, and autonomic nerve fibers. Structural and functional abnormalities include Schwann cell dysfunction, impaired nerve conduction, and progressive distal axonal degeneration. Adapted from Mauermann M et al [80].

2) Dorsal root ganglion (DRG) involvement: a stressed but largely surviving neuronal population

The DRG is mechanistically important but relatively less investigated in DSPN. DRGs have distinctive vascular and barrier properties, including a barrier less restrictive than blood-brain or blood-nerve barriers [81], and specific physiological characteristics that may confer susceptibility to diabetic microvascular dysfunction. Despite this vulnerability, chronic experimental models demonstrate little or no DRG sensory neuron loss even after prolonged diabetes (e.g., preservation in long-duration rat models; modest loss reported in some mouse models) [76, 77]. Human post-mortem studies are limited and have reported variable degenerative changes and mostly mild loss of neurons in some cases [82-84]. In summary, most studies suggest that DSPN is driven primarily by neuronal dysfunction, atrophy and distal axon/terminal degeneration rather than great loss of sensory neuron cell bodies (**Figure 5**).

3) A paradox of distal axon loss with an inappropriate regenerative transcriptional response

Following axotomy, sensory neurons typically undergo an "axon reaction" involving chromatolysis and the induction of regeneration-associated genes (RAGs) such as tubulin, GAP43, and c-Jun in response to retrograde injury signals [85-87]**.** In DSPN, the distal terminals undergo degeneration in an 'axotomy-like' fashion, but the typical induction of RAGs is reduced or absent; growth-related proteins (tubulin and GAP43, in particular) do not increase as expected in experimental diabetes [76]. This mismatch-terminal degeneration without the typical accompanying switch to a pro-regenerative phenotype-shows that diabetes is characterized by reduced neuronal growth and impaired regenerative capacity.

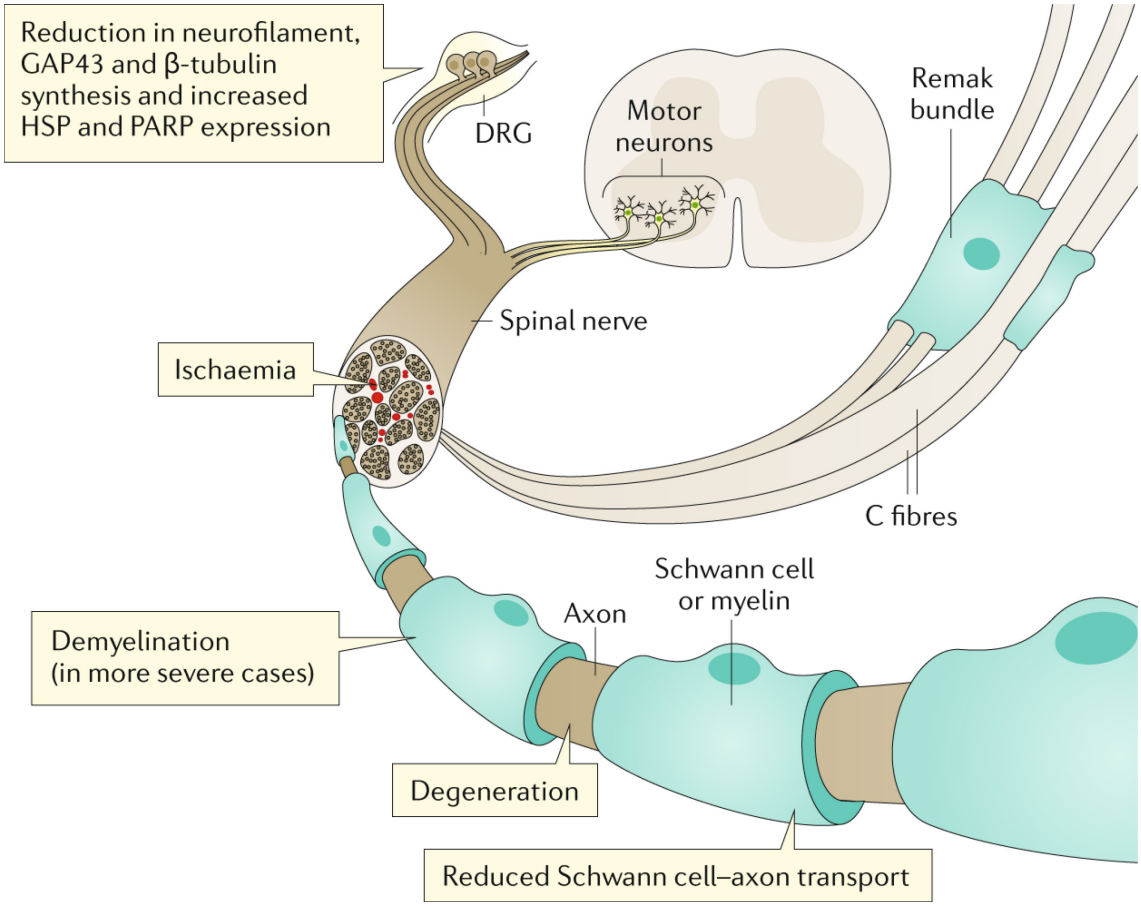


Figure 5 Integrated pathophysiological mechanisms underlying diabetic DSPN

Sensory neurons transmit sensory information from peripheral nerve terminals to the dorsal horn of the spinal cord, while their cell bodies are located in the dorsal root ganglia (DRG). Small unmyelinated sensory fibers are grouped in Remak bundles by non-myelinating Schwann cells, whereas larger sensory axons are myelinated by Schwann cells that preserve axonal structure and function. In diabetic DSPN, pathological changes include Schwann cell dysfunction, altered DRG protein expression, demyelination, oxidative stress, mitochondrial dysfunction, and progressive distal axonal degeneration. GAP43, growth-associated protein 43; HSP, heat shock protein; PARP, poly (ADP-ribose) polymerase. Adapted from Feldman et al [88, 89].

4) Microvascular contribution: DRG ischemic sensitivity and vasoconstrictor injury

In diabetes, blood flow to the DRG may be reduced in both T1D and T2D experimental models suggesting altered perfusion dynamics and/or metabolic demand [90, 91]. A strong mechanistic signal comes from endothelin-1 (ET-1): ET-1-induced vasoconstriction leads to transient

ischemia in non-diabetic nerves but causes significantly more severe damage in diabetes, including endoneurial infarction and axonal degeneration [91-93]. Regarding ganglionic vasculature, ET-1 results in more profound and prolonged reductions in DRG perfusion in diabetes, accompanied by biomarkers of sensory neuron injury and downstream sensory axon degeneration [94]. These findings support microvascular dysregulation as a permissive or amplifying factor in sensory neuron stress and distal axonopathy (**Figure 6**).

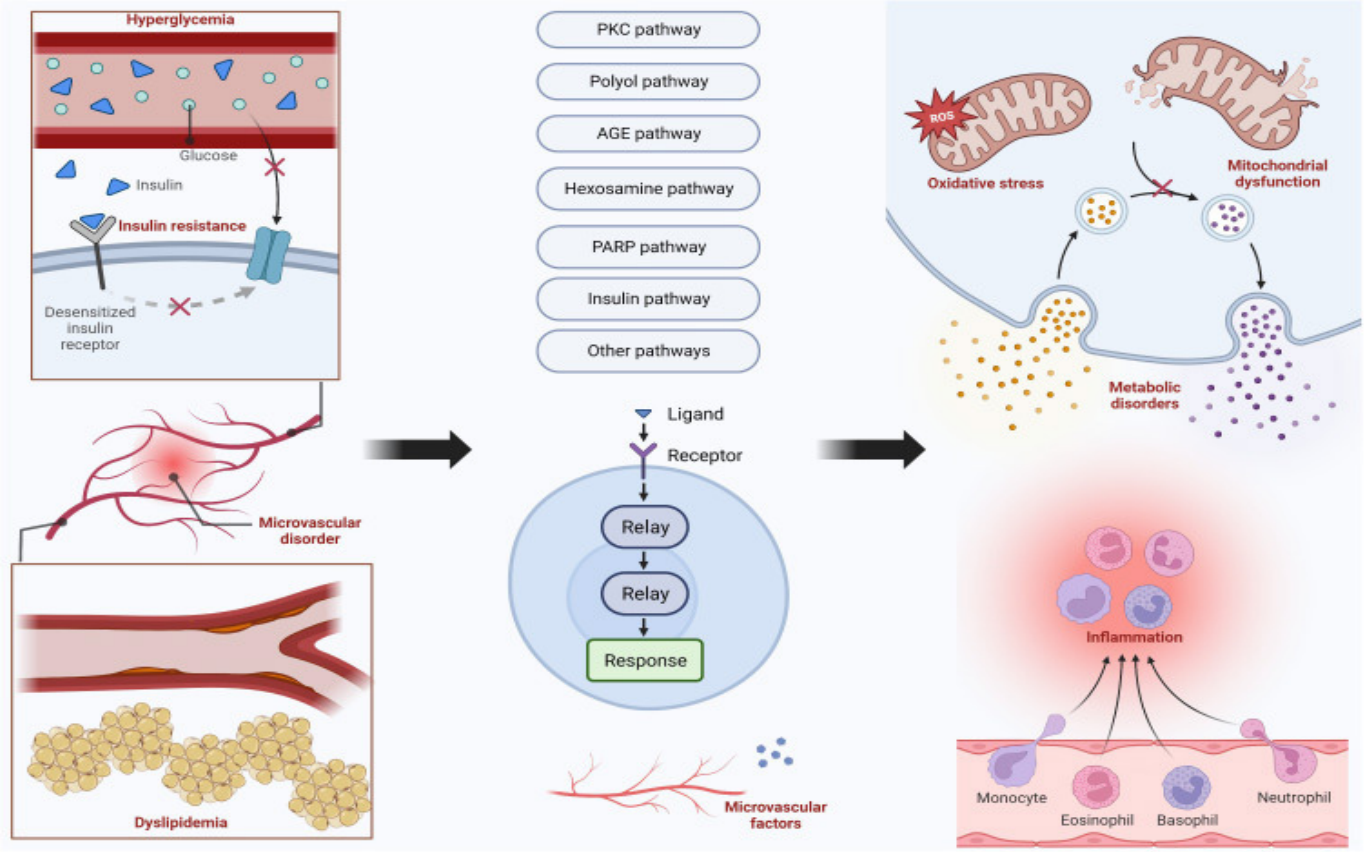


Figure 6 Important pathways and microvascular disorder contributing to diabetic DSPN

DSPN results from oxidative stress, mitochondrial dysfunction, and other metabolic pathways. Hyperglycemia, dyslipidemia, insulin resistance, and microvascular disorder are the four main factors that lead to Distal Symmetric Polyneuropathy (DSPN). Hyperglycemia and dyslipidemia are the most common two factors that can trigger the Protein Kinase C (PKC) pathway, polyol pathway, Advanced Glycation End-products (AGE) pathway, hexosamine pathway, and Poly(ADP-ribose) Polymerase (PARP) pathway. Insulin pathways, microvascular disorders, and other pathways are also activated to bring some harmful nervous effects, including inflammation, metabolic disorders, oxidative stress, and mitochondrial dysfunction. Adapted from Zhu J, et al [95].

5) Molecular phenotype: structural/growth downregulation with stress and excitability remodeling

The molecular phenotype described for diabetic sensory neurons includes the downregulation of structural and growth-associated genes (e.g., neurofilament subunits, tubulin, neuropeptides such as CGRP/SP, neurotrophins receptors, GAP43) and the up-regulation of stress- and injury-linked pathways (e.g., PARP activation, activated caspase-3, Mitogen-Activated Protein Kinase [MAPK] signaling pathway, HSP27) together with changes in ion channel expression [96]. Reduced neurofilament mRNA expression and diminished distal neurofilament content lead to structural instability of the axon, resulting in distal axonal atrophy [79], but genetic evidence (neurofilament knockout mice develop DSPN) argue against neurofilament loss as a primary driver, instead suggests that the loss of neurofilaments is not a causative factor but rather a marker of the pathological state [76]. Transcriptomic profiling in chronic diabetes also implicates abnormalities in RNA processing (GW bodies) and mRNA expression, consistent with the notion that post-transcriptional regulation is involved in the global "down-tuning" of neuronal maintenance programs [97, 98].

A notable mechanistic observation is the involvement of the spliceosome. Upregulation of the spliceosome-associated factor CWC22 in diabetic DRG nuclei is linked to suppressed neurite growth, while its knockdown increases neurite outgrowth in vitro and improves DSPN parameters (such as sensory conduction velocity and thermal sensation) in vivo in chronic diabetic mice, supporting the pathogenic role of splicing abnormalities in diabetic neuronal damage [99]. In parallel, emerging data suggests that the programmed axon degeneration (e.g., SARM1-linked pathways) may play a role in DSPN axonal loss [100-102].

6) Painful DSPN: channelopathy and ectopic excitability as a subtype biology

A key feature of diabetic DSPN is its phenotypic heterogeneity; notably, not all cases are painful, suggesting that excitability changes probably define a mechanistic subtype superimposed on distal axon degeneration rather than a general mechanism. There are studies showing the hyperexcitability of DRG neurons and axons [103-105].

2.4 Neurophysiological changes

Diabetic DSPN has traditionally been regarded as a disorder of the peripheral nervous system (PNS), primarily characterized by distal axonal degeneration and impaired nerve conduction [88]. However, advances in neurophysiology, neuroimaging, and translational neuroscience have fundamentally reshaped this view [106]. Emerging evidence demonstrates that diabetes induces widespread neurophysiological alterations that extend beyond peripheral nerves to the spinal cord and brain [88, 107]. These changes evolve dynamically over time and play a role in symptoms development, disease heterogeneity, and treatment resistance [106].

2.4.1 Large-fiber dysfunction

At the peripheral level, large-fiber involvement is characterized by progressive abnormalities in motor and sensory nerve conduction. Accumulated evidence demonstrates reduced conduction velocities, prolonged distal latencies, and decreased compound potential amplitudes, reflecting axonal degeneration with secondary demyelination rather than primary myelin pathology [108, 109]. Large-fiber neurophysiological abnormalities detected by conventional nerve conduction studies (NCS) typically represent relatively late manifestations in the natural history of diabetic DSPN, as these techniques primarily assess the functional integrity of large, myelinated fibers that are affected only after prolonged exposure to metabolic, vascular, and oxidative stress [108, 109]. Consequently, substantial peripheral nerve injury may already be present before measurable changes in conduction velocity or action

potential amplitude become apparent, leading to important diagnostic and conceptual limitations. In early diabetic DSPN, particularly among patients presenting with sensory symptoms or neuropathic pain, large-fiber conduction parameters may remain within normal limits despite significant neural dysfunction, contributing to the under-recognition of early disease and the misclassification of symptomatic individuals as having 'normal' neurophysiology [110, 111]. Furthermore, painful diabetic DSPN is frequently characterized by a weak correlation between symptom severity and large-fiber abnormalities, with patients experiencing severe burning pain, allodynia, or dysesthesia in the absence of marked conduction slowing or amplitude loss [108, 112]. In conclusion, these findings show the underdiagnosis of large-fibre nerve dysfunction and highlight the significance for broader neurophysiological assessments of small-fiber function and central nervous system (CNS) to reflect more accurately the heterogeneous and progressive nature of diabetic DSPN.

2.4.2 Small-fiber neurophysiology

In contrast to large fibers, small-diameter sensory fibers show an early and excessive susceptibility to the metabolic stress of diabetes. Studies using quantitative sensory testing, laser-evoked potentials, and intraepidermal nerve fiber density (IENFD) have shown an early impairment of thermal and nociceptive pathways, despite normal NCS [110, 111].

Emerging evidence demonstrates the intrinsic biological vulnerability of small sensory fibers, which are characterized by high metabolic demands, limited or absent myelination, and a strong dependence on intact microvascular perfusion. These features show the selective vulnerability of small-fibers to the metabolic, ischemic, and oxidative stresses associated with diabetes [113-115].

2.4.3 Spinal and supraspinal neurophysiological alterations

Beyond the PNS, there is strong evidence of the involvement of the spinal cord and brain in diabetic DSPN. Neurophysiological and imaging techniques have shown changes in the ascending sensory pathways, such as the dorsal columns and spinothalamic tracts, suggesting an abnormality in the transmission and modulation of somatosensory signals [116, 117]. This finding does not support the “dying- back” hypothesis but rather a parallel process of injury due to diabetes to the peripheral and central parts of the nervous system [116, 117].

Structural magnetic resonance imaging has consistently shown reductions in grey matter volume within regions to be crucial for sensory processing and pain regulation, such as the primary and secondary somatosensory cortices, thalamus, anterior cingulate cortex, insula, and prefrontal cortex (**Figure** *7*) [116, 117]. White matter abnormalities involving thalamocortical, corticospinal, and spinothalamic fibers further indicate impairments of sensorimotor integration [116, 117]. Notably, these changes are observed in both painful and painless diabetic DSPN, showing a generalized neurodegenerative process [116].

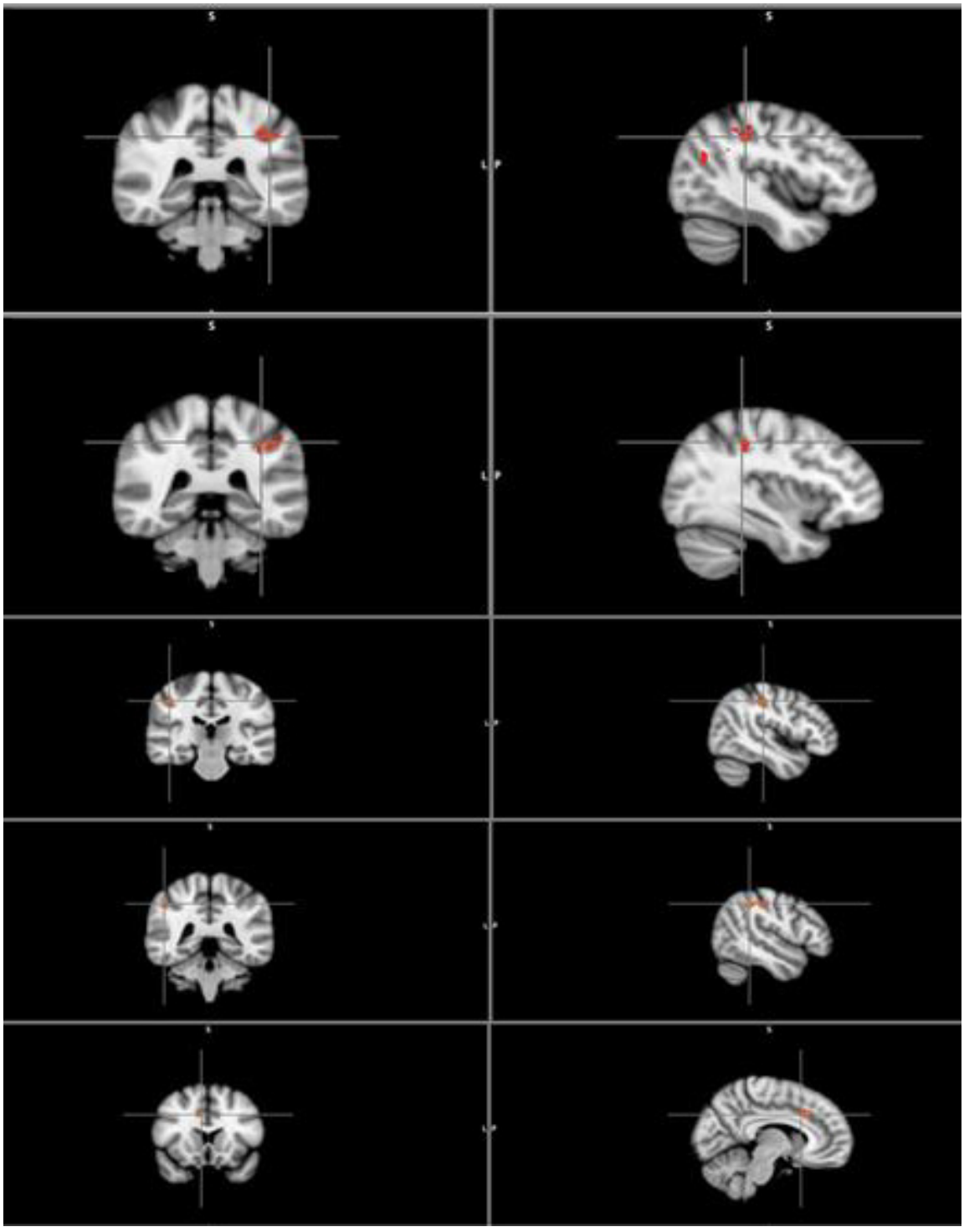


Figure 7 Structural brain alterations in DSPN

Structural magnetic resonance imaging demonstrates reduced grey matter volume in regions involved in sensory processing and pain modulation, including the somatosensory cortex, supramarginal gyrus, and cingulate cortex, in individuals with diabetic DSPN compared with subjects without neuropathy and healthy controls. Adapted from Selvarajah D, et al [112].

2.5 Aetiological factors

2.5.1 Hyperglycaemia and diabetes duration

DSPN accounts for 75% of diabetic neuropathies [118]. The principal role of hyperglycaemia in the onset and the progression of DSPN is well established (**Figure 8**). The Rochester study has demonstrated that duration of hyperglycaemia is associated with the severity of DSPN [119]. Optimised glycaemic control by intensified insulin in T1D, as in the Diabetes Control and Complications trial (DCCT), reduces the incidence of DSPN by 64% and improves nerve function, as assessed by NCS (faster sensory and motor conduction velocities and shorter F-wave latencies) [120]. However, this was not the case in patients with T2D in the UKPDS [69], Veterans Affairs Cooperative Study on Glycemic Control and Complication in NIDDM (VACSDM)[121], Steno-2 [122] and in the ACCORD [67]. The paramount importance of diabetes duration has been shown in other studies as well [62, 120, 123, 124].

2.5.2 Hypertension

In a study of subjects with T1D, hypertension was associated with deficits in NCS and decreased vibration perception, as compared with normotension [125]. After adjusting for age, gender, diabetes duration, HbA1c, baseline TGs, and body-mass index (BMI), tibial compound motor action potential (TCMAP) and tibial motor nerve conduction velocity (TMNCV) remained independently associated with systolic blood pressure [125].

Similarly, in a retrospective trial of 1262 subjects with diabetes, hypertension was associated with DSPN prevalence (odds ratio [OR]: 1.829, 95% CI: 1.146-2.920, p=0.011)[126]. The Maastricht study, a cross-sectional analysis of 2401 adults (25.3% T2D), demonstrated that antihypertensive medication (suggesting prior exposure to elevated arterial blood pressure) was related with lower peroneal motor action potential amplitude (CMAP) and nerve conduction velocity (NCV) (β = -0.13 [-0.23 - -0.03] and β = -0.16 [-0.25 - -0.0], respectively) [127].

According to experimental studies, streptozotocin-induced diabetic rats with or without hypertension developed nerve ischaemia, thermal hyperalgesia, reduced NCV and axonal atrophy [62, 120, 123, 124]. However, hypertensive diabetic rodents exhibited thinly myelinated fibers with supernumerary Schwann cells, indicative of demyelination and remyelination, along with reduced nerve levels of myelin basic protein [128]. In addition, the combination of hypertension and hyperglycaemia in rats with T1D is linked with reduced number of small unmyelinated sensory nerve fibers in the skin and mild axonal atrophy in myelinated tibial and sural nerve fibers in rats with T1D [129].

2.5.3 Dyslipidaemia

In several large observational studies, dyslipidaemia has emerged as a major factor in the pathogenesis of DSPN. In the EURODIAB study (n=1407 patients with T1D), elevated total cholesterol, Low-Density Lipoprotein cholesterol (LDL-C), TGs, and low HDL-C levels were associated with higher VPT values [130]. Of all lipid fractions, elevated TGs had the greatest association with DSPN risk (OR: 1.35, 95% CI: 1.15-1.57, p=0.002) over 7.3 years [130]. In a meta-analysis of 39 studies in diabetes, higher serum TGs and LDL-C levels were associated with increased risk of DSPN (OR: 1.36, 95% CI: 1.20-1.54, $p< 0.001$ and OR: 1.10, 95% CI: 1.02-1.19, p=0.274, respectively) [131].

Moreover, decreased baseline serum medium-chain acylcarnitines and increased free fatty acids were associated with 10-year incident DSPN development in a human study of serum lipidomics (69 patients with T2D) [132]. Conversely, high HDL-C levels were associated with decreased risk of DSPN (OR: 0.85, 95% CI: 0.75-0.96, $p <0.001$) [131]. Similarly, the Utah Diabetic Neuropathy study showed a positive association between plasma TGs ≥150 mg/dL and the risk of DSPN in patients with T2D (relative risk [RR]: 2.3, 95% CI: 1.1-4.7) [133]. In this context, lipid-lowering therapies were shown to reduce DSPN risk in some clinical trials. Especially, in the Fenofibrate and Event-Lowering in Diabetes (FIELD) study of 9795 patients

with T2D, fenofibrate significantly reduced non-traumatic amputations (hazard ratio [HR]: 0.62, p=0.011), an indirect index of DSPN[134]. In the Fremantle Diabetes Study, statins were associated with a significant 35% reduction of DSPN incidence in T2D [135]. In an exploratory analysis of the PROMINENT trial including 10,497 participants with T2D, allocation to pemafibrate was associated with a 37% relative reduction in incident lower extremity ischemic ulceration and gangrene compared with placebo (HR: 0.63; 95% CI 0.41–0.96; p=0.03) over a median follow-up of 3.4 years. No significant reduction in major amputation events was observed, likely due to low event rates and limited statistical power; importantly, pemafibrate was not associated with an increased risk of amputation, supporting a safety profile [136].

2.5.4 Obesity and insulin resistance

In a population-based study in Germany, obesity was identified as a predisposing factor for DSPN (OR: 3.47, 95% CI: 1.72-7.00) in 513 subjects with or without T2D [137]. In a study of 7442 participants with T2D, obesity was linearly correlated with DSPN development: HR: 1.29, 95% CI: 1.09-1.53 for men and HR: 1.66, 95% CI: 1.30-2.12 for women when BMI was 30.0-34.9 kg/m^2; HR: 1.52, 95% CI: 1.27-1.83 for men and HR: 1.77, 95% CI: 1.37-2.27 for women when BMI was 35.0-39.9 kg/m^2; HR: 1.31, 95% CI: 1.04-1.65 for men and HR: 1.51, 95% CI: 1.15-1.99 for women when was BMI ≥ 40 kg/m^2, all p<0.001] [138]. Similarly, abdominal obesity as defined by waist circumference (WC) ≥102 cm for men and ≥88 cm for women was associated with increased risk of incident DSPN [127, 138].

In this context, bariatric surgery was associated with improvement in symptoms of sensory, motor and autonomic neuropathy in a prospective study of 26 obese subjects with T2D after 12 months [139]. In a study of adults ≥40 years, obesity with or without T2D was associated with increased risk of DSPN (OR: 2.20, 95% CI 1.43-3.39), while greater prevalence was observed after excluding individuals with T2D [140].

Notably, in a 6-year follow-up study of Korean patients with T2D, insulin resistance showed a positive correlation (r=0.629, p=0.001) with abnormal sural sensory nerve action potential after adjustment for age, gender and height, even in patients with mean HbA_{1c} <7% over the past 6 years [141]. In a cross-sectional analysis of 982 subjects with T2D, homeostatic model assessment for insulin resistance (HOMA-IR) was an independent risk factor for DSPN (OR: 1.20, 95% CI: 1.07-1.34, p <0.001) [124].

2.5.5 Metabolic syndrome and prediabetes

DSPN prevalence increased as the number of metabolic syndrome (MetS) components increased (p=0.03) independently of glycaemic status in a large cross-sectional study of 2382 individuals (21% with T2D) over 11 years [142]. In the Prospective Metabolism and Islet Cell Evaluation (PROMISE) study, the prevalence of DSPN was not significantly different between those with and without metabolic syndrome (33% vs. 37%, respectively, p=0.4), but average VPT was higher among participants with vs without MetS (p=0.01) [143].

DSPN has been reported in individuals with prediabetes, suggesting that nerve injury may begin before the onset of overt diabetes. In the population-based MONICA/KORA Augsburg study including 1006 participants, the prevalence of DSPN was 7.4% in individuals with normal glucose tolerance (NGT), compared with 11.3% in IFG and 13.0% in IGT [144]. Similarly, in the San Luis Valley Diabetes Study in the United States, the prevalence of DSPN was 3.9% in individuals with NGT, compared with 11.2% among those with IGT, prior to diabetes diagnosis [145]. Together, these epidemiological data indicate that peripheral nerve damage may develop during earlier stages of dysglycemia, highlighting the importance of early detection and metabolic risk modification (**Figure 8**).

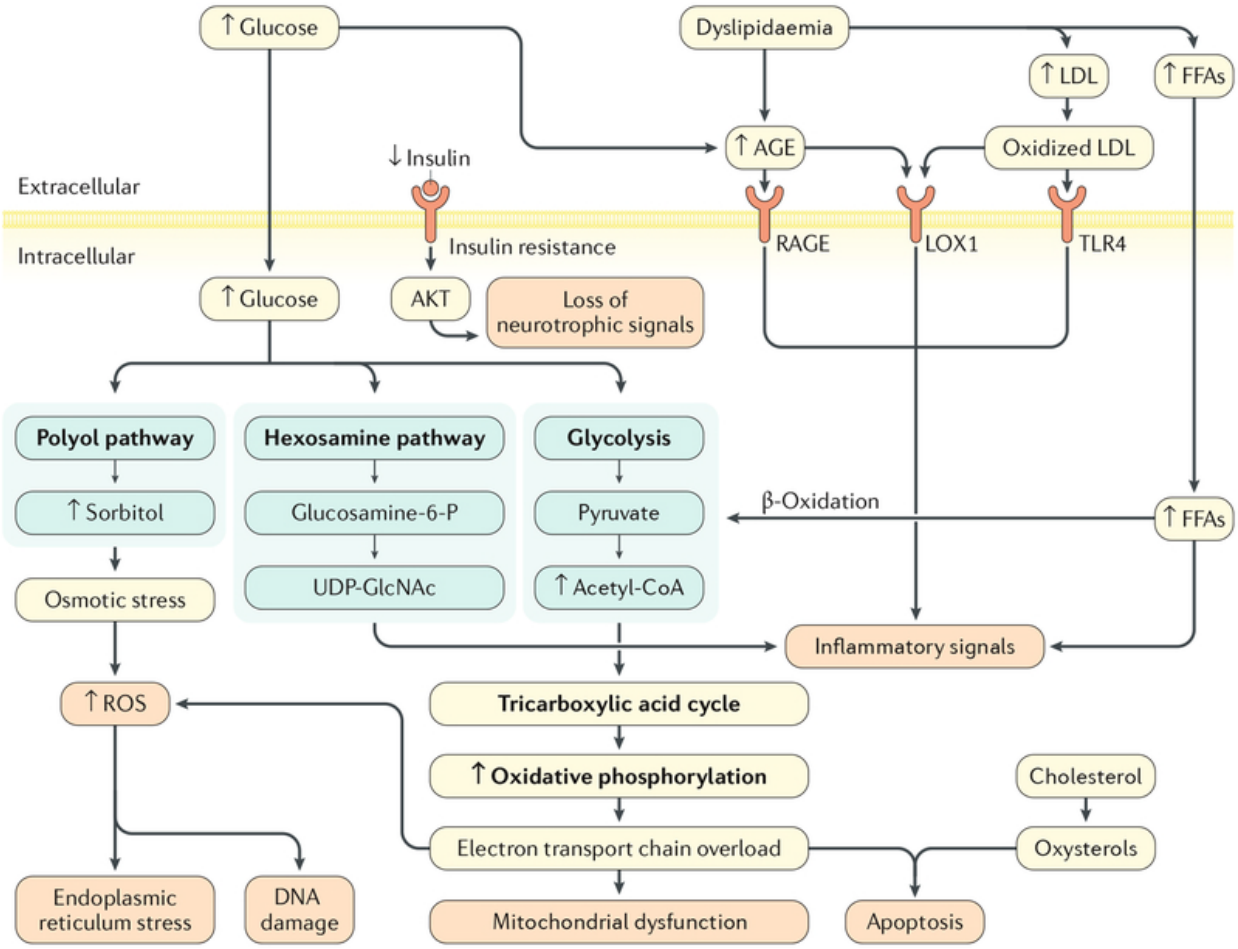


Figure 8 DSPN pathogenesis

Hyperglycaemia, dyslipidaemia, insulin resistance, obesity, oxidative stress, inflammation, and microvascular dysfunction contribute to Schwann cell injury, mitochondrial dysfunction, and progressive axonal degeneration in DSPN. Adapted from Feldman et al [88].

Abbreviations: AGE, Advanced Glycation End-products; Akt, Protein Kinase B; FFAs, Free Fatty Acids; LDL, Low-Density Lipoprotein; LOX1, Lectin-Like Oxidized Low-Density Lipoprotein Receptor-1; RAGE, Receptor for Advanced Glycation End-products; ROS, Reactive Oxygen Species; TLR4, Toll-Like Receptor 4; UDP-GlcNAc, Uridine Diphosphate N-Acetylglucosamine.

2.5.6 Hypoinsulinaemia

In a study of 133 patients with newly diagnosed T2D, low serum insulin concentrations before and after the oral administration of glucose were associated with the development of DSPN, even after adjustment for FPG values or HbA_{1c} ($p=0.03$) [146].

2.5.6 Smoking

There is evidence that current or past smoking [127, 130, 142] is a risk factor of DSPN in subjects with diabetes. In a large study of 9914 Japanese subjects with T2D, a significant correlation between DSPN-related sensory symptoms/signs and smoking status (OR: 2.04 for current and 1.64 for former smoking, $p<0.001$ and $p=0.002$, respectively) was found [147]. Likewise, in a cross-sectional study of 473 subjects with T2D, smoking was significantly associated with the risk of DSPN (OR: 1.64, 95% CI: 1.03-2.62, $p=0.037$) [148]. In a large observational, cross-sectional study of the Australian National Diabetes Audit (ANDA) data, current or past smokers with diabetes had greater risk of DSPN compared to never smokers (OR: 1.54, 95% CI: 1.36-1.74 and OR: 1.38, 95% CI: 1.26-1.51, respectively, all $p<0.0001$) [149].

2.5.7 Non-modifiable risk factors: age and height

Age is a well-recognised, non-modifiable risk factor of DSPN [127, 130]. According to a cross-sectional study, age is significantly correlated with DSPN (per 5 years increase) (OR: 1.12, 95% CI: 1.04-1.21, $p=0.003$) in subjects with T2D [124]. Multivariable logistic regression analysis in a study of 2382 participants with or without diabetes showed that age was significantly associated with abnormal 10-g (heavy) monofilament (OR: 1.14, 95% CI: 1.08-1.20, $p<0.05$) and 1.4 (light) monofilament (OR: 1.08, 95% CI: 1.05-1) [142]. Emerging data show that height is positively associated with risk of DSPN in subjects with or without diabetes (OR: 1.39, 95% CI: 1.24-1.54, $p <0.05$) [130, 142].

2.6 Clinical presentation of diabetic DSPN

Diabetic DSPN is characterized by a length-dependent neuropathic pattern, with symmetric distal (toe) involvement and proximal stocking-like distribution [80, 109, 150, 151]. As disease

progresses, the upper limbs may become affected, typically with distal onset at the fingertips and proximal extension in a glove-like pattern (**Figure *9a)*** [80, 108, 109, 150, 152].

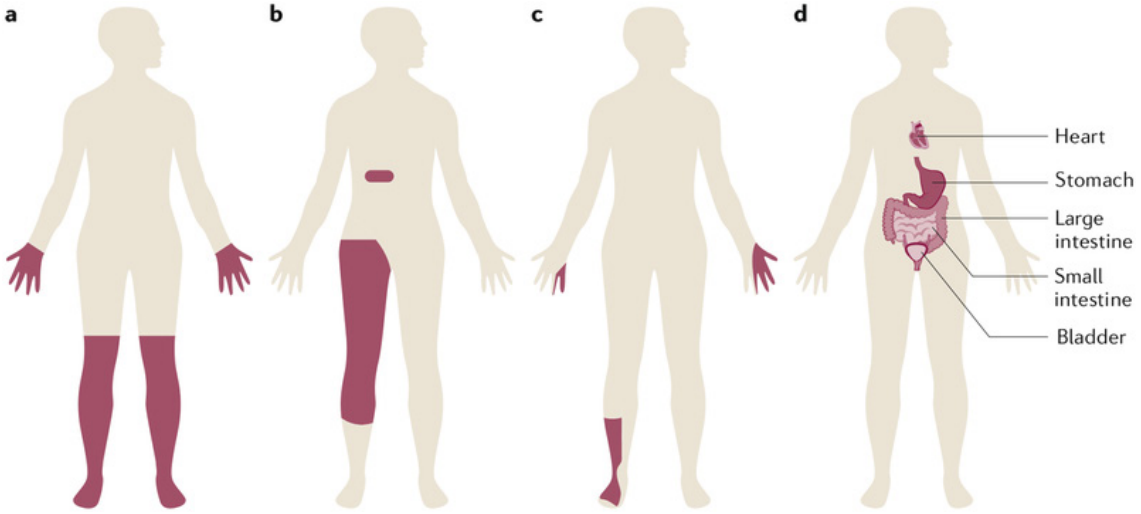


Figure 9 Patterns of nerve injury in diabetic neuropathy

Different neuropathic patterns may occur in individuals with diabetes, including distal symmetrical polyneuropathy (DSPN), small-fiber-predominant neuropathy, radiculoplexopathy, mononeuropathy, autonomic neuropathy, and treatment-induced neuropathy. DSPN is the most common phenotype and typically presents with a length-dependent stocking-glove distribution. Adapted from Peltier, Goultman, and Callaghan and Feldman et al [88, 153].

In rare cases, neuropathic involvement of the trunk or cranial regions has been reported [80, 150]. Both small and large nerve fibers may be involved; although some studies suggest early small-fiber dysfunction [154]. Isolated small-fiber neuropathy -characterized by impairment of pain and thermal sensation with relative preservation of motor function and large-fiber modalities- is uncommon [155]. Early motor manifestations include reduced ankle reflexes and distal muscle wasting, particularly in the hands and feet, predisposing to deformities such as claw toes and pes cavus [156]. These anatomical and functional alterations are associated with an increased risk of complications, including foot ulceration and Charcot neuroarthropathy [156].

Painless DSPN represents the most common clinical phenotype of diabetic neuropathy and is characterized by an insidious onset, progressive length-dependent sensory loss, and an absence of prominent neuropathic pain [58, 156]. Many patients remain asymptomatic, underscoring the importance of routine foot screening, as ulceration may be the first clinical manifestation. Sensory symptoms are commonly classified as positive (tingling, burning, pain) or negative (numbness, reduced sensation)[154]. They usually follow a length-dependent stocking-glove distribution, though pain may affect the upper limbs while sensory loss predominates in the lower limbs [151, 152]. With disease progression, impairment of motor function becomes evident, affecting tasks such as gripping, typing, or opening containers [156]. Autonomic nervous system involvement further contributes to morbidity, with reduced sweating leading to dry, fissured skin and an increased susceptibility to infection [115]. Consequently, comprehensive foot assessment is essential and should include evaluation of skin integrity, vascular health, and footwear, as poorly fitting shoes and abnormal pressure distribution are common precipitants of trauma and subsequent ulceration [61, 115].

Painful DSPN is characterized by neuropathic pain that may be severe and disabling [108, 154]. Patients commonly describe hyperalgesia or allodynia, although objective findings such as reproducible brush-evoked allodynia are uncommon [115, 157]. Pain may arise at any stage of nerve injury and shows marked interindividual variability [154]. Some patients experience severe pain despite profound sensory loss, the so-called “painful-painless leg” [154]. Burning pain and electric shock-like sensations predominate, frequently worsening at night and disrupting sleep [156, 158]. Despite its clinical burden, the natural history of painful DSPN remains poorly defined.

2.7 Diagnostic methods

Diagnosis of DSPN is mainly clinical after exclusion of other causes. Screening is recommended at the time of diagnosis of T2D and 5 years after the diagnosis of T1D on an

annual basis [58]. A combination of typical symptoms and symmetrical distal sensory loss or typical signs without symptoms in a person with diabetes is highly suggestive of DSPN [58]. The ADA proposes that patient evaluation should include a careful general medical and neurological history, foot inspection and a basic neurological examination of sensory function (10g Semmes-Weinstein monofilament for light touch, Tiptherm rod for temperature, calibrated Rydel Seiffer tuning fork for vibration [**Figure 10**], pin-prick for pain), as well as motor function and tendon reflexes (ankle and knee) [58].

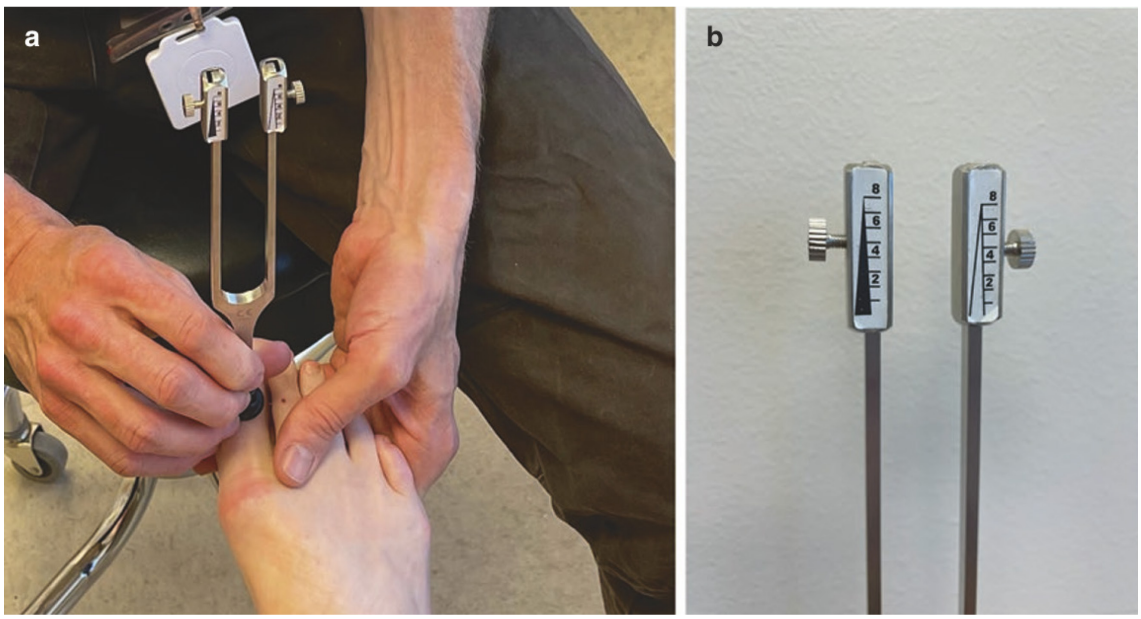


Figure 10 Assessment of vibration perception using the Rydel-Seiffer tuning fork

The Rydel-Seiffer tuning fork is a semiquantitative tool to assess vibration sensation. The tuning fork is placed on the great toe (a) and the two arms of the tuning fork are pinched and released. The triangles formed by the movement allow definition of the number when the sensation is no longer perceived (b)

Two tests assess DSPN and help quantify the risk of future foot ulceration: the 10-g Semmes Weinstein Monofilament, which evaluates protective sensation on the plantar aspect of the foot, and the biothesiometer [159, 160]. The latter is a medical device assessing sensation by

measuring the VPT on the foot: values >25 V are suggestive of increased risk for foot ulcer and amputation [159, 160].

Neuropathic symptoms are classified into *positive* (burning, shooting or pain, paraesthesias, dysaesthesias) and *negative* (numbness). Symptoms are distal, symmetrical, and often associated with nocturnal exacerbations [58].

The abovementioned neuropathic deficits may be assessed by established standardised clinical tools. These include the Michigan Neuropathy Screening Instrument (MNSI), the Neuropathy Symptom Score (NSS) and the Neuropathy Disability Score (NDS) [161, 162]. Quantitative sensory testing and NCS are used in expertized centres. Especially the latter are useful for differential diagnosis of DSPN, for cases with atypical symptoms and for use in research [58].

There are also newer diagnostic tools to enable earlier DSPN detection. These include the NC Stat DPNCheck (a hand-held device measuring sural sensory conduction velocity and amplitude), the SUDOSCAN (a non-invasive, test of sudomotor function of the feet and hands) and the Neuropad (a diagnostic tool assessing sudomotor function by an indicator pad on the plantar surface of the foot) [163-165].

More precise methods include skin biopsy with IENFD quantification (**Figure 11**) and corneal confocal microscopy (measuring corneal nerve fiber characteristics) (**Figure 12**) [155]. These yield good specificity and sensitivity, positive and negative predictive value, and are very useful for research purposes [166] .

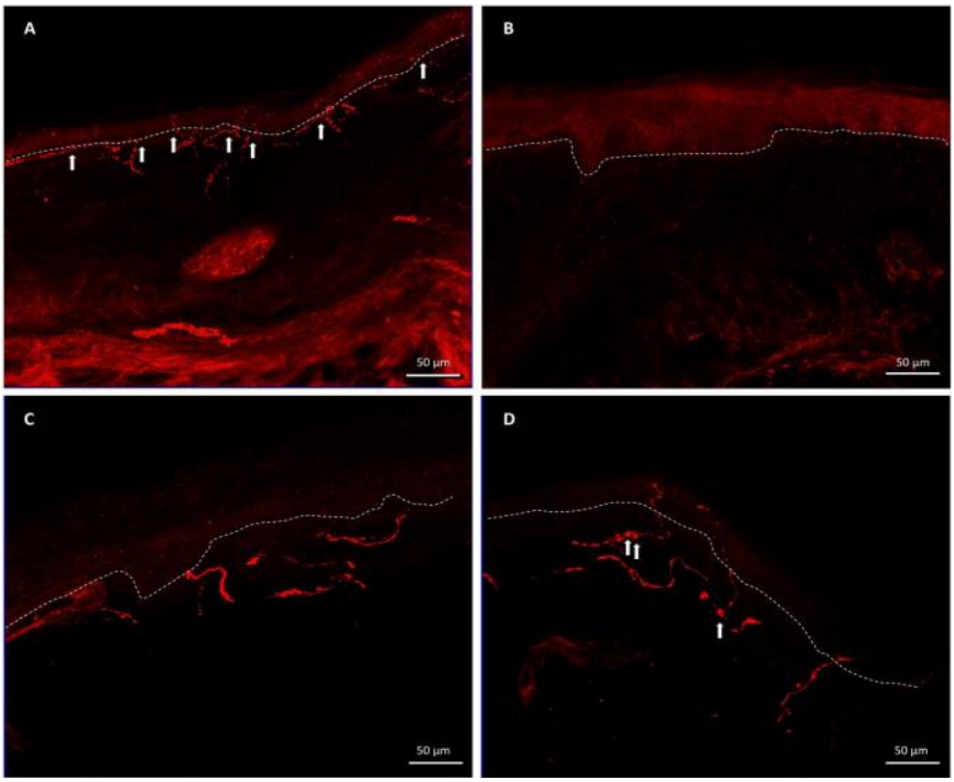


Figure 11 Skin nerve fiber changes in diabetic DSPN

(A) Intraepidermal nerve fibers (*arrows*) in a healthy individual. (**B**) Distal symmetric polyneuropathy (DSPN) patient with severe fiber loss. (**C**) High magnification of intraepidermal nerve fibers in a healthy individual (note absence of axonal swellings). (**D**) High magnification of intraepidermal nerve fibers in a DSPN patient (presence of axonal swellings, arrows). Adapted from Jensen et al [117]

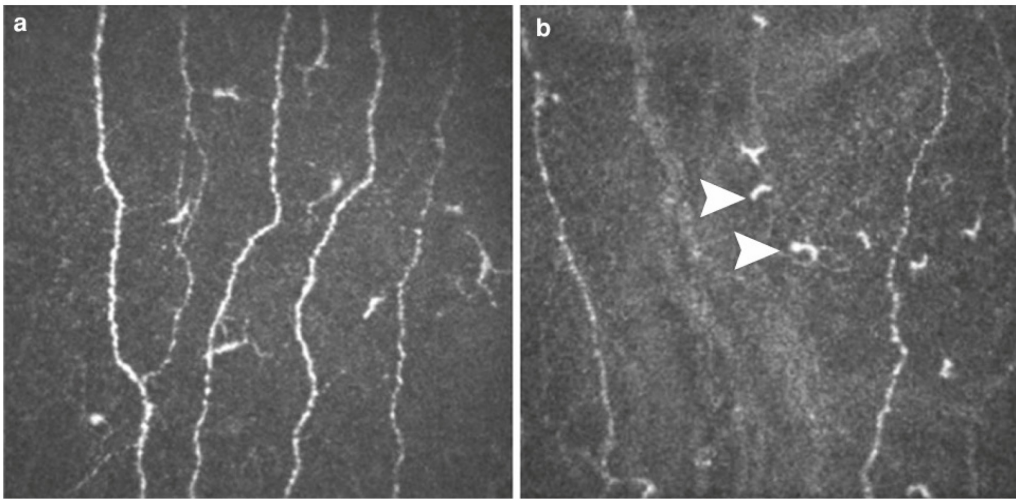


Figure 12 Corneal confocal microscopy

Corneal confocal microscopy (CCM) from a normal subject demonstrates normal nerve fiber length and density (a). CCM from a patient with diabetic DSPN demonstrates reduced nerve fiber length and density as well as proliferation of Langerhan's Cells (arrowheads) (b). Adapted from Davalos L et al [167].

2.8 Therapeutic Approaches

Management of patients with DSPN

No pathogenesis-oriented therapy has yet been approved by the U.S. Food and Drug Administration (FDA) or the European Medicine Association (EMA). Currently, treatment primarily aims at symptom relief and interventions are classified into: **A) symptomatic therapy**, **B) prevention of DSPN progression,** and **C) early detection and treatment of foot complications [168].** Therefore, proactive prevention and early intervention are crucial to mitigate the impact of this condition (**Figure 13**).

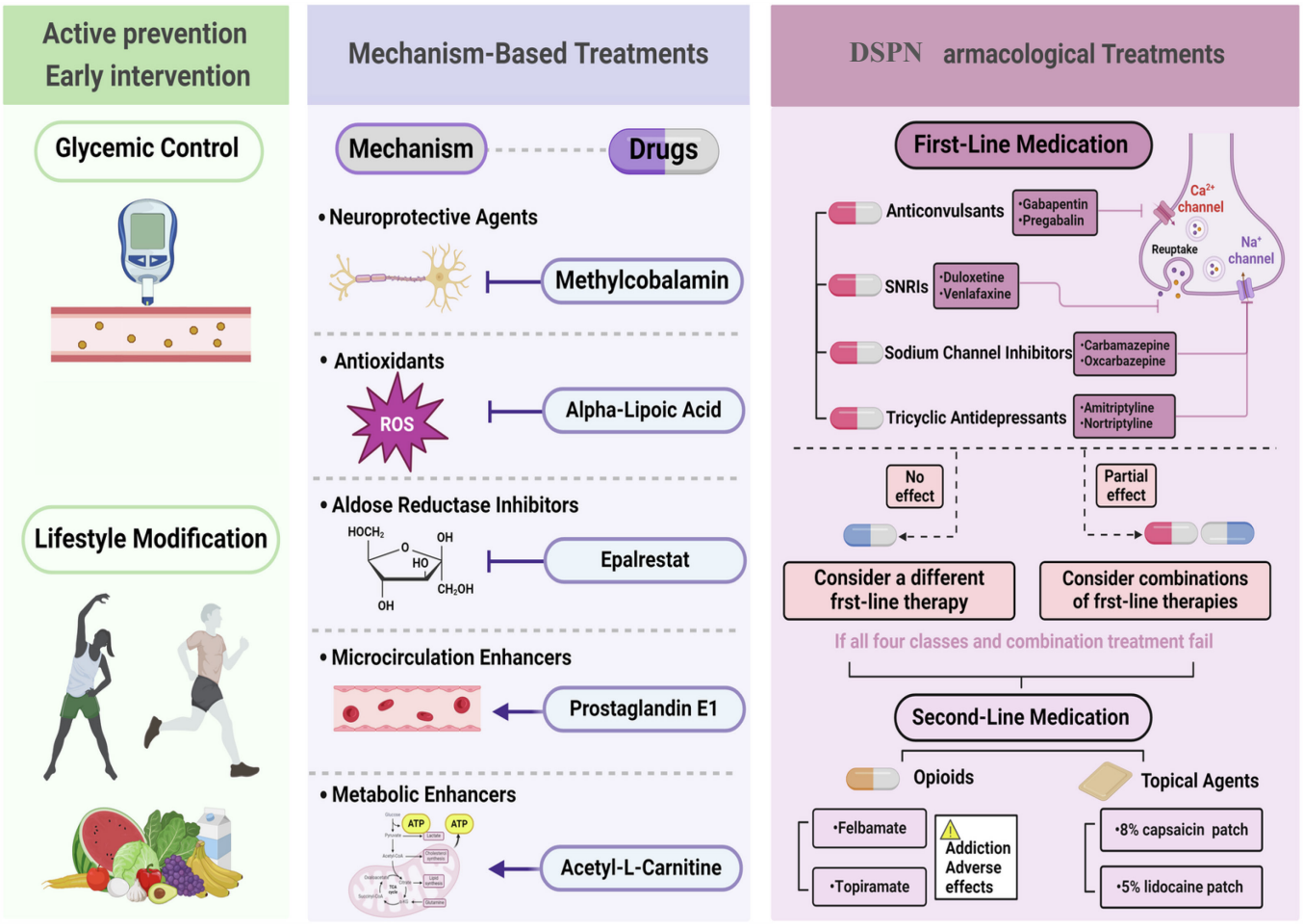


Figure 13 Mechanism-Based and Pharmacological Management

Glycemic control and lifestyle modification remain central to the prevention and management of distal symmetric polyneuropathy (DSPN). Mechanism-based therapies target oxidative stress, metabolic dysfunction, and microvascular impairment, whereas symptomatic treatment of painful DSPN includes gabapentinoids, serotonin-norepinephrine reuptake inhibitors (SNRIs), sodium channel blockers, and tricyclic antidepressants. Adapted from Feldman et al [106]

2.8.1 Symptomatic therapy: treatment of painful DSPN

The aim of symptomatic treatment is to reduce pain in the lower limbs and thus improve the quality of life [58]. Current treatments for painful DSPN are symptomatic and do not alter the disease progress [169]. According to the 2026 ADA Guidelines and the 2022 American Academy of Neurology Guidelines, first-line drugs for painful DSPN include α2δ ligands (gabapentin and pregabalin), serotonin-norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants (TCAs), and sodium channel blockers (Level A) [170-172]. Recently, Optimal Pathway For Treating Neuropathic Pain In Diabetes Mellitus Trial (OPTION-DM), a head-to-head trial showed bioequivalence for pregabalin, duloxetine and amitriptyline in pain resolution in DSPN (n=130 patients) (average mean reduction in pain as assessed by pain numerical rating scale (NRS) was 2.6 [98.3% CI: 2.2-3.0] over 6 weeks) [173]. Remarkably, OPTION-DM showed better pain control with combination therapy vs monotherapy. Recommended pharmacological treatment is presented in **Table 4** [58].

2.8.1.1 Anticonvulsants (α2δ ligands)

Pregabalin is U.S FDA approved and is regarded as the first-line drug [58, 174]. The efficacy and superiority of pregabalin in management of painful DSPN compared with placebo has been demonstrated in several clinical trials [58, 174, 175].

2.8.1.2 Tricyclic antidepressants

Amitriptyline is the most commonly administered agent from this drug class [174]. However, caution is required in cases of ischaemic heart disease or glaucoma. Remarkably, 1 in 3 patients cannot endure the minimal dose of amitriptyline due to adverse events [176].

2.8.1.3 Serotonin-noradrenaline reuptake inhibitors

Of SNRIs, duloxetine is FDA approved and the most commonly used agent for painful DSPN [58, 170]. Various randomised controlled trials have verified its effectiveness and head-to-head trials have demonstrated comparable efficacy to other agents, such as pregabalin and gabapentin [174].

2.8.1.4. Opioids

Tramadol is shown to have some efficacy in the management of painful DSPN, especially in combination with acetaminophen [177]. However, according to recent ADA clinical compendia, given modest efficacy in pain alleviation and the high risk of addiction, overdose and subsequent sedation, this drug class should not be administered in the treatment of painful DSPN [173, 178].

2.8.1.5 Combination therapy

Synergistic properties of 2 or 3 agents probably result in pain management at lower dosages and minimization of adverse effects. American Academy of Neurology recommendations (2022) suggest that when patients have partial amelioration with initial drug class, healthcare practitioners should initiate a medication sourced from alternative class or combination therapy with a medication derived from a distinct effective class (Level B) [172]. The COMBO-DN trial indicated that combination of duloxetine (60 mg/d) and pregabalin (300 mg/d) is possibly no more likely than either high-dose duloxetine (120 mg/d) or high-dose pregabalin (600 mg/d) to reduce pain (standardised mean difference -0.10; 95% CI: -0.33 to 0.13) [179]. However, the superiority of combination therapy over monotherapy for pain management in DSPN has been demonstrated in a recent, large, and long-term, head-to-head trial [173]. In OPTION-DM

crossover trial, 140 patients with DSPN and NRS ≥ 4 were randomly assigned to complete 6 sequences of these 3 different treatment pathways: amitriptyline with pregabalin added after 6 weeks in case of suboptimal respond NRS >3 at week 6 in monotherapy with amitriptyline (A-P), pregabalin with amitriptyline added in case of suboptimal response in monotherapy with pregabalin (P-A), or duloxetine with pregabalin added in case of suboptimal response in monotherapy with duloxetine (D-P) [173]. NRS score was significantly reduced from a baseline mean 6.6 to 3.3 at week 16 in all 3 pathways ($p<0.0001$) (no significant difference between them) [173]. Greater average pain reduction was demonstrated with combination treatment vs monotherapy [173].

2.8.2 Prevention of DSPN progress: management of cardiometabolic risk factors

2.8.2.1 Glycaemic control

Optimised glycaemic control seems to be more efficient in reducing the risk of DSPN development in T1D than in T2D. The DCCT study clearly showed that tight glycaemic control reduce the incidence of DSPN in patients with T1D [180]. However, residual risk of DSPN persisted after intensive glycaemic control in DCCT. Furthermore, good glycaemic control after pancreatic transplantation in T1D was shown to inhibit DSPN progression and reverse neuropathic deficits [181, 182]. In contrast to T1D, a large body of evidence support that enhanced glycaemic control in T2D mitigates the risk of DSPN development modestly (5-9% relative risk reduction) [67, 150]. Large studies such as ACCORD and UKPDS showed that intensive glycaemic control did not inhibit DSPN progress [67, 69]. However, in a recent study with neurophysiological parameters, tight glucose control significantly reduced the prevalence of DSPN and NDS, enhanced the outcomes of all neurophysiological tests (i.e. median nerve conduction velocity, conduction velocity and amplitude of ulnar and sural nerves, vibration and warm perception thresholds) in patients with T2D and those with IGT [183].

A recent meta-analysis of 10 observational studies in subjects with diabetes (n = 72,565) showed that patients with high glucose variability (GV) had greater risk of incident DSPN (risk ratio: 1.51, 95% CI: 1.23 to 1.85) compared with those with low GV (mean follow-up: 7.1 years) [184]. Similarly, GV, evaluated by mean amplitude of glycaemic excursions, was significantly correlated with DSPN prevalence (OR: 2.05, 95% CI: 1.36-3.09, p=0.001) in 90 subjects with T2D [185]. In addition to GV indices, continuous glucose monitoring (CGM) metrics such as time in range (TIR) has been inversely associated with DSPN presence (β = -0.106, p=0.033) in 261 subjects with T2D [186]. Highest TIR tertile (TIR≥77%) was associated with lower risk of slowing conduction velocity (OR: 0.26, 95% CI: 0.18-0.40, p<0.01), lower risk of amplitude reduction (OR: 0.60, 95% CI: 0.41-0.88, p=0.01) and greater rate of reduced latency (OR: 1.71, 95% CI: 1.16-2.53, p=0.01) [187]. Similarly, DSPN prevalence was inversely related with TIR in a cross-sectional study of 105 patients with T2D [188].

Despite the beneficial effect of glucose control in DSPN prevalence, treatment-induced neuropathy in diabetes (TIND) (also referred to as 'insulin neuritis') may rarely develop after rapid amelioration of glycaemic control in the setting of prolonged hyperglycemia. In a retrospective study of 954 patients with diabetes, 10.9% developed TIND occurring within 2-6 weeks of improvement in glucose control, and the absolute risk of TIND development was positively related with the magnitude and rate of HBA_{1c} decrease over 3 months [189]. The exact prevalence of TIND is unknown. Previous to that study, only some cases have been described [169].

2.8.2.2 Lifestyle and weight management

Supervised exercise in people with DSPN significantly reduced pain and neuropathic symptoms and increased intra-epidermal nerve fibre branching [190]. Interestingly, in the LOOK AHEAD study, long-term intensive lifestyle intervention for weight loss in overweight/obese patients with T2D resulted in a significant decrease in questionnaire-based

DSPN, which was related with the weight loss [191]. Moreover, bariatric surgery results in improvements in small nerve fibers in obese people with T2D [139]. However, the Diabetes Prevention Program Outcomes Study (DPPOS) showed no significant change in DSPN prevalence after long-term healthy lifestyle intervention in prediabetic patients [192].

2.8.2.3 Dyslipidaemia and hypertension

Statins may potentially prevent DSPN development in patients with T2D through dual mechanisms: lipid-lowering functions and pleiotropic effects (endothelial activation, anti-inflammatory and antioxidative effects) [193, 194]. In a large population-based cohort study of 37, 894 Taiwanese patients with T2D and dyslipidaemia, statin therapy was associated with reduced new-onset DSPN (HR: 0.85, 95% CI: 0.82-0.89) and new-onset diabetic foot ulcers (HR: 0.73, 95% CI: 0.68-0.78) [195]. Similarly, in a study of 259,625 individuals with T2D (48% non-statin users, 23% statin-naïve, 29% statin-prevalent users) the incidence rate of DSPN events per 1,000 person-years was similar in new users (4.0, 95% CI: 3.8-4.2), prevalent users (3.8, 95% CI: 3.6-3.9) and nonusers (3.8, 95% CI: 3.7-4.0) [196]. As already mentioned, in the FIELD study, fenofibrate decreased the incidence of non-traumatic lower-limb amputations, especially in patients without evidence of large-vessel disease at baseline [134]. Similarly, as previously reported, an exploratory analysis of the PROMINENT trial showed that pemafibrate was associated with a 37% relative reduction in incident lower extremity ischemic ulceration and gangrene; however, pemafibrate was not associated with an increased risk of amputation, supporting a neutral safety profile [136].

Data show that statins may increase the risk of DSPN. Many observational, case control studies reassure the absence of association between statins and DSPN development [197, 198]. A TriNetX-based retrospective analysis including 3,237,051 individuals with T2D and hypercholesterolemia (972,929 statin users) reported a higher incidence of DSPN among statin

users (5.6% vs 3.7%; RR 1.50, 95% CI 1.48-1.52) [199]. A systematic review including 66 studies evaluating the association between statins and peripheral neuropathy in both diabetic and non-diabetic settings reported that statins may exert neuroprotective effects in DSPN, whereas prolonged exposure has been minimally associated with idiopathic neuropathy, primarily in non-diabetic populations [194].

Hypertension treatment has shown beneficial effects on DSPN. A study demonstrated that combined calcium channel blocker (CCB) and angiotensin-converting-enzyme (ACE) inhibitor or ACE inhibitor treatment alone were associated with reduced risk of incident DSPN compared with placebo in hypertensive patients with T2D [200].

2.8.3 Disease-modifying treatment-Nutraceuticals

2.8.3.1 A-lipoic acid

A-lipoic acid has been suggested as potential therapy for DSPN due to the antioxidant effects [162, 201]. Studies showed that intravenous α-lipoic acid ameliorated neuropathic symptoms and signs after 3 weeks, and oral treatment for 5 weeks improved pain, numbness, and paraesthesia [162, 202]. Multiple meta-analyses confirmed that a-lipoic acid administration is beneficial and efficient in alleviating of neuropathic symptoms and pain [203]. In the NATHAN 1 trial (Neurological Assessment of Thioctic Acid in Diabetic Neuropathy), which encompassed 460 patients with diabetes and mild to moderate, largely asymptomatic DSPN, 4-year treatment with a-lipoic acid (600 mg/ daily) resulted in improvement of neuropathic symptoms, inhibition of neuropathic signs aggravation and was well-tolerated [203, 204]. Interestingly, in a study of 72 patients with painful DSPN, treatment with a-lipoic acid (600 mg/ daily) for 40 days reduced neuropathic symptoms as assessed by NSS (mean NSS score decreased from 7.9 at baseline to 5.3 at day 40, $p < 0.001$) and Subjective Peripheral Neuropathy

Screen Questionnaire (SPNSQ) (mean SPNSQ score decreased from 8.8 at baseline to 4.4 at day 40, $p < 0.001$) and improved quality of life [93]. A-lipoic acid is approved and suggested by guidelines in many countries with primary indication symptomatic DSPN, except for United States and Canada. [205].

2.8.3.2 Benfotiamine

Benfotiamine, a lipid-soluble synthetic S-acyl prodrug of thiamine that inhibits the major metabolic pathways of hyperglycemia (such as Advanced Glycation End-products [AGE] formation, the Protein Kinase C [PKC] pathway, Nuclear Factor kappa-light-chain-enhancer of activated B cells [NF-κB] activation and the hexosamine pathway), is approved and recommended by regional guidelines as treatment of DSPN, but not in the United States or Canada [176, 205]. The BENDIP (Benfotiamine in Diabetic Polyneuropathy) study demonstrated that benfotiamine in a dose of 300 mg twice daily reduced neuropathic pain and symptoms after 6 weeks of therapy (n= 165 patients with DSPN) [206]. B-complex vitamins of B1 (thiamine), B6 (pyridoxine), and B12 (cobalamin) play a crucial role for nerve health and function [206].

2.8.3.3 Actovegin

Actovegin, a deproteinised hemoderivative of calf blood, is currently used to treat DSPN in Russia and Eastern Europe [207]. In a multi-centre trial, 567 subjects with DSPN were randomly assigned to receive either 20 intravenous actovegin administrations (2000 mg/day) followed by 3 daily actovegin tablets (1800 mg/day) or a placebo over a 140-day period [207]. Actovegin reduced neuropathic pain and vibration perception threshold, with a safety profile comparable to placebo [208]. Similarly, in a study of 567 patients with DSPN actovegin reduced neuropathic symptoms and/or deficits after 6 months of treatment [209].

2.8.3.4 Metanx

Metanx is a medical food including including L-methylfolate (a form of vitamin B9), methylcobalamin (a form of vitamin B12), and pyridoxal 5'-phosphate (a form of vitamin B6). In a multi-centre, randomised clinical trial of 214 subjects with DSPN, Metanx significantly reduced neuropathic pain at week 16 ($p<0.05$ vs placebo) and week 24 ($p<0.05$ vs placebo), while had no impact on VPT [210]. Real-world data confirm the efficacy of metanx in alleviation of neuropathic pain [211].

2.8.3.5 Vitamins (vitamin B12, vitamin D and vitamin E)

Vitamin B12

In a 12-month randomised controlled trial, oral vitamin B12 supplementation at a daily dosage of 1000 μg in patients with DSPN and low vitamin B12 levels (<400 pmol/L) on metformin improved neurophysiological parameters, pain scores, sudomotor function, quality of life but not MNSI score [212]. Interestingly, in a smaller pilot study, vitamin B12 supplementation reduced pain and improved electrochemical skin conductance in the foot [213].

Vitamin D

Accumulating evidence suggests a correlation between low vitamin D levels and DSPN [214]. In subjects with DSPN (many of whom were vitamin D deficient), 24 weeks of high-dose vitamin D (40,000 IU/week) improved neuropathic symptoms compared with lower dose vitamin D (5,000 IU/week) [215]. Interestingly, there is an ongoing multicentre, randomized, double-blinded, placebo-controlled trial which aims to investigate the efficacy and safety of intramuscular injection of high-dose vitamin D in patients with DSPN and vitamin D insufficiency [216].

Vitamin E

Vitamin E did not ameliorate neuropathic symptoms after 1 year of treatment in patients with DSPN [217] in one study, although 3 randomised control trials showed beneficial effects of vitamin E in lancinating neuropathic pain in patients with DSPN [218].

Table 4 Current pharmacological agents for painful DSPN

Pharmacotherapy	**FDA Approval for DSPN**	**EMA Approval for DSPN**	**Up-titration and daily dosage**	**Adverse effects**
Anticonvulsants				
Pregabalin	Yes	Yes	Initial: 50 mg/ day Titration up to 100 mg/day per week A total daily dose of 300 mg is the maximum dose approved by the FDA for diabetes-associated neuropathic pain (some patients may require up to 600 mg/day for pain relief)	Somnolence, vertigo, peripheral edema, water retention, visual disturbances

			It can be given twice, or, on occasion, three times daily	
Tricyclic antidepressants				
Amitriptyline	No	No	Initial dose: 25 mg/day Increase if needed to a maximum of 150 mg/ day It can be given once daily, usually early evening	Xerostomia, water retention, increased appetite, weight gain, constipation, vertigo
Serotonin-noradrenaline reuptake inhibitors				
Duloxetine	Yes	Yes	Initial dose: 60 mg/day (in single daily dose or divided q12h) Target dose: 60 mg/day, not to exceed 60 mg/day	Nausea, somnolence, xerostomia, vertigo, decreased appetite
Opioids				
Tramadol	No	No	Initial dose: 25 mg/ day	Vertigo, nausea, somnolence,

			Titrate in 25 mg as separate doses every 3 days to 100 mg/ day (25 mg 4 times per day) After titration, 50-100 mg q4-6h for pain relief Not to exceed 400 mg/day	constipation, headache, central nervous system stimulation
Pathogenesis-oriented treatment				
α-Lipoic acid	No	No	600-1800 mg orally or 600 mg/day for 3 weeks, excluding weekends	Nausea, vomiting, abdominal discomfort, diarrhea

Abbreviations: FDA; Food and Drug Administration, EMA; European Medicines Agency

Limitations and room for improvement

Currently, our insights into DSPN are limited by the various definitions, variable diagnostic criteria, study designs, and assessment tools across studies [218]. Moreover, little is known on the cost-effectiveness of novel diagnostic tools, as well as their use in large scale [219].

There are also limitations regarding treatment. Currently, there is no generally established disease modifying therapy for DSPN. Except for NATHAN 1, therapeutic trials, generally, had

short follow-up [109]. Hence, comprehensive evaluation of long-term outcomes, efficacy and safety are constrained. In addition, while diabetes management is characterised by digital technology solutions, DSPN is yet to undergo a more substantial shift toward digital methodologies, which will improve the quality of patients' life [220]. Currently, comparing to our understanding of other diabetic complications such as retinopathy and nephropathy, the application of genomic technologies to DSPN and especially neuropathic pain is in its early stages [221]. A further limitation is inherent in the subjective nature of neuropathic pain. The latter is hard to be phenotyped, and therefore large cohorts that phenotype DSPN and neuropathic pain have not been fully developed [222]. However, these issues are now increasingly being addressed in large cohorts in UK-Biobank [222]. Such cohorts, along with advancements in sequencing technology and the creation of human cell models, hold promise for improved insights into genomics concerning DSPN, neuropathic pain and potential response to analgesics.

3. DSPN IN PREDIABETES

3.1 Definition

DSPN in prediabetes is initially a small-fiber, peripheral neuropathy in individuals with IFG and/or IGT, in the absence of established diabetes [89, 223, 224]. This disorder primarily affects the sensor nerve fibers in a symmetrical distal distribution and represents an early manifestation of DSPN prior to the onset of overt diabetes.

3.2 Epidemiology

DSPN has been increasingly recognized in individuals with prediabetes, including those with IFG and/or IGT, suggesting that peripheral nerve dysfunction develop during early

dysglycemic states [225-227]. Due to a variety of study populations and methods of assessing DSPN, there is marked heterogeneity in the prevalence and incidence estimates. Most studies report a higher prevalence of DSPN in prediabetes compared with the general population, predominantly characterized by small-fiber involvement. In a recent systematic review of 29 studies (9351 participants) prevalence varied substantially, ranging from 2% (95% CI: 0-4%) in a study conducted in the USA in women to 77% (95% CI: 54-100%) in a Brazilian study [226]. Most studies reported DSPN prevalence estimates of ≥10%, primarily based on small nerve fiber tests [226]. Among hospital-based cross-sectional studies, 10 of 12 (83%) reported prevalence estimates ≥20% [226]. Notably, the 3 studies reporting the highest prevalence used plantar thermography, quantitative sensory testing (QST), and NCS respectively, reflecting the impact of diagnostic sensitivity on reported rates [226]. Meta-analysis of epidemiological data is not feasible due to substantial clinical and statistical heterogeneity across studies investigating prevalence estimates, driven by variability in DSPN assessment techniques, study design, sampling strategies and population characteristics. Further stratification by geographic region or prediabetes subtype is also not feasible due to inconsistent methodology. For example, when DSPN was defined as “possible” by the Toronto criteria [228], in which the prevalence reached 17.4% in the NGT population, but when more stringent definitions (“probable” or “confirmed”) were applied, the prevalence decreased to 4.2% for the combined categories, highlighting the strong influence of diagnostic criteria on reported prevalence estimates [229]. Another potential source of bias is the inconsistent definition of prediabetes across studies, including different diagnostic thresholds (e.g., ADA vs WHO criteria) and the lack of differentiation between IFG and IGT, despite their distinct pathophysiological profiles [230]. Despite these discrepancies, a recent systematic review reported that the prevalence of DSPN among population-based studies ranged from 4.2% to 11.3% in IFG, 1.5% to 26.0% in IFG/IGT, 7.7% to 29.0% in those without prediabetes, and 7.7% to 49.0% in known diabetes

[227]. Only the KORA F4 study reported separate prevalence estimates for isolated IGT and combined IFG+IGT, 14.8% and 23.9%, respectively [231].

Several hospital-based studies have reported an increased prevalence of DSPN in prediabetes [226]. A cross-sectional analysis was conducted at the 3-year follow-up in a cohort of 458 subjects of the PROMISE study, of whom 336 had NGT, 100 had IGT, and 22 had diabetes at baseline [143]. Prevalence rates according to the MNSI >2 criteria for DSPN were 29% in NGT, 49% in prediabetes, and 50% in newly diagnosed diabetes, showing a significant increasing trend ($p < 0.001$) [143].

3.3 Pathophysiological Mechanisms

3.3.1 Hyperglycemia and IR

Human studies demonstrate that even modest elevations in glucose are sufficient to disrupt peripheral nerve homeostasis, particularly in the context of IR. In subjects with IGT, modest but chronic hyperglycemia is sufficient to activate metabolic and vascular pathways that contribute to the development of DSPN [223, 232].

At the cellular level, dysglycemia promotes metabolic stress within peripheral nerves through increased flux of glucose into alternative metabolic pathways, including the polyol pathway, hexosamine biosynthetic pathway, and PKC activation [88]. Although these pathways have been well-described in diabetes, experimental evidence indicates that partial activation occurs under prediabetic glycemic conditions, leading to early mitochondrial dysfunction, increased production of reactive oxygen species (ROS) and impaired axonal transport within peripheral nerves [88, 222] (**Figure 14**). Importantly, these metabolic abnormalities appear to preferentially affect small unmyelinated and thinly myelinated fibers, consistent with the predominantly small-fiber phenotype observed in prediabetic DSPN in humans.

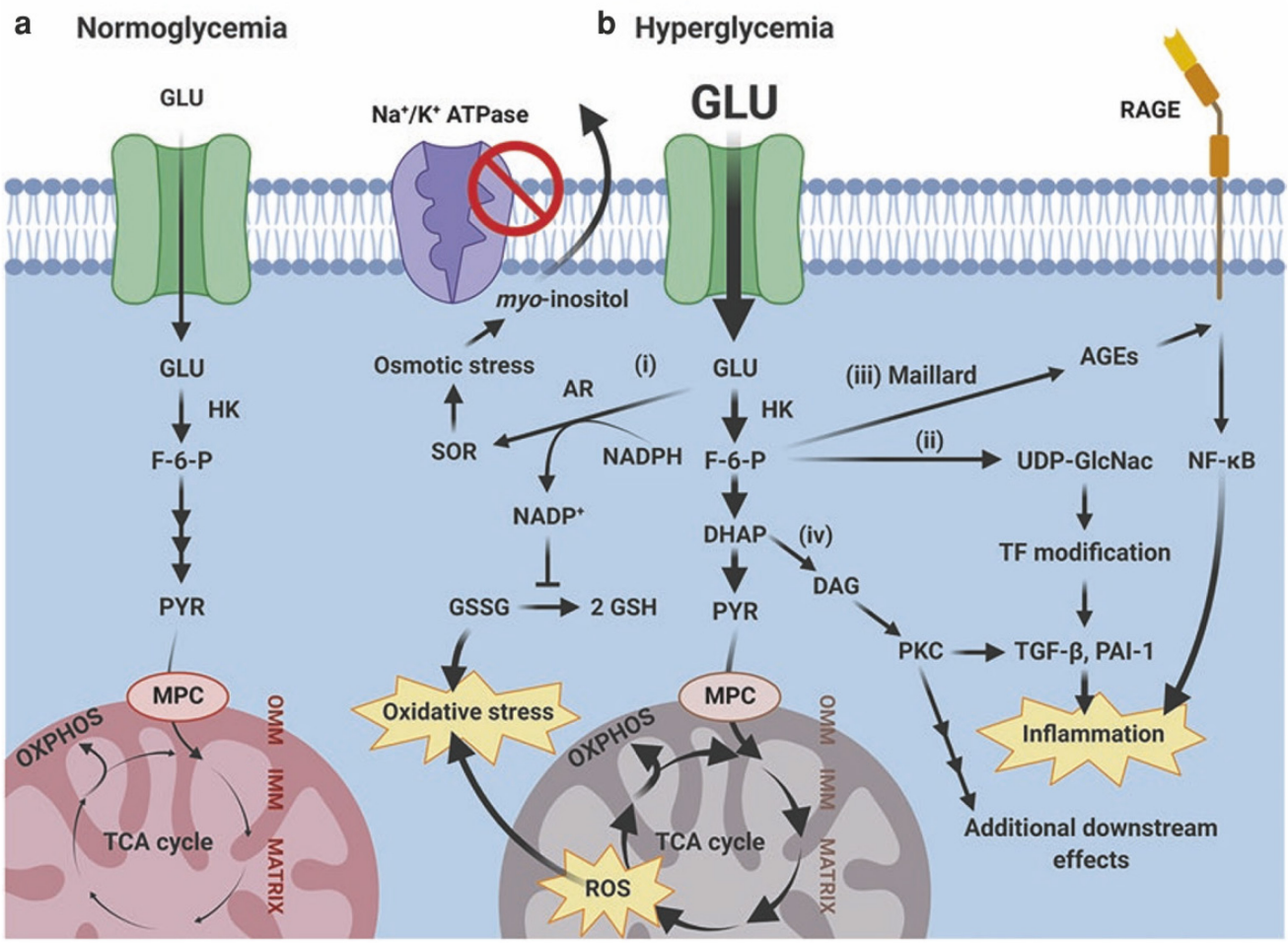


Figure 14 Hyperglycaemia-induced metabolic and inflammatory pathways involved in DSPN

Under physiological conditions, glucose (GLU) enters axons through insulin-independent glucose transporter 1 (GLUT1) and undergoes glycolysis via hexokinase (HK) to form fructose-6-phosphate (F-6-P), followed by mitochondrial tricarboxylic acid (TCA) cycling and oxidative phosphorylation (OXPHOS) to generate cellular energy while maintaining normal mitochondrial integrity. In chronic hyperglycaemia, excessive intracellular glucose increases mitochondrial reactive oxygen species (ROS) production, resulting in oxidative stress and mitochondrial dysfunction. Surplus glucose is diverted into several pathogenic pathways, including the polyol pathway mediated by aldose reductase (AR), the hexosamine pathway, the advanced glycation end products (AGEs) pathway, and the protein kinase C (PKC) pathway. Activation of these pathways disrupts osmotic balance, impairs sodium–potassium adenosine triphosphatase (Na+/K+-ATPase) activity, depletes antioxidant defenses such as glutathione (GSH), alters transcription factor (TF) regulation, and promotes inflammatory signaling through nuclear factor-kappa B (NF-κB), transforming growth factor-beta (TGF-β), and plasminogen activator inhibitor-1 (PAI-1). Additionally, AGEs interact with the receptor for advanced glycation end products (RAGE), amplifying inflammation and vascular dysfunction. Collectively, these mechanisms contribute to axonal dysfunction, microvascular injury, inflammation, and progressive peripheral nerve damage characteristic of distal symmetrical polyneuropathy (DSPN). Adapted from Eid, et al [233].

Rodent models provide critical mechanistic insight into the effects of early dysglycemia on peripheral nerves. In diet-induced obesity and IGT models, animals develop peripheral nerve dysfunction in the absence of overt diabetes [234, 235]. Such experimental models of prediabetes demonstrate early reductions in IENFD, impaired thermal and mechanical nociception, and alterations in nerve conduction properties, supporting a direct pathogenic role of mild dysglycemia combined with IR [234, 235].

In humans, IR appears to be a central determinant of dysglycemia-related nerve injury in prediabetes. Clinical studies in individuals with IGT demonstrate that IR is strongly associated with early DSPN, even in the absence of sustained hyperglycemia, suggesting that impaired insulin signaling contributes directly to nerve dysfunction [224]. Peripheral nerves express insulin receptors, and insulin signaling plays a critical role in neuronal survival, axonal maintenance, and mitochondrial function. Consequently, disruption of insulin signaling in insulin-resistant states may impair neuronal metabolism and increase the susceptibility of peripheral nerves to injury [106, 236]. Experimental animal models provide supportive mechanistic evidence for these observations [88, 236]. Rodent studies have shown that impaired insulin signaling is associated with reduced neurotrophic support, increased oxidative stress, and higher vulnerability of sensory neurons to metabolic insults [237]. These findings are consistent with the human phenotype observed in prediabetes, characterized by early, predominantly small-fiber DSPN [133, 227]. Taken together, clinical and experimental evidence suggests that dysglycemia in prediabetes initiates a cascade of metabolic, oxidative, and vascular pathways that contribute to peripheral nerve injury.

3.3.2 Dyslipidemia

In humans, dyslipidemia has emerged as a metabolic determinant of DSPN in prediabetes, independent of glycemic status, suggesting that lipid abnormalities may contribute directly to nerve injury [224]. Evidence indicates that dyslipidemia amplifies metabolic stress in

peripheral nerves through lipid accumulation, mitochondrial dysfunction, and oxidative stress, impairing axonal integrity and Schwann cell function [236]. Moreover, dyslipidemia commonly accompanies IR, creating a metabolic environment that may exacerbate peripheral nerve injury beyond the effects of dysglycemia alone (**Figure 15**) [88, 224].

Experimental animal models provide supportive mechanistic evidence, demonstrating that lipid excess can induce peripheral nerve dysfunction through oxidative stress and impaired mitochondrial bioenergetics, even in the absence of marked hyperglycemia [236, 238]. However, direct evidence in humans with prediabetes remains scarce.

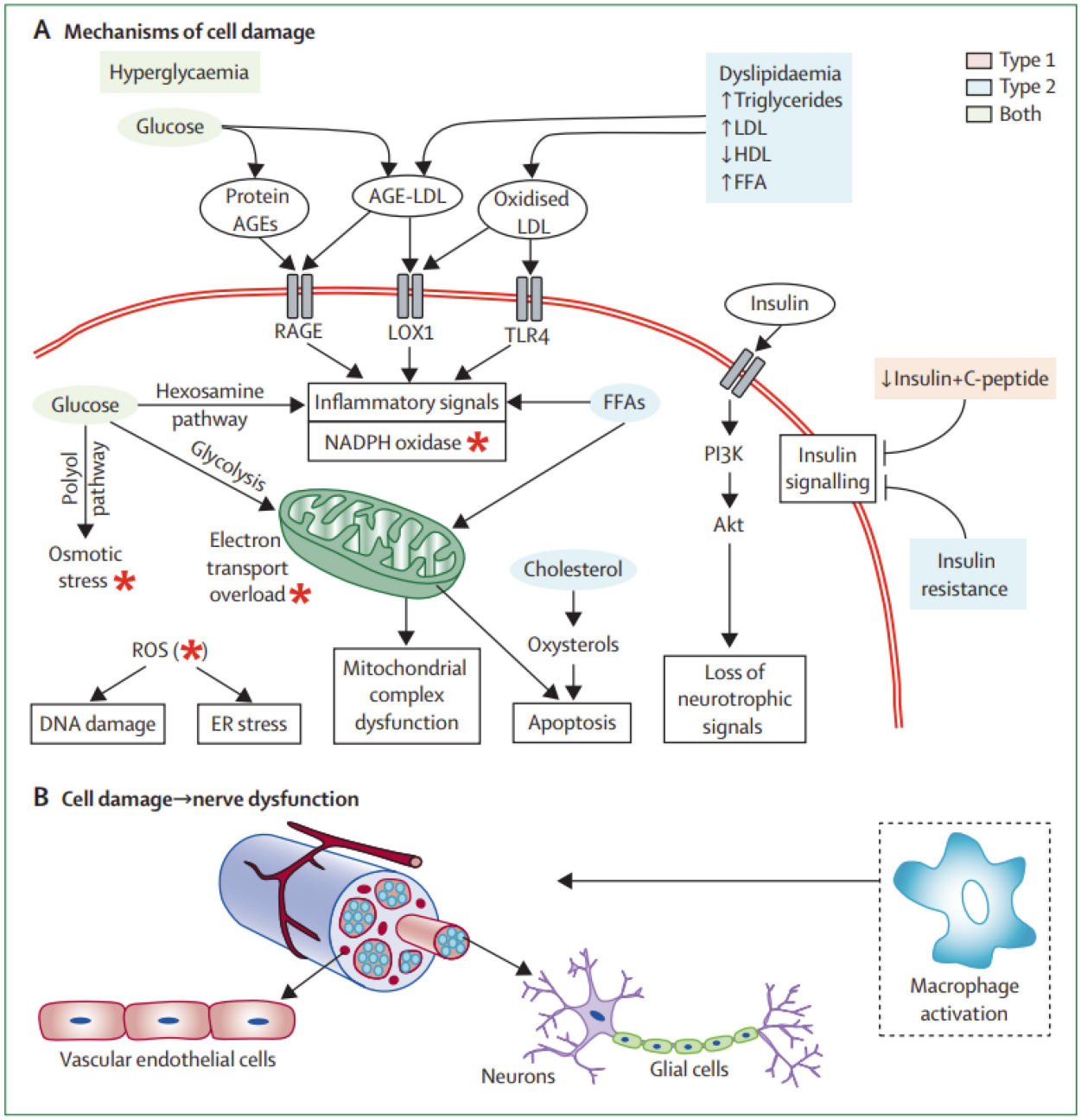


Figure 15 Hypeglycemia and dyslipidemia in DSPN

Chronic hyperglycemia and dyslipidemia are key metabolic drivers in the pathogenesis and progression of distal symmetrical polyneuropathy (DSPN). Persistent hyperglycemia induces excessive intracellular glucose accumulation, resulting in activation of the polyol, protein kinase C, hexosamine, and advanced glycation end product (AGE) pathways. These mechanisms increase oxidative stress, mitochondrial dysfunction, inflammation, and microvascular impairment, ultimately leading to neuronal and Schwann cell injury. In parallel, dyslipidaemia, characterized by elevated triglycerides, free fatty acids, and oxidised low-density lipoprotein (LDL) cholesterol, contributes to lipotoxicity, endothelial dysfunction, and

enhanced inflammatory signalling. The combined effects of glucotoxicity and lipotoxicity disrupt neuronal energy metabolism, impair neurovascular function, reduce neurotrophic support, and promote axonal degeneration and demyelination. Together, these metabolic and vascular abnormalities contribute to progressive nerve fiber damage and impaired nerve conduction. Adapted from Callaghan B et al [150].

3.3.3 Obesity

In humans, obesity is a significant risk factor for DSPN in prediabetes, primarily through its strong association with IR, dyslipidemia, and chronic metabolic stress rather than hyperglycemia alone (**Figure 16**) [150, 224]. Clinical studies provide evidence linking adiposity to early neuropathic abnormalities in prediabetes. Obesity promotes a systemic pro-inflammatory and IR state that disrupts peripheral nerve metabolism, impairs mitochondrial function, and compromises axonal integrity, thereby increasing the susceptibility of peripheral nerves to neuropathic injury [88, 236]. Moreover, increased adiposity promotes oxidative stress and microvascular dysfunction, further exacerbating metabolic and vascular injury to peripheral nerves in prediabetes [224, 236].

Experimental animal models provide additional mechanistic insight, showing that diet-induced obesity alone can lead to peripheral nerve dysfunction and loss of small nerve fibers even in the absence of overt diabetes, largely through inflammatory mechanisms rather than hyperglycaemia [238]. However, similar to findings related to dyslipidemia, these experimental observations mainly support the biological plausibility of obesity-mediated DSPN in prediabetes, rather than definitive evidence of causality between obesity and DSPN [239].

3.3.4 Hypertension and vascular factors

Vascular dysfunction is an important contributor to DSPN in prediabetes, as peripheral nerves have high metabolic and oxygen requirements and are therefore particularly vulnerable to microvascular injury [150, 225]. Clinical studies in individuals with IGT have shown early endothelial dysfunction and reduced nerve blood flow even before the development of overt diabetes, indicating that vascular abnormalities may occur prior to sustained hyperglycemia [232]. Hypertension, which frequently coexists with IR and prediabetes, can further impair microvascular integrity by worsening endothelial dysfunction and disrupting autoregulatory mechanisms in the vasa nervorum, thereby increasing the susceptibility of peripheral nerves to ischemic injury [224]. Evidence also suggests that vascular dysfunction and metabolic disorders interact in a complex manner, and thus hemodynamic abnormalities amplify the degenerative effects of dysglycemia, dyslipidemia, and IR on peripheral nerves [227].

3.3.5 Chronic low-grade inflammation and oxidative stress

Low-grade chronic inflammation is increasingly acknowledged as a pathophysiological contributor to DSPN in prediabetes, reflecting the inflammatory process associated with insulin resistance, IGT, and the MetS rather than hyperglycemia per se [224]. Individuals with IGT have elevated inflammatory markers and endothelial activation, which correlate with early nerve dysfunction and microvascular abnormalities prior to overt diabetes [225, 232].

Oxidative stress represents a downstream mechanism linking metabolic inflammation to peripheral nerve injury in prediabetes. Evidence indicates that IR and low-grade inflammation promote the overproduction of ROS, leading to mitochondrial dysfunction, impaired axonal transport, and reduced neuronal integrity even in the absence of sustained hyperglycemia [225]. Evidence further suggests that oxidative stress can be detected early in prediabetes and preferentially affects small sensory fibers, which may contribute to the predominance of small-fiber neuropathy observed in prediabetes [227, 236].

3.3.6 Genetic susceptibility

Genetic susceptibility may contribute to the interindividual variability observed in the development of DSPN across the dysglycemic spectrum. Although cardiometabolic factors such as IR, dyslipidemia, and obesity play a central role in DSPN pathogenesis, genetic variation may influence neuronal vulnerability to metabolic and oxidative stress [106, 150].

Certain polymorphisms are associated with an increased risk of DSPN, particularly in genes involved in oxidative stress, polyol pathway activity, inflammation, and vascular regulation. Variants in the aldose reductase gene (AKR1B1) have been associated with increased susceptibility to DSPN in diabetes [240, 241]. Similarly, polymorphisms in genes related to vascular and inflammatory signaling, including ACE and Vascular Endothelial Growth Factor (VEGF), have been implicated in microvascular dysfunction and increased DSPN risk in diabetes [242, 243]. However, genetic studies have been conducted in cohorts with overt diabetes, and targeted genetic studies examining susceptibility to DSPN specifically in prediabetes are lacking, highlighting the need for research in this early stage of dysglycemia.

3.3.7 Aging and lifestyle factors

Aging is a well-recognized modifier of peripheral nerve vulnerability and increases the risk of DSPN in prediabetes [224]. Advancing age is associated with reduced regenerative capacity of peripheral nerves, progressive microvascular dysfunction, and increased oxidative stress. Several studies indicate that older individuals with IGT are more likely to manifest DSPN than younger individuals with comparable metabolic profiles, suggesting an age-dependent susceptibility to nerve injury [225].

Lifestyle factors further modulate DSPN risk in prediabetes. Physical inactivity exacerbates IR, dyslipidaemia, and endothelial dysfunction, thereby amplifying metabolic and vascular stress on peripheral nerves [224]. Smoking has been associated with impaired microvascular perfusion, oxidative stress, and endothelial dysfunction [149, 227].

3.4 Vascular theory and endoneurial microcirculation impairments

Peripheral nerves are supplied by the vasa nervorum, a specialized microvascular network responsible for oxygen and nutrient delivery required for axonal metabolism and neuronal integrity. The vascular theory of DSPN proposes that chronic impaired endoneural blood flow and microvascular dysfunction are central to the development and progression of peripheral nerve damage. Hyperglycemia and dyslipidemia trigger endothelial damage, basement membrane thickening, and pericyte degeneration in nerve microvasculature, reducing perfusion and causing hypoxia-ischemia in vasa nervorum [244, 245]. This leads to oxidative stress, inflammation, and nerve fiber degeneration, with capillary temporal heterogeneity further disrupting oxygen delivery patterns (**Figure 16**) [244, 245].

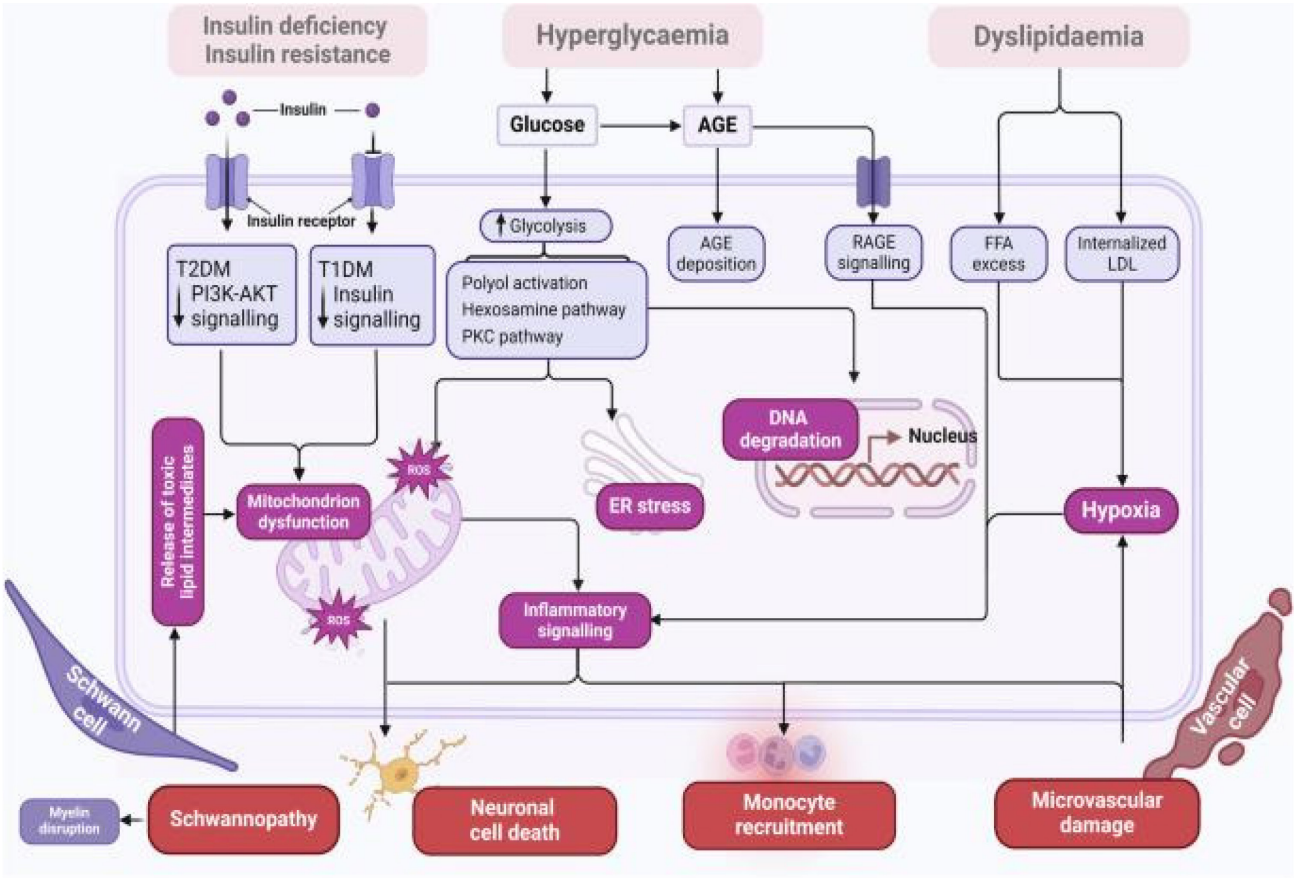


Figure 16 Metabolic and microvascular mechanisms contributing to DSPN

DSPN develops through the combined effects of hyperglycaemia, dyslipidaemia, and impaired insulin signaling, which activate multiple metabolic and inflammatory pathways, including the polyol, hexosamine, protein kinase C (PKC), and advanced glycation end-product (AGE)/receptor for advanced glycation end-products (RAGE) pathways. In type 1 diabetes mellitus (T1D), insulin deficiency, and in type 2 diabetes mellitus (T2D), insulin resistance with impaired phosphatidylinositol-3-kinase/protein kinase B (PI3K-AKT) signaling, contribute to neuronal, Schwann-cell, and microvascular injury. These mechanisms promote oxidative stress, mitochondrial dysfunction, endoplasmic reticulum (ER) stress, DNA damage, inflammation, impaired neurotrophic support, and microvascular dysfunction, ultimately leading to axonal degeneration and peripheral nerve damage. Adapted from Yang Y, et al [151].

Experimental data further indicate that dysglycemia and IR promote endothelial dysfunction and reduced nitric oxide (NO) bioavailability, which may impair vasodilation of the vasa nervorum and reduce endoneurial blood flow [115]. Reduced nerve perfusion may expose peripheral nerves to chronic low-grade ischemia and hypoxia, disrupting axonal transport and mitochondrial energy metabolism [95].

Structural alterations of the microvasculature may exacerbate these functional changes. Histopathological studies have demonstrated capillary basement membrane thickening, endothelial cell damage, and increased vascular permeability in DSPN, changes that increase diffusion distances for oxygen and nutrients within the endoneurial space [246]. Although most histological data derive from diabetes, these mechanisms are thought to begin during the prediabetic stage of dysglycemia, when metabolic and vascular abnormalities are already present [225, 227]. Overall, accumulating evidence suggests that microvascular dysfunction contributes to early nerve injury along the dysglycemic continuum [225, 227]. Human sural nerve biopsies reveal narrowed lumens and swollen endothelium in DSPN, while animal models confirm reduced nerve oxygen tension precedes functional loss [95].

3.5 Clinical phenotype

DSPN in prediabetes is a peripheral neuropathy in individuals with IFG and/or IGT, in the absence of established diabetes. This disorder primarily affects the distal lower limbs in a symmetrical distribution. Motor function is typically preserved in early disease [88, 89].

Neuropathic manifestations in prediabetes are classically divided into positive (gain-of-function) and negative (loss-of-function) sensory phenomena. Positive symptoms reflect peripheral nerve hyperexcitability and include burning pain, electric shock-like or stabbing sensations, painful cold, and tingling, typically in a length-dependent “stocking” distribution [58, 227, 247]. Negative symptoms indicate fiber loss and include numbness, reduced sensation, impaired proprioception, and sensory ataxia, often described by patients as “walking

on thick socks" or "cotton" [58, 227, 247]. Paresthesias and dysesthesias may occur spontaneously or be stimulus-evoked [58, 227, 247]. A characteristic feature of prediabetic DSPN is the predominant involvement of small nerve fibers, which mediate pain and temperature sensation [95, 217]. Patients commonly report burning pain, dysesthesia, and impaired thermal perception [225].

On clinical examination, pinprick and temperature sensation are often reduced, while large-fiber modalities such as vibration perception and proprioception may initially remain preserved [225, 248]. Consistent with this pattern, conventional electrophysiological testing is often normal or only mildly abnormal in early prediabetic DSPN, as these methods primarily assess large, myelinated fibers [111, 224].

3.6 Screening and diagnostic tools

Early detection of DSPN in individuals with prediabetes is challenging because neuropathic changes at this stage are often subtle and predominantly involve small nerve fibers, which are not well detected by conventional electrophysiological tests [224, 225]. As a result, screening strategies typically rely on a combination of clinical assessment, symptom-based questionnaires, neurological examination, and specialized diagnostic testing [58, 228].

Diagnosis of prediabetic DSPN follows the guidelines used for diabetic DSPN as discussed above [58, 249]. A detailed clinical history together with a focused neurological examination remains the cornerstone of diagnosis. This evaluation typically includes assessment of neuropathic symptoms, bedside neurological tests, and careful foot inspection [58, 249].

In clinical routine, prediabetic DSPN is primarily a diagnosis of exclusion. Alternative causes of DSPN must be systematically considered, including chronic alcohol use, nutritional deficiencies (e.g., vitamins B6, B12, and E, thiamine, folate, copper, phosphate), thyroid dysfunction, autoimmune disorders such as systemic lupus erythematosus and rheumatoid arthritis, hematologic malignancies (notably multiple myeloma), infectious etiologies

including Human Immunodeficiency Virus (HIV) and Lyme disease, exposure to chemotherapeutic agents, and other neurotoxins [58].

3.6.1 Clinical assessment and symptom questionnaires

Initial screening commonly involves assessment of neuropathic symptoms using validated clinical tools such as the NSS or the MNSI, which evaluate symptoms including burning pain, paresthesia, numbness, and dysesthesia [58, 228]. Symptom-based instruments may be particularly useful in prediabetes because neuropathic pain and sensory symptoms frequently precede objective neurological deficits [224].

3.6.2 Bedside neurological examination

Large-fiber dysfunction is assessed by reduced vibration perception using a 128-Hz tuning fork, impaired pressure detection with the 1g or 10g monofilament, diminished light touch with wisp of cotton, and loss of ankle and patellar deep tendon reflexes [58, 247]. Small-fiber involvement is evaluated through pinprick and temperature discrimination testing, while sensory gain phenomena such as allodynia (pain triggered by normally non-painful stimuli such as the contact of socks, shoes, or bedclothes) and hyperalgesia (exaggerated response to painful stimuli) may be elicited clinically [58, 247]. Motor involvement, although typically mild in early disease, may manifest as weakness of ankle dorsiflexion or great toe extension. Balance and fall risk assessment (Romberg test, normal gait, and tandem gait) and comprehensive foot inspection for deformities, ulcers, fungal infection, muscle wasting, hair distribution or loss, and the presence or absence of pulses are components of examination for ulcer prevention [58, 247]. According to the ADA Standards of Care-2026, clinical screening for DSPN should include assessment of pinprick and temperature sensation to evaluate small-fiber dysfunction, as well as examination of lower-extremity reflexes, particularly the Achilles reflex, and vibration perception using a 128-Hz tuning fork to assess large-fiber function [249].

3.6.3 Nerve conduction studies

NCS and electromyography (EMG) remain the gold standard for confirming large-fiber DSPN. They provide objective, reproducible, and minimally variable measures of neuropathic severity and progression over time (**Figure 17**) [250-253]. However, their diagnostic sensitivity in prediabetes is limited because early neuropathic changes predominantly involve small fibers [224]. Consequently, many individuals with symptomatic prediabetic DSPN may demonstrate normal or only mildly abnormal nerve conduction findings [225]. These tests are also labor-intensive, costly, and difficult to integrate into clinical routine [112, 247].

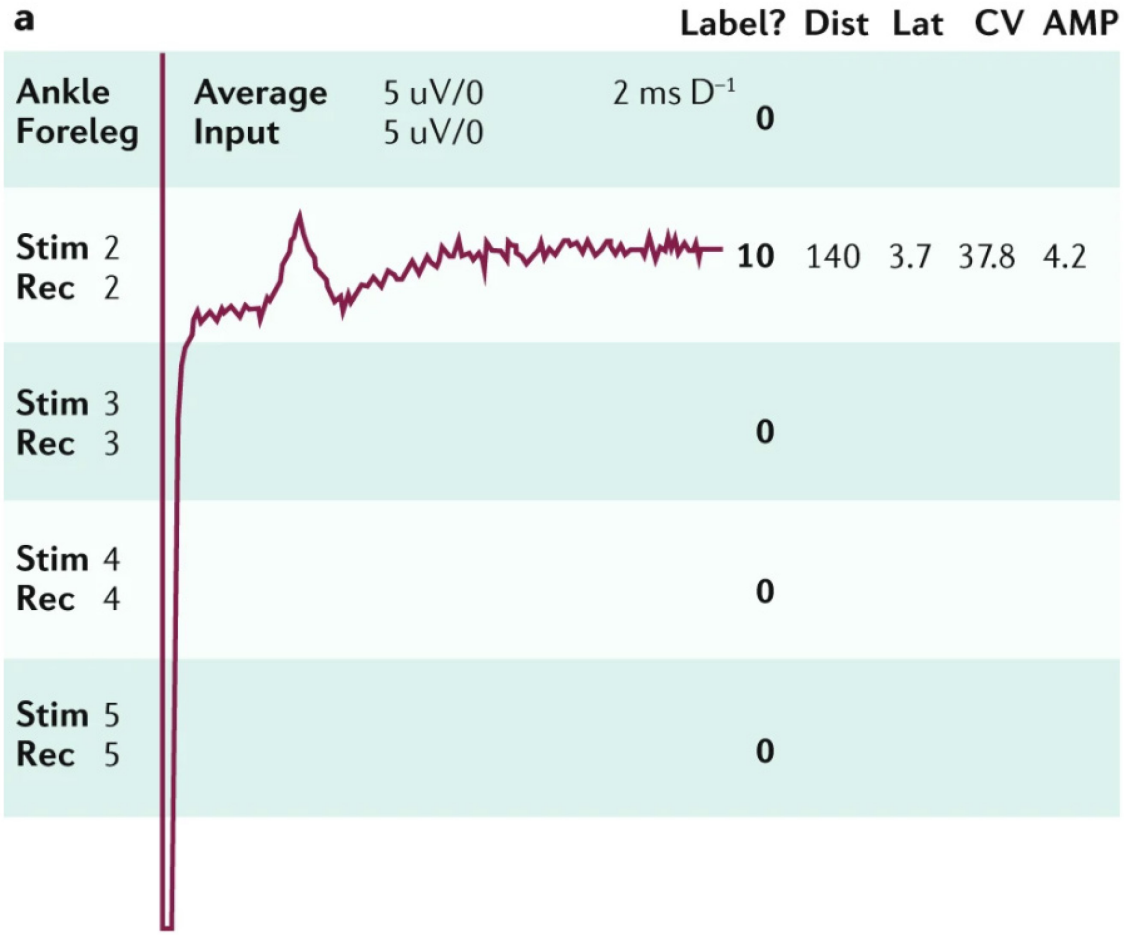


Figure 17 Abnormal sural nerve recording from a patient with distal symmetric polyneuropathy (DSPN) showing a decreased sural sensory nerve action potential amplitude (normal>6 μV) and slow sural sensory nerve conduction velocity (normal >39 m s^{-1}). Adapted from Feldman et al [88]

3.6.4 Small-fiber diagnostic methods

Small-fiber dysfunction is a characteristic feature of DSPN in prediabetes. QST evaluates thermal and pain perception thresholds and can detect early small-fiber abnormalities in individuals with IGT [224]. Skin biopsy, through quantification of IENFD, is the gold standard to identify early, small-fiber DSPN in prediabetes (**Figure 18**) [58, 225, 254]. However, its invasive nature limits the utility for longitudinal monitoring or evaluation of therapeutic efficacy in daily clinical practice.

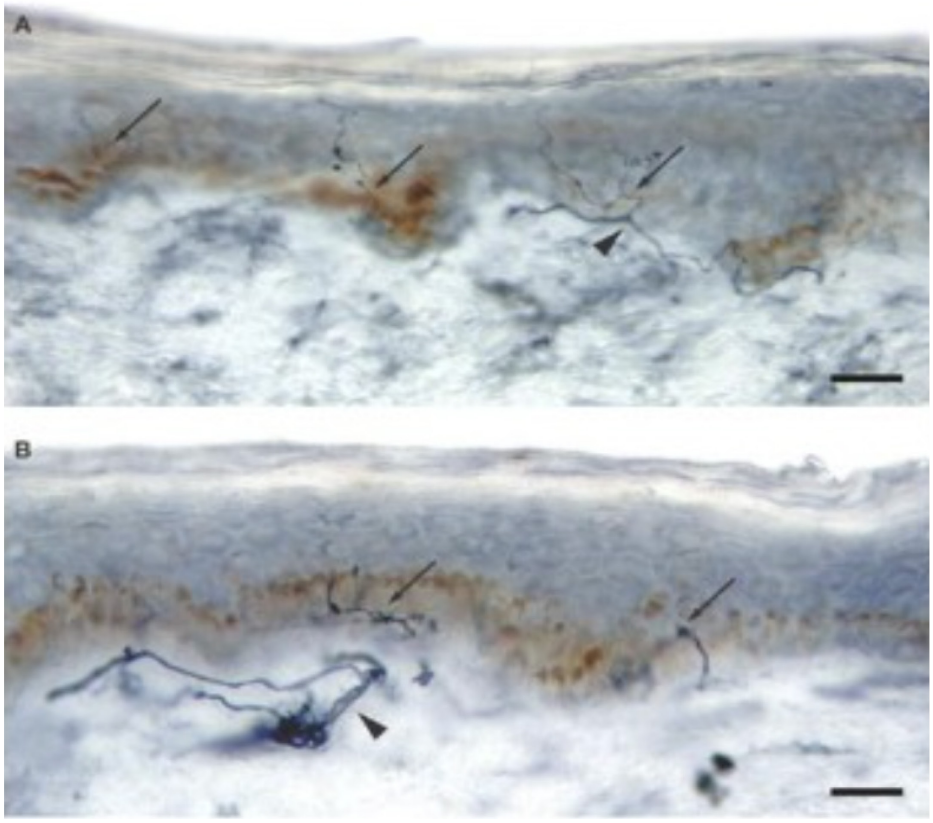


Figure 18 Skin biopsy assessment of IEFND in small-fiber DSPN

(A) Intraepidermal nerve fibers (arrows) and branched fibers (arrowhead) in a skin biopsy sample from a patient with small-fiber DSPN. Adapted from Feldman et al [88]
(B) Reduced and morphologically abnormal intraepidermal nerve fibers with altered branching and swelling (arrowhead), consistent with small-fiber distal symmetric polyneuropathy (DSPN)

Confirmatory testing are not routinely required for the diagnosis of typical DSPN when clinical criteria are fulfilled, as emphasized by ADA [249]. In patients with a characteristic length-dependent pattern and no atypical features, additional investigations rarely change management. However, further evaluation becomes appropriate when clinical features are atypical-such as asymmetry, rapid progression, predominant motor involvement, early upper-limb involvement, or diagnostic uncertainty-where alternative etiologies must be excluded [255-257]. In such cases, detailed neurological work-up may include large-fiber assessment with electrodiagnostic studies (NCS and EMG) and small-fiber assessment via measurement of IENFD from skin biopsy. These tests provide objective confirmation and phenotyping of neuropathy but are primarily reserved for atypical presentations or research settings rather than routine clinical diagnosis [152, 258, 259]. Currently, there is no single gold standard test for objective assessment and early identification of DSPN in prediabetes in routine clinical practice [250, 260]. A narrative consensus suggests that DSPN should be considered in a patient with prediabetes who manifests neuropathic symptoms (involve both sides symmetrically) and/or signs of neuropathy in the presence of DSPN risk factors (i.e., advancing age, obesity, hypertension, dyslipidemia, poor glycemic control) [58]. The proposed “screening and diagnostic” algorithm is provided in **Figure 19** [261].

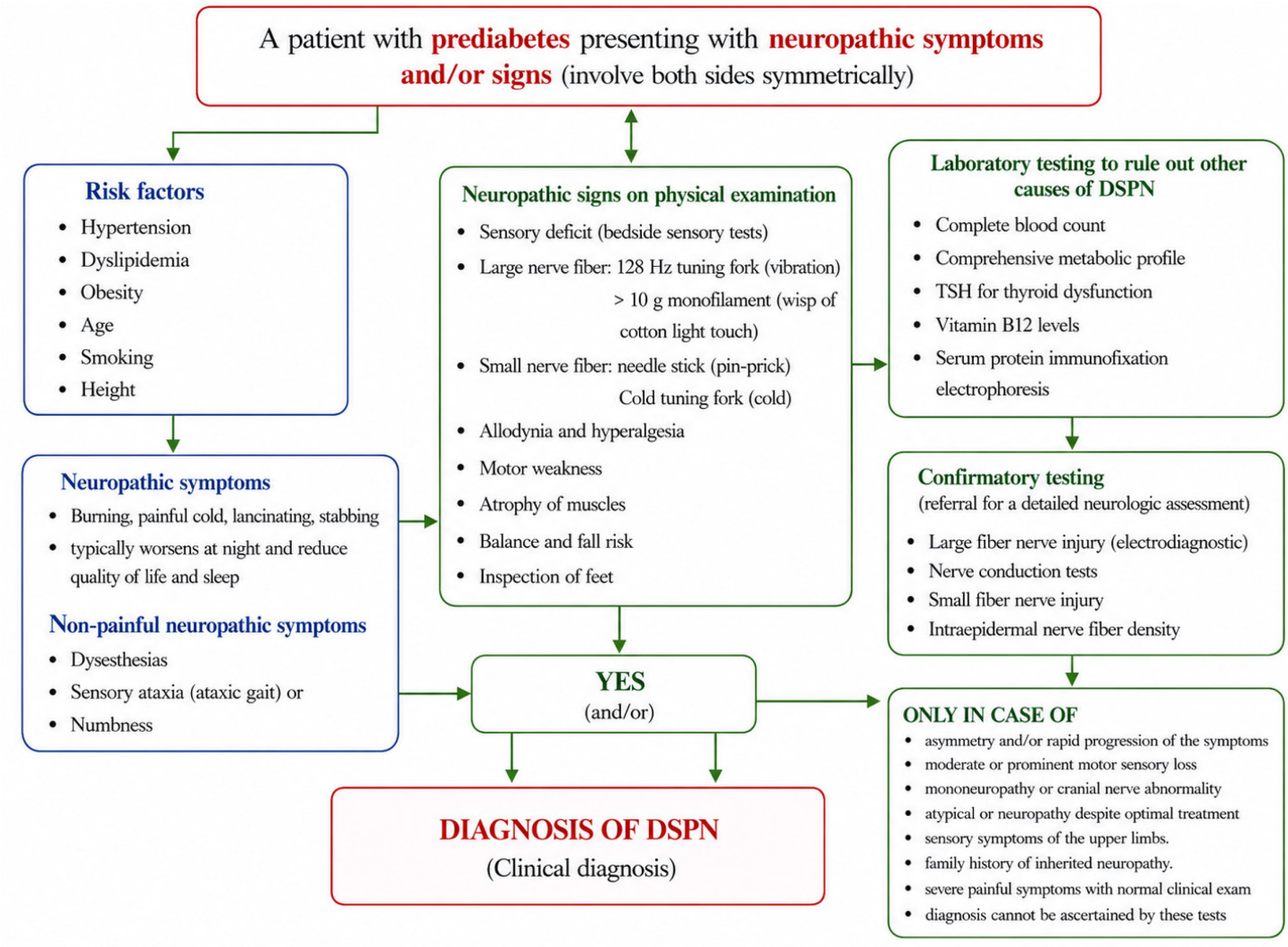


Figure 19 Diagnostic algorithm of DSPN in prediabetes

Schematic diagnostic algorithm for the evaluation of DSPN in individuals with prediabetes presenting with neuropathic symptoms and/or signs. The approach integrates clinical history, neurological examination, laboratory evaluation to exclude alternative etiologies, and confirmatory testing when clinically indicated. Bedside sensory testing includes assessment of large-fiber function (vibration perception with a 128-Hz tuning fork and 10-g monofilament) and small-fiber function (pin-prick and temperature perception). In typical symmetric presentations with compatible clinical findings, DSPN can be diagnosed clinically, whereas electrophysiological or structural testing may be used in atypical or uncertain cases. **Abbreviations:** DSPN, distal symmetric polyneuropathy; TSH, thyroid-stimulating hormone. Adapted from Atmaca A, et al [261].

3.7 Cardiometabolic and cardiovascular risk factors and predictors

Several cardiometabolic and cardiovascular risk factors, including central adiposity and IR states, dyslipidemia, hypertension, and smoking, as well as non-modifiable factors such as age and height have been associated with prevalent DSPN in individuals with prediabetes. However, most available evidence is demonstrated from cross-sectional human studies, and prospective data investigating independent predictors of DSPN in prediabetes remain scarce.

Obesity and central adiposity

Obesity, and particularly central adiposity, have been identified as risk factors for DSPN in prediabetes. In the MONICA/KORA Augsburg Surveys (n=393, age 25-74 years), the prevalence of DSPN varied from 7.4% in NGT to 11.3% in IFG and 13.0% in IGT. Waist circumference (OR 1.03, 95% CI 1.00-1.05) remained independently associated with DSPN in the overall cohort including those with prediabetes ($p<0.05$) [144]. Similarly, in the PROMISE cohort (n=467), participants with DSPN had a greater mean waist circumference than those without (101.0 vs 98.2 cm, p=0.04) [143]. These results highlight that central obesity is associated with increased risk of DSPN even in prediabetes.

Data from the KORA F4/FF4 study (mean follow-up 6.5 years, 127 incident DSPN cases) showed that the OR (95% CI) of DSPN were 3.06 (1.57; 5.97) for overweight, 3.47 (1.72; 7.00) for obesity (reference: normal BMI), while every 5 cm increase in waist circumference raised the risk of DSPN by 22% (OR 1.22, 95% CI 1.07-1.38) [137]. Mediation analysis in the same cohort demonstrated that inflammatory markers explained partially this relationship, with proinflammatory cytokines previously identified as predictors of incident DSPN, suggesting a biological pathway from visceral adiposity through low-grade inflammation to nerve damage [137, 262].

Dyslipidemia

Lipid disorders have also been identified as a potential risk factor for DSPN along with the dysglycemic continuum but to date findings in prediabetes are inconsistent [263]. Prospective series of individuals with idiopathic DSPN confirm the high prevalence of dyslipidemia in this population [264, 265]. An analysis of data from 2 randomized controlled trials (RCTs) showed that elevated TGs have been associated with loss in sural nerve myelin fiber density in diabetes (R=-0.110, p=0.02) [266]. Similarly, hypertriglyceridemia and obesity were independent risk factors for early DSPN (risk ratios 2.3, 95% CI: 1.1-4.7 and 2.1, 95% CI: 1.1-4.3, respectively) in diabetes [133]. In the MONICA/KORA study, TGs were associated with DSPN in prediabetes in univariate analysis (OR 1.61, 95% CI: 1.09–2.38)[144]. Notably, in a cross-sectional study (n=2383 of whom 30% with prediabetes), TGs and HDL-C levels were inversely associated with abnormal light monofilament testing (OR 0.93, 95% CI 0.88-0.99 and OR 0.92, 95% CI 0.86-0.98, respectively) but not significantly linked with DSPN [142]. The different anatomical locations of motor and sensory neuron cell bodies may contribute to the susceptibility of sensory neurons to lipotoxicity [89]. Unlike motor neurons, DRG sensory neurons are exposed to circulating metabolites, including fatty acids in IR states, making them vulnerable to metabolic stressors and axonal injury in prediabetes [89]. Unmyelinated C nerve fibers are also particularly sensitive to metabolites, such as fatty acids, because of the absence of a myelin sheath [89].

Age, height and smoking

Age and height are well-established, non-modifiable determinants of DSPN risk across the spectrum from prediabetes to overt diabetes. In the MONICA/KORA Augsburg surveys, age remained an independent risk factor, with each additional year of age increasing neuropathic pain risk by 8% (OR 1.08, 95% CI 1.02-1.14, p=0.0085) [144]. Age-related neuronal

degeneration, mitochondrial dysfunction, and impaired nerve regeneration may further exacerbate susceptibility to peripheral nerve damage [88, 115]. Similarly, previous population-based studies identifying height and smoking as risk factors for DSPN [267]. The association between height and DSPN risk is commonly attributed to the "length-dependent" nature of DSPN, whereby longer axons are more vulnerable to metabolic, oxidative, and microvascular injury [88, 115].

A recent meta-analysis of cross-sectional and case-control (n=228,699 participants with diabetes) showed that smoking is associated with increased risk of DSPN in diabetes (OR: 1.29, 95% CI: 1.17-1.41) and OR: 1.48, 95% CI: 1.23-1.72) [268]. In the Maastricht study, current smoking (OR 2.13, 95% CI 1.38, 3.29) was significantly associated with increased neuropathic pain, while the same covariate along with increased age (beta= 0.08 [0.04, 0.13], $p<0.05$) were correlated with higher VPT [127]. Smoking may contribute through endothelial dysfunction, oxidative stress, and impaired microvascular perfusion, which may aggravate nerve ischemia and metabolic injury [88, 115].

3.8 Prevention

Lifestyle modification is considered the cornerstone of management for individuals with prediabetes. Several small uncontrolled studies showed that lifestyle intervention was related to an increase in IENFD in heterogeneous groups with prediabetes or MetS with or without diabetes [269, 270]. It has been suggested that exercise may improve cutaneous nerve regenerative capacity, neuropathic pain, and functional performance measures of gait in diabetes, but studies are limited especially in prediabetes [270, 271]. The DPP which included 2776 participants with prediabetes (88% of the total cohort) showed that intensive lifestyle intervention reduced diabetes incidence by 27% ($p<0.0001$), and metformin reduced it by 18% ($p=0.001$) compared with placebo over a 15-year follow-up [272]. Although overall microvascular outcomes (DSPN, nephropathy, and retinopathy) did not significantly differ

between treatment groups, individuals with prediabetes who did not progress to diabetes had a lower prevalence of DSPN than those who developed diabetes [272]. Notably, among women (n=1887), lifestyle intervention was associated with a lower prevalence of microvascular complications (8.7%) compared with placebo (11%, p=0.03) and metformin (11.2%, p=0.02) [272]. Likewise, in a prospective study of 32 individuals with IGT, there was a 0.3 ± 1.1-fiber/mm improvement in distal IENFD and a 1.4 ± 2.3-fiber/mm improvement in proximal IENFD ($p < 0.004$) after 1 year of diet and physical activity [269]. The change in proximal IENFD linked with decreased neuropathic pain ($p<0.05$) and a change in sural sensory amplitude ($p<0.03$) [269]. However, improvements in small-fiber nerve function were not sustained after 3 years, suggesting that the effects of lifestyle intervention on DSPN may be temporary without continued metabolic control. In contrast, in the Da Qing study, lifestyle intervention for 6 years in participants with IGT at baseline did not result in a reduction in DSPN incidence after 20 years [273]. However, in both studies DSPN assessment was relatively limited, relying only on the 10 g Semmes-Weinstein monofilament as the single diagnostic measure.

Bariatric surgery results in substantial and sustained weight loss and has been associated with significant metabolic benefits, including remission of T2D. A recent meta-analysis including 32,756 individuals with obesity and T2D reported a markedly lower incidence of DSPN following bariatric surgery compared with nonsurgical treatment (OR 0.27, 95% CI 0.22-0.34) [274]. In addition, 2 small uncontrolled studies demonstrated improvements in small-fiber pathology assessed by CCM after 12 months in obese individuals with or without diabetes who underwent bariatric surgery [275, 276]. However, larger studies specifically involving individuals with prediabetes are needed to confirm these findings. Potential benefits of bariatric surgery should also be balanced against possible complications, including subacute axonal neuropathy related to micronutrient deficiencies, particularly thiamine deficiency, as

well as orthostatic hypotension due to autonomic dysfunction [227, 277]. These findings suggest a potential role for lifestyle intervention in preventing DSPN along the dysglycemic continuum, although robust evidence in prediabetes is still lacking.

3.9 Therapeutic approaches and pharmacological interventions

Evidence on the treatment of DSPN in individuals with prediabetes remains limited. A pilot study demonstrated a 36% reduction in neuropathic pain after one month of single-blind treatment with pregabalin in individuals with prediabetes and painful DSPN [278]. To date, no drugs have been approved by the FDA specifically for the treatment of DSPN in prediabetes, and most treatment approaches are based on data derived from studies in diabetes. In the absence of randomized clinical trials in prediabetes, pharmacological management remains off-label and generally follows therapeutic strategies established for painful diabetic DSPN, as discussed above. First-line pharmacological options therefore include centrally acting agents such as SNRIs and TCAs, as well as anticonvulsants including pregabalin or gabapentin [247]. Topical therapies, particularly the capsaicin 8% patch, may also be considered for localized neuropathic pain [247].

In addition to symptomatic pain management, pathogenetic approaches targeting metabolic and oxidative stress pathways have been proposed. As discussed above, agents such as α-lipoic acid and benfotiamine may be considered because of their antioxidant properties and potential to modulate glucose-related metabolic pathways implicated in nerve injury [247]. However, the evidence on their efficacy in prediabetic DSPN remains scarce.

4. ARTERIAL STIFFNESS AND ENDOTHELIAL DYSFUNCTION

4.1 Molecular and metabolic drivers of arterial stiffness and endothelial dysfunction

Arterial stiffness is characterized by structural and functional changes of the arterial wall due to endothelial dysfunction and remodeling of the tunica media [279-281]. The gradual loss of elasticity of the large central arteries, particularly the aorta, is recognized as an early hallmark of vascular aging [282]. At the molecular and structural level, arterial stiffening is driven by oxidative stress, persistent low-grade inflammation, medial calcification, fragmentation and crosslinking of elastin fibers, and progressive collagen accumulation within the extracellular matrix. Together, these changes result in increased arterial stiffness [281]. Endothelial dysfunction further amplifies this process due to impaired endothelium-dependent vasodilation through reduced NO bioavailability and increased chemokine production and vascular inflammatory signaling (**Figure 20**) [283]. These alterations progressively increase arterial wall rigidity and create a vicious cycle that reduces organ perfusion and promotes end-organ damage. Cardiovascular risk factors, including hypertension, diabetes, central obesity and IR-states, smoking and dyslipidemia accelerate arterial stiffening and are associated with chronic low-grade systemic inflammation, which in turn promotes endothelial dysfunction and progressive vascular remodeling characterized by extracellular matrix alterations and fibrosis [284, 285]. Specifically, chronic low-grade inflammation in these metabolic disorders contributes to arterial stiffening by promoting endothelial dysfunction through increased ROS generation and reduced endothelial NO synthase (eNOS) activity, leading to diminished NO bioavailability [286]. Reduced NO availability upregulates adhesion molecules such as Vascular Cell Adhesion Molecule 1 (VCAM-1) and Intercellular Adhesion Molecule 1 (ICAM-1) and facilitates leukocyte adhesion and transmigration into the vascular wall [286]. In parallel, activation of the renin-angiotensin-aldosterone system further amplifies oxidative

stress through mineralocorticoid receptor-dependent nicotinamide adenine dinucleotide phosphate (NADPH) oxidase signaling [287]. Mineralocorticoid receptor activation increases endothelial sodium channel activity, promoting intracellular sodium accumulation and consequently endothelial dysfunction [287]. In addition, perivascular adipose tissue undergoes a phenotypic shift from a monocyte chemoattractant protein-1 (MCP-1), which further amplifies vascular inflammation and arterial stiffening [286]. In IR-states such as prediabetes, vascular dysfunction develops early and affects both the macro- and microcirculation through disruption of insulin-mediated endothelial signaling (**Figure 21**) [287].

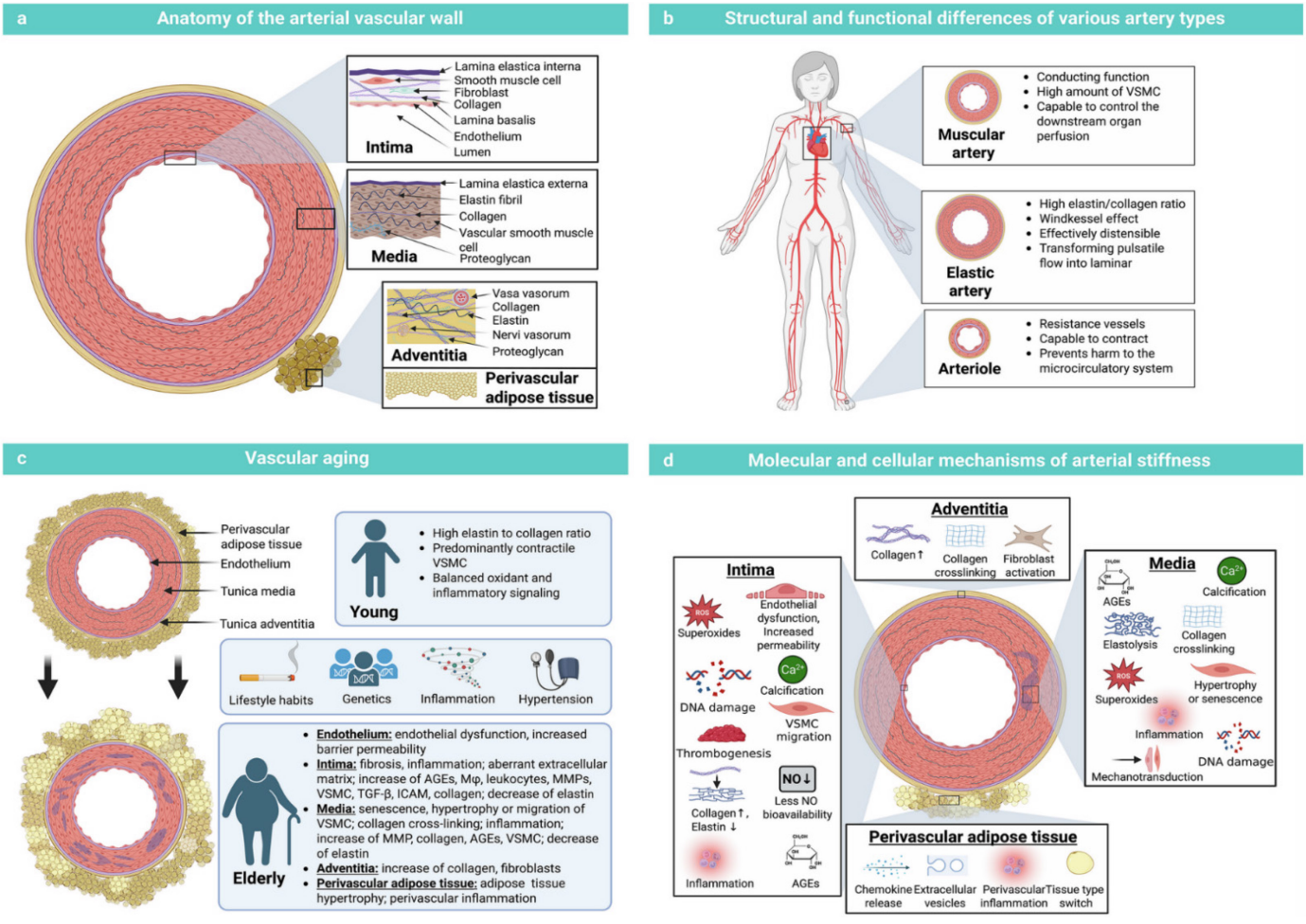


Figure 20 Structural organization of the vascular wall and mechanisms contributing to arterial stiffness and vascular aging

(a) Cross-sectional anatomy of the vascular wall demonstrating the tunica intima, tunica media, tunica adventitia (externa), and surrounding perivascular adipose tissue (PVAT). (b) Structural and functional characteristics of elastic arteries, muscular arteries, and arterioles. (c) Age-related vascular alterations contributing to vascular aging and increased arterial stiffness, including endothelial dysfunction, elastin fragmentation, collagen accumulation, inflammation, and vascular calcification. (d) Overview of the principal molecular and cellular mechanisms involved in arterial stiffening across the different layers of the arterial wall. Adapted from Herzog M, et al [2].

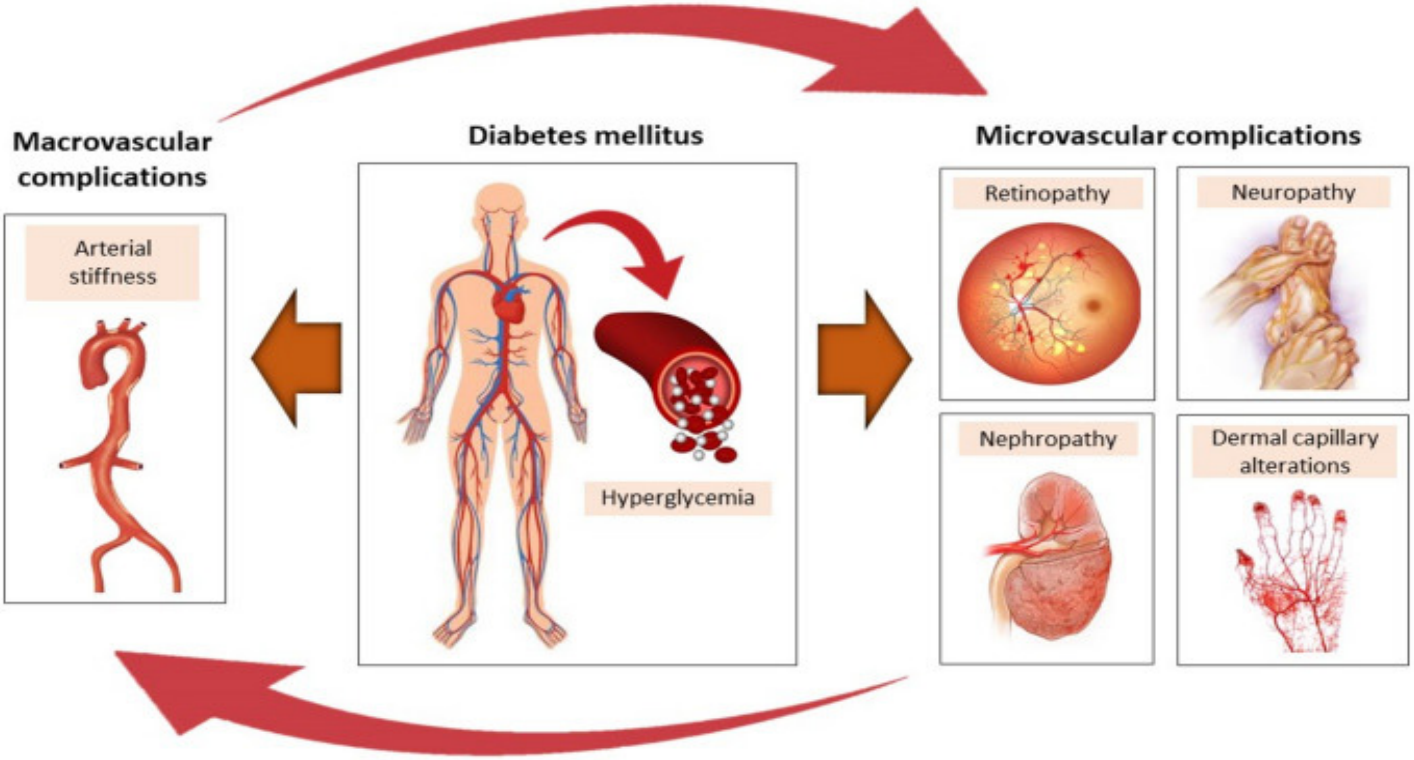


Figure 21 Interaction between macrovascular and microvascular dysfunction in diabetes

Schematic illustration of the bidirectional relationship between large artery stiffness and microvascular complications in diabetes. Chronic hyperglycemia induces widespread vascular injury through multiple mechanisms, including endothelial dysfunction, oxidative stress, chronic low-grade inflammation, and accelerated atherosclerosis. Both the macro- and microcirculation are simultaneously exposed to these metabolic disturbances, contributing to progressive vascular dysfunction. Increased large artery stiffness enhances central blood pressure, pulse pressure, and pulsatile flow transmission into small resistance vessels, thereby promoting microvascular injury affecting the retina, peripheral nerves, kidneys, and dermal capillaries. In parallel, structural and functional abnormalities within the microcirculation increase total peripheral resistance and mean arterial pressure, further exacerbating large artery stiffening. This interaction establishes a vicious cycle linking macrovascular dysfunction with microvascular damage in diabetes. Adapted from Partalidou et al [288].

Under physiological conditions, insulin stimulates eNOS via the Phosphoinositide 3-Kinase (PI3K-Akt) pathway, increasing NO production and promoting vasodilation [288]. In IR- and hyperglycemic states, this pathway becomes selectively impaired, and the MAPK signaling pathway remains preserved or relatively overactive. This imbalance reduces NO bioavailability while increasing ET-1 generation, vascular smooth muscle proliferation, and vasoconstrictive

signaling, thereby promoting endothelial dysfunction, impaired microvascular reactivity (e.g. reduced flow-mediated dilation, capillary rarefaction) and dysregulated vascular tone [288-291].

Persistent hyperglycemia further exacerbates arterial stiffness and endothelial dysfunction through non-enzymatic glycation of proteins and the accumulation of AGEs [292]. Specifically, within the arterial wall, structural proteins such as collagen are particularly susceptible to glucose-induced cross-linking, which increases extracellular matrix rigidity and reduces arterial elasticity, directly contributing to arterial stiffening [292]. AGEs also activate inflammatory signaling pathways by stimulating macrophage release of pro-inflammatory cytokines, increasing ET-1 production, and reducing NO availability, thereby aggravating endothelial dysfunction [292, 293]. Hyperglycemia simultaneously enhances ROS production through increased glycolytic flux and mitochondrial oxidative phosphorylation, leading to excess superoxide generation and amplification of oxidative stress within the vascular wall [292, 293].

4.2 Pulse Wave Velocity (PWV)

Arterial stiffness has emerged as an indicator of vascular aging and cardiovascular risk. Increased arterial stiffness is considered as target organ damage and has emerged as an important marker of cardiovascular risk, morbidity and mortality in individuals with cardiometabolic disorders [294]. The assessment using PWV has increasing importance in both research and clinical practice.

PWV has progressively been established as a robust prognostic indicator of cardiovascular outcomes [295-297]. Among available techniques, carotid-femoral PWV (cfPWV) is considered the gold-standard method for assessing arterial stiffness as demonstrated in population-based studies [298]. In 2007, the European Society of Hypertension (ESH) guidelines first recommended the measurement of arterial stiffness [298]. Recently, the 2023

ESH and the 2024 ESC hypertension guidelines recommend PWV measurement for cardiovascular risk stratification in individuals with hypertension [294, 299]. Regional differences in arterial compliance create a physiological stiffness gradient along the aorta, and current expert consensus recommends a threshold of 10 m/s for increased arterial stiffness [300].

There are several invasive and noninvasive methods for measuring arterial stiffness. Structural changes primarily involve the aorta and are quantified by aortic PWV. In the invasive reference method, a pigtail catheter records pressure waves in the ascending aorta and at the bifurcation; the measured distance and transit time between sites are used to calculate PWV [1]. Cf PWV is a reliable noninvasive surrogate of invasive aortic PWV (**Figure 22**) [1, 301, 302]. It is calculated by dividing the distance between the carotid and femoral measurement sites by the pulse transit time [1, 301, 302]. The blood pressure at the time of measurement must always be considered.

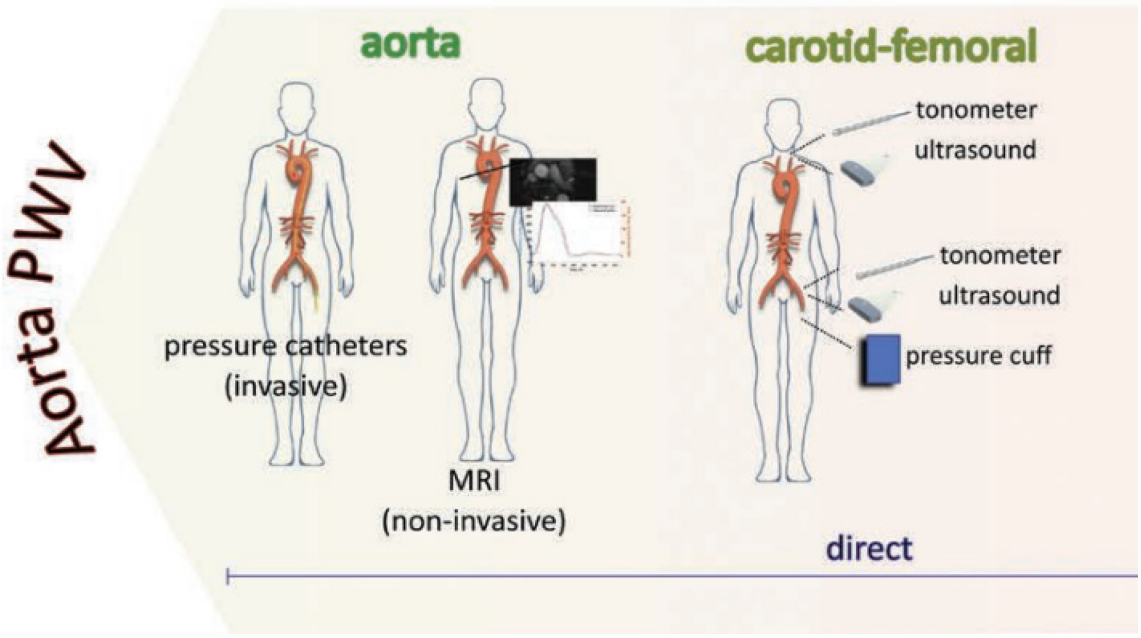


Figure 22 Visual overview of methods to assess larger artery stiffness

Pulse wave velocity (PWV) assessment techniques are categorized into invasive methods and noninvasice approaches. Adapted from Segers P et al [1]

CfPWV is already elevated in individuals with prediabetes compared with those with normoglycemia, indicating the presence of early subclinical aortic stiffening before overt diabetes develops [290]. A recent systematic review and meta-analysis including 37 studies demonstrated significantly higher cfPWV values in prediabetes, with mean differences of approximately 0.5-1.0 m/s vs those with normoglycemia and a progressive increase across the continuum from prediabetes to overt diabetes [290]. Several studies have proposed cfPWV thresholds of approximately 6.9 to 8.3 m/s to identify increased arterial stiffness in individuals with prediabetes, with higher values associated with obesity, dyslipidemia and IFG [303].

4.3 Hemodynamic alterations and nerve blood flow

Arterial stiffness generates important alterations in central hemodynamics beyond large arteries and directly affects microcirculation. Progressive stiffening of the aorta reduces its Windkessel function, leading to increased PWV, elevated pulse pressure, and increased transmission of

pulsatile energy into peripheral microvascular beds [304, 305]. Under physiological conditions, the elastic properties of the aorta buffer systolic pressure waves and protects distal microvascular networks from excessive pulsatile stress. Under conditions of increased arterial stiffness, this protective mechanism is impaired, and consequently greater pulsatile pressure and flow energy is transmitted into microcirculation [304, 305]. This abnormal transmission of increased pulsatile energy into microcirculation triggers structural remodeling of small vessels, increases total peripheral resistance and contributes to impaired tissue perfusion [304, 305]. The consequent rise in total peripheral resistance further elevates systemic blood pressure, which in turn accelerates pressure-dependent arterial stiffening, creating a self-reinforcing vicious cycle of vascular dysfunction [304, 305].

Growing evidence indicates a bidirectional interplay between macrovascular and microvascular dysfunction. Elevated PWV has been associated with the development of microvascular complications in diabetes, suggesting that large-artery stiffening and microvascular injury may share common glucotoxic and inflammatory pathophysiological mechanisms [289, 291, 303]. These mechanisms include endothelial dysfunction, oxidative stress, and chronic low-grade inflammation, which are already present during the prediabetic stage and contribute to impaired vascular reactivity across the arterial tree [291].

4.4 Associations with DSPN in prediabetes and diabetes

Accumulating evidence suggests that arterial stiffness is associated with microvascular complications of diabetes, including DSPN [291, 303]. Cross-sectional studies evaluating the relationship between arterial stiffness and DSPN have shown heterogeneous findings [306-310]. In a cohort of 447 individuals with T2D without peripheral arterial disease, of whom 53% had DSPN and 66% hypertension, cfPWV values above the 90th percentile of age- and blood pressure-adjusted reference ranges were significantly associated with DSPN only among normotensive individuals [307]. Similarly, a study in individuals with T1D have demonstrated

significantly higher cfPWV values in those with DSPN compared with those without (8.6 ± 1.5 m/s vs 7.9 ± 1.3 m/s, p<0.01) [311]. In a study including patients with well-controlled T2D, DSPN was significantly associated with ankle-brachial PWV (OR 1.002, 95% CI 1.001-1.003, p=0.002) [308]. In patients with T2D, higher ankle-brachial PWV values were associated with increased risk of prevalent DSPN (OR 12.8, 95% CI 1.1-149.8, p=0.042 for PWV 15.5-19.0 m/s and OR 15.2, 95% CI 1.0-221.3, p=0.047 for PWV>1900 cm/s) compared with PWV≤1550 cm/s [309]. Other studies in patients with T2D have reported that ankle-brachial PWV is associated with several microvascular complications, including DSPN, cardiac autonomic dysfunction, albuminuria, and diabetic retinopathy, while heart-carotid PWV was not significantly correlated with these complications [309]. However, brachial-ankle PWV is a composite measure of aortic stiffness and peripheral arterial stiffness of the lower limbs and provides information mostly about muscular arteries, unlike cfPWV that provides information mostly about elastic arteries [309]. Moreover, this measurement predominantly reflects the stiffness of muscular arteries, in contrast to carotid-femoral PWV, which primarily reflects the stiffness of central elastic arteries.

Prospective data provides stronger evidence supporting a relationship between arterial stiffness and DSPN in diabetes. In a longitudinal cohort of 473 individuals with T2D, increased aortic stiffness (cfPWV>10 m/s) independently predicted the development or progression of DSPN (incidence rate ratio 2.04, 95% CI 1.28-3.23; p= 0.002) during a median follow-up of 6.2 years [306]. However, evidence on the association between arterial stiffness and DSPN in individuals with prediabetes remains scarce. Therefore, whether the macro-microvascular interplay observed in overt diabetes is already present at the prediabetic stage remains unclear, highlighting an important gap in the current literature. Prospective studies evaluating arterial stiffness and DSPN development and progression in prediabetes are therefore needed to clarify the temporal relationship and effect of vascular stiffening in early neural injury.

RESEARCH PROTOCOL AND RESULTS

5. AIM AND STUDY DESIGN

5.1 Background and Aim

As discussed above in detail, the global burden of prediabetes is rapidly increasing, with an estimated 635 million people affected by IGT and 488 million by IFG worldwide in 2024 as per the IDF [17]. Beyond its role as a precursor of T2D, prediabetes is associated with microvascular and macrovascular complications, including nephropathy, neuropathy, retinopathy, and cardiovascular disease [312]. DSPN is the most common form of diabetic neuropathy and the leading cause of peripheral nerve dysfunction in clinical practice [80]. In established diabetes, DSPN is associated with substantial clinical burden, including neuropathic pain, impaired mobility, reduced quality of life, and increased morbidity [313]. Notably, neuropathic changes may begin in the prediabetic stage, with several studies suggesting that individuals with prediabetes are at high risk of developing DSPN [143, 227]. However, reported prevalence estimates vary widely in prediabetes, from 2% in U.S. women to 77% in a Brazilian study, reflecting heterogeneity in diagnostic approaches and study populations [226]. Consequently, the true burden and natural history of DSPN in prediabetes remain insufficiently defined.

Prediabetes is characterized by a clustering of cardiometabolic abnormalities, including IR, central adiposity, and dyslipidemia. Emerging evidence suggests that these factors-rather than hyperglycemia per se-may contribute more to early nerve injury, supporting a cardiometabolic model of DSPN pathogenesis [314, 315]. Nevertheless, prospective data examining the relationship between these risk factors and the development of DSPN in prediabetes are limited

The present study was designed to address these gaps using both a cross-sectional and a prospective design. Specifically, we aimed to (i) determine the prevalence of DSPN in individuals with prediabetes at baseline; (ii) assess the incidence of DSPN over a 24-month follow-up; (iii) evaluate the association between baseline cardiometabolic risk factors, including measures of obesity and adiposity, insulin resistance, lipid profile, smoking, arterial stiffness as well as nonmodifiable factors (age, sex and height) with prevalent DSPN; (iv) examine the association between baseline cardiometabolic and cardiovascular risk factors along with the nonmodifiable parameters with the development of DSPN during a 24-month follow-up; and (v) examine the association between longitudinal changes in these cardiometabolic and cardiovascular risk factors over follow-up with the development of DSPN.

5.2 Materials and methods

5.2.1 Study design and study population

Consecutive adults with prediabetes who attended the Outpatient Lipid and Obesity Clinic of the University Hospital of Ioannina in Greece from 2019 to 2023 were recruited and followed in a prospective observational study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the University hospital of Ioannina (Ethical Approval Code 30/29-11-2018, approved on 29 November 2018). Participants gave their signed informed consent.

Prediabetes was defined as the presence of IFG, defined as FPG 100-125 mg/dL (5.6-6.9 mmol/L); IGT, defined as 2h PG 140-199 mg/dL (7.8-11.0 mmol/L) during a 75-g OGTT and/or HbA1c 5.7-6.4% (39-47 mmol/mol) in accordance with 2019 ADA Guidelines [316]. MetS was diagnosed when at least 3 of the following criteria were present: waist circumference ≥ 102 cm in men or ≥ 88 cm in women, TGs levels ≥ 150 mg/dL, HDL-C levels < 40 mg/dL in men or < 50 mg/dL in women, FPG ≥ 100 mg/dL, and blood pressure ≥ 130/85 mm Hg,

based on the American Heart Association/National Heart, Lung, and Blood Institute Scientific guidelines [317].

Participants with secondary causes of neuropathy were excluded. Specifically, individuals were not eligible if they had any of the following: A history of malignancy and/or chemotherapy, use of neurotoxic drugs, excessive alcohol consumption (>2 units/day for men, >1 unit/day for women), vitamin B12 deficiency, hypothyroidism, estimated glomerular filtration rate (eGFR) <30 mL/min/1.73 m^2, systemic vasculitis, paraproteinemia, amyloidosis, HIV infection, hepatitis B virus infection, Lyme disease, chronic inflammatory demyelinating polyneuropathy, or a history of hereditary motor, sensory, or autonomic neuropathy. The participant selection process is illustrated in the study flow diagram (**Figure 23**).

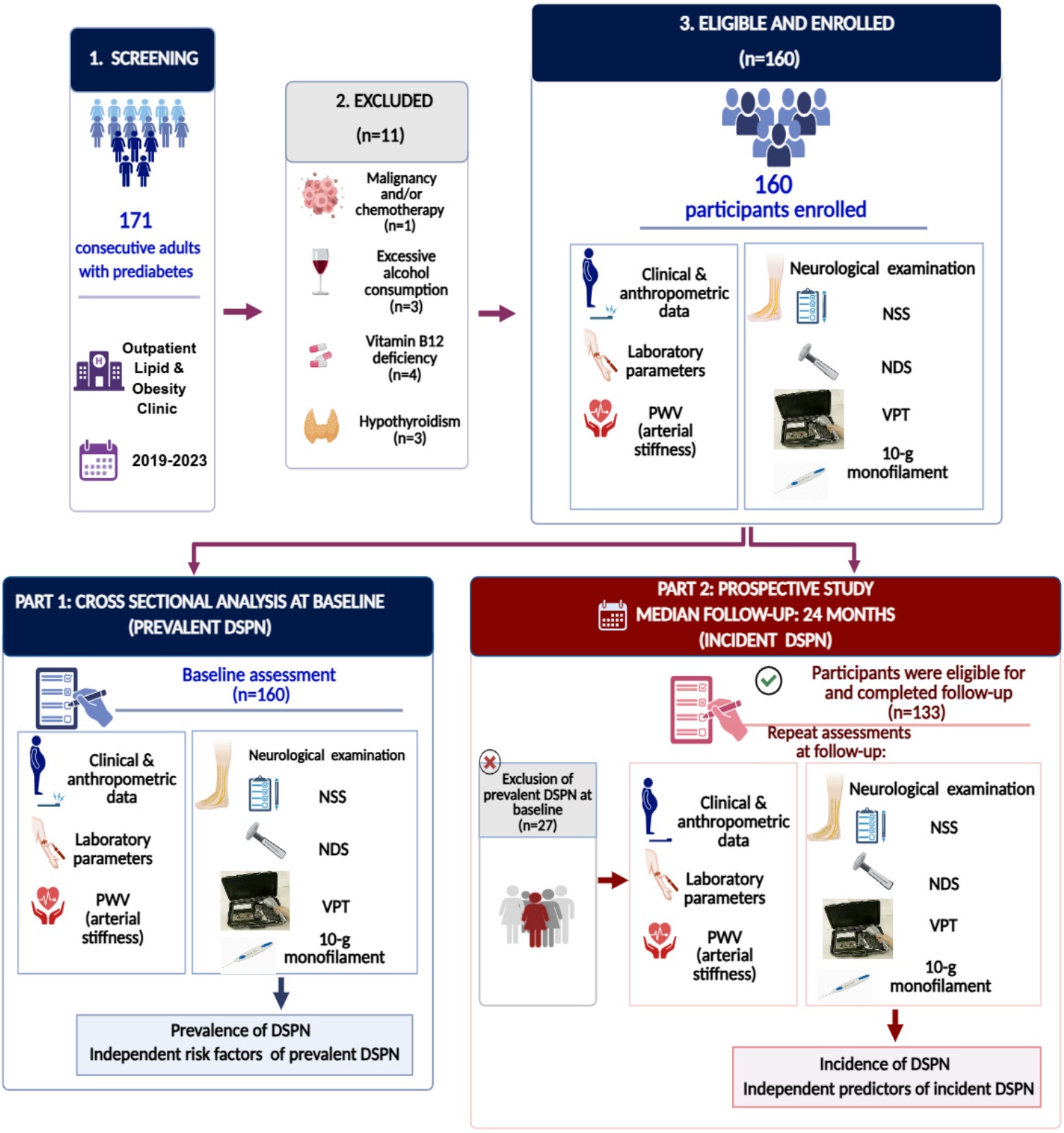


Figure 23 Study flow diagram of participant selection and follow-up

5.2.2 Clinical and laboratory measurements

Data was collected at enrollment (baseline) and at 24-month follow-up visit. A comprehensive assessment of the clinical and laboratory profile was conducted, including sex, age, BMI, blood pressure measurement (MICROLIFE Watch BP Office device, ABI, Switzerland) and body composition [measured by bioelectrical impedance analysis (BIA) (Tanita MC-780MA, Japan)]. Laboratory assessments were performed after an overnight fast (≥8 hours) and included: FPG, serum 25(OH) vitamin D, creatinine, total cholesterol, HDL-C, TGs, LDL-C, apolipoprotein A1 (apoA1), apolipoprotein B (apoB), and lipoprotein(a) [Lp(a)] as well as urinary albumin-to-creatinine ratio (UACR). A standard oral glucose tolerance test (2-hour plasma glucose following a 75-g OGTT) was performed at enrollment. HOMA-IR was calculated based on FPG and fasting insulin as follows: [Fasting insulin (μU/mL) × FPG (mg/dL)]/ 405 [318].

5.2.3 Arterial stiffness assessment

Both at enrollment and at 24-month follow-up visit, arterial stiffness was evaluated by PWV as measured by the Sphygmocor system (Version 7.01, At Cor Medical, Sydney, Australia) (**Figure 24**). PWV ≥10 m/s was considered abnormal [319]. Ankle-brachial index (ABI) was evaluated by the MICROLIFE Watch BP Office device (ABI, Switzerland) and adherence to the Mediterranean diet was assessed by the Med Diet Score [320, 321].

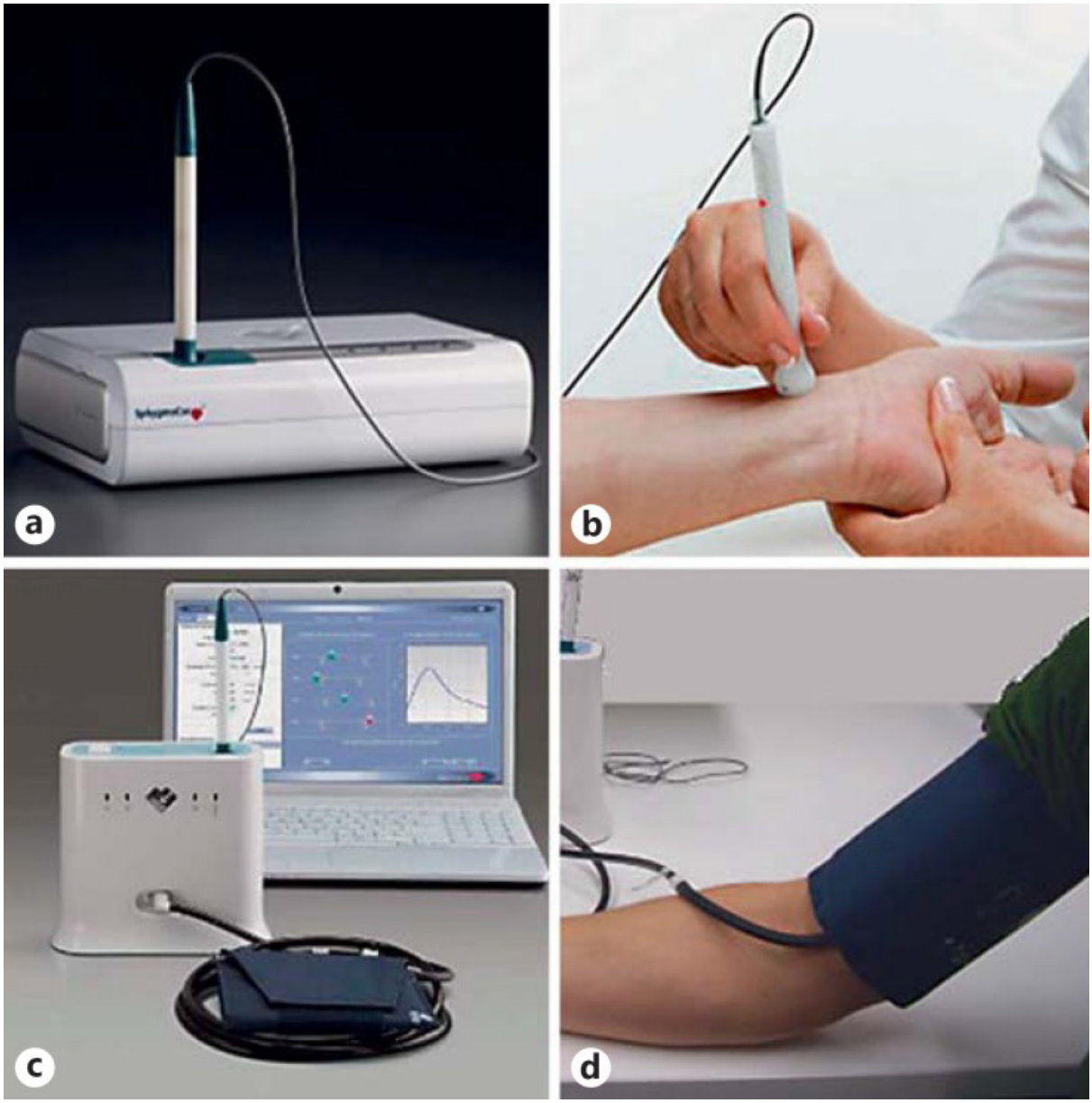


Figure 24 Non-invasive assessment of arterial stiffness using PWV
a, b The SphygmoCor system, which acquires peripheral arterial waveforms using applanation tonometry (b)
c, d The SphygmoCor system, which records peripheral vascular waveforms through cuff-based volumetric displacement technology (d). Adapted from [322]

5.2.4 DSPN diagnosis

All participants underwent examination for DSPN at enrollment and follow-up visits. Neuropathic symptoms were assessed using the NSS [66, 323]. Neuropathic signs were quantified by the NDS (**Figure 25**) [66]. The severity of NSS was graded as mild (scores: 3-4), moderate (scores: 5-6), and severe (scores: 7-9) [66, 324]. Severity of NDS was graded as mild (scores: 3-5), moderate (scores: 6-8), and severe (scores: 9-10) [123].

Figure 25 Neurological examination tools used for assessment of the NDS

The figure illustrates the standardized bedside neurological examination tools used for the evaluation of large- and small-fiber peripheral nerve function as part of the Neuropathy Disability Score (NDS). Achilles tendon reflexes were evaluated using a reflex hammer. Vibration perception, reflecting large-fiber function, was assessed with a 128-Hz tuning fork applied to the distal hallux. Thermal discrimination (warm/cold sensation), indicative of small-fiber integrity, was examined using the Tip-Therm device, while pin-prick sensation was assessed using a sterile neurological pin/needle

VPT was measured by a biothesiometer (Bio-medical Instrument Co, Newbury, Ohio, USA) (**Figure 26**). Age-specific abnormal VPT was defined as values above the upper reference limit for each age category (20-45 years: >16.6 V; 45-60 years: >19.4 V; 60-77 years: >24.3 V) and

for participants aged >77 years, the threshold for the oldest age category was applied due to lack of reference data [325].

Figure 26 An analog biothesiometer

DSPN was diagnosed based on the following criteria: NSS ≥5 + NDS ≥3 or NDS ≥6 or age-specific, abnormal VTP + NSS ≥3 + NDS ≥3 [66, 326, 327]. Large myelinated Aβ fibers, which mediate protective and pressure sensation, were examined with the 10-g Semmes-Weinstein monofilament (**Figure 27**) [328].

Figure 27 10-g Semmes-Weinstein monofilament

Large myelinated Aβ fibers, which mediate protective and pressure sensation, were assessed using the 10-g Semmes-Weinstein monofilament.

5.3 Statistical analysis

Statistical analysis was performed with SPSS Version 28.0 (IBM Corp., Armonk, NY). Graphs were generated with GraphPad Prism for Windows (Version 9.3.1). The data are presented as the mean ± standard deviation (SD) and median [interquartile range (IQR)] for data with normal distribution and non-normal distribution, respectively. For normally distributed continuous variables, between-group differences were assessed using the independent samples t-test. For continuous variables that were not normally distributed, Mann-Whitney U test was applied. For categorical values, frequency counts and percentages were applied. A chi-square test was performed for the interactions between the categorical values.

For prevalent DSPN at baseline, associations with cardiometabolic and cardiovascular variables were assessed using univariate and multivariable binary logistic regression models (cross-sectional analysis of the prospective observational cohort).

For incident DSPN, participants with DSPN at baseline were excluded. Predictors of incident DSPN were evaluated using univariate and multivariable binary logistic regression analyses. Changes in continuous variables were calculated as the difference between follow-up and

baseline values (Δ). Multivariate analyses was adjusted for baseline variables and their 24-month changes with $p<0.10$ in univariate analyses, as well as clinical important confounders: age, sex, smoking, waist circumference, total body fat mass (kg) and ΔTotal body fat mass (kg), HOMA-IR and ΔHOMA-IR, LDL-C and ΔLDL-C, TGs, PWV, VPT, and statin +/- ezetimibe initiation during follow-up (the backward conditional method was used). Results are presented as ORs with 95% CIs.

Longitudinal analyses were performed to assess the association between changes in cardiometabolic and cardiovascular risk factors over follow-up and the development of incident DSPN.

Significance was set at 0.05 for all analyses. It was estimated using G*power (version 3.1) that a sample size of 150 subjects would yield 90% power to detect significant associations at an α-level lower than 0.05 [329]. We initially included 160 subjects to allow for a dropout rate of ~6% (**Figure 23**).

6. RESULTS

6.1 Baseline

6.1.1 Baseline demographic and clinical characteristics of study population and prevalence of DSPN

The study population consisted of 160 Caucasian adults with prediabetes (mean age 62 years, median BMI 28.9 kg/m2). Of them, 68.8% had IFG, 31.2% both IFG and IGT, 60.6% were male and 45.0% current/previous smokers (**Table 5**).

Overall, 27 subjects (16.9%) were diagnosed with DSPN at baseline. **Table 5** summarizes demographic and clinical characteristics based on presence of DSPN. Individuals with DSPN were older (67 vs 61 years, $p=0.017$) and had a higher prevalence of current/previous smoking

(55.5 vs 42.9%) (p=0.028), higher BMI (30.3 vs 28.2 kg/m^2, p=0.011) and increased waist circumference (98.0 vs 89.0 cm, p=0.002) compared with no DSPN. Also, a higher percentage of participants with DSPN had hypertension (77.8 vs 33.0%, p<0.001) and MetS (51.9 vs 34.6%, p=0.033) vs those without (**Table 5**).

Demographic and clinical characteristics grouped by glycemic status (IFG vs IFG+IGT) are presented in **Table 6**. Individuals with IFG+IGT had significantly higher prevalence of DSPN compared to those with IFG (24.0 vs 13.6%, p=0.031) (**Figure 28**). Participants with IFG+IGT were significantly older than those with IFG (68 vs 59 years, p<0.001) and had higher waist circumference (94.5 vs 90.0 cm, p<0.001), total body fat percentage (31.1 vs 28.6%, p<0.001) and total body fat mass (26.8 vs 23.7 kg) (p<0.001). PWV was higher in the IFG+IGT group vs IFG (9.4 vs 8.1 m/s, p<0.001) with or without DSPN. No significant differences in hypolipidemic and antihypertensive therapies were observed between the 2 groups (**Table 7**).

Table 5 Demographic and clinical characteristics of study participants

	Overall	**Without DSPN**	**With DSPN**
	N=160	**N=133**	**N=27**
Age, years	62 ± 13	61 ± 14	67 ± 8*
Male gender, N (%)	97 (60.6)	81 (60.9)	16 (59.3)
Never smoking, N (%)	88 (55.0)	76 (57.1)	12 (44.4)*
Previous smoking, N (%)	45 (28.1)	33 (24.8)	12 (44.4)*
Current smoking, N (%)	27 (16.9)	24 (18.1)	3 (11.1)*
Height, cm	167 (160-173)	166 (159-173)	170 (162-174)
Weight, kg	80.5 ± 19.3	79.0 ± 19.8	88.0 ± 14.0*
BMI, kg/m^2	28.9 (26.3-31.9)	28.2 (26.1-31.9)	30.3 (27.9-34.2)*
Waist circumference, cm	90.0 (82.0-99.0)	89.0 (81.0-98.0)	98.0 (92.0-105.0)*
Waist to height ratio	0.54 (0.54-0.62)	0.53 (0.49-0.60)	0.59 (0.56-0.65)
Total body fat mass, %	30.8 ± 7.8	29.5 ± 7.9	32.4 ± 7.4
Total body fat mass, Kg	25.6 ± 9.6	24.8 ± 9.5	28.9 ± 9.5
Lean body mass, %	69.9 ± 7.8	70.5 ± 7.0	67.6 ± 7.4
Lean body mass, Kg	54.7 ± 9.6	53.8 ± 14.6	58.4 ± 13.4
Glycemic status IFG only, N (%) IFG + IGT, N (%)	 110 (68.8) 50 (31.2)	 95 (71.4) 38 (28.6)	 15 (55.6)* 12 (44.4)*
Hypertension, N (%)	65 (40.6)	44 (33.0)	21 (77.8)*
Dyslipidemia, N (%)	111 (69.4)	91 (68.4)	20 (74.1)
MetS, N (%)	60 (37.5)	46 (34.6)	14 (51.9)*
Stroke, N (%)	11 (6.9)	9 (6.8)	2 (7.4)
Family history of T2D, N (%)	46 (28.8)	37 (27.8)	9 (33.3)
Mini mental score	28 (26-29)	28 (26-29)	28 (27-29)
MedDiet score	28 (26-32)	28 (25-32)	28 (27-30)
ABI-Right lower extremity	1.2 (1.0-1.3)	1.2 (1.0-1.3)	1.2 (0.9-1.3)
ABI-Left lower extremity	1.2 (1.0-1.3)	1.2 (1.0-1.3)	1.2 (0.8-1.3)

PWV (carotid-femoral), m/s	8.2 (7.4-9.5)	8.0 (7.3-9.0)	8.8 (7.5-10.7)*
PWV carotid-femoral ≥ 10 m/s, N (%)	23 (14.4)	15 (11.3)	8 (29.6)*

Abbreviations: DSPN; distal symmetrical polyneuropathy**,** BMI; body mass index, MetS; metabolic syndrome, T2D; type 2 diabetes, MedDiet; mediterranean diet, ABI; ankle-brachial index, PWV; pulse wave velocity

Parametric variables are expressed as mean ± SD, non-parametric variables as median (IQR), and categorical variables as number (percentage). *p<0.05 vs participants without DSPN

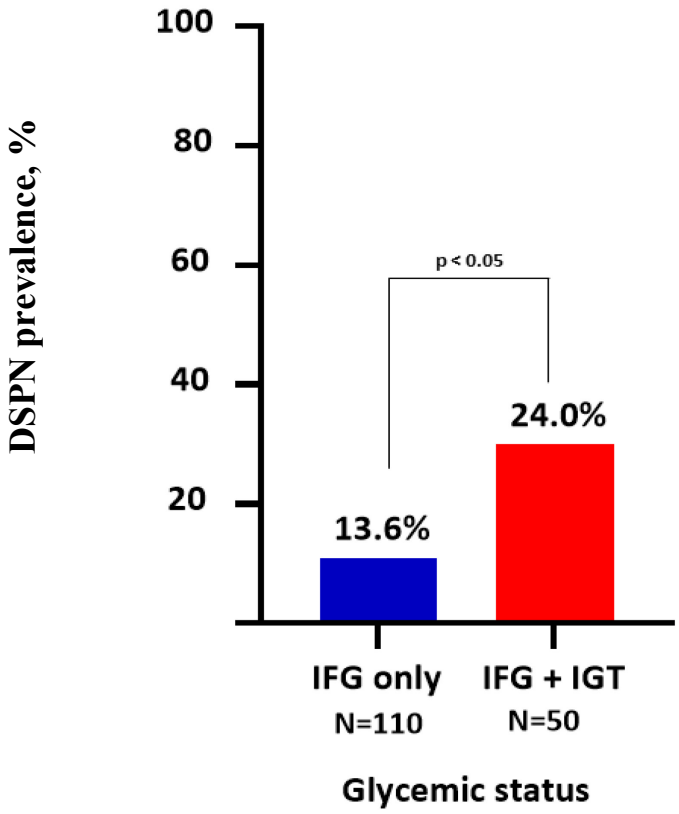


Figure 28 DSPN prevalence in individuals with IFG vs IFG + IGT at baseline

Abbreviations: DSPN; distal symmetrical polyneuropathy, IFG; impaired fasting glucose, IGT; impaired glucose tolerance.
Data are presented as percentages with group sizes indicated below each bar.

Table 6 Demographic and clinical characteristics of study participants grouped by glycemic status

	Overall (N=160)		**Without DSPN (N=133)**		**With DSPN (N=27)**	
	IFG (N=110)	**IFG+IGT (N=50)**	**IFG (N=95)**	**IFG+IGT (N=38)**	**IFG (N=15)**	**IFG+IGT (N=12)**
Age, years	59 ± 13	68 ±10*	58 ± 14	67 ± 10^	65 ± 8	70 ± 9#
Male gender, N (%)	69 (62.7)	28 (56.0)	61 (64.2)	21 (55.3)	8 (53.3)	7 (58.3)
Never smoking, N (%)	55 (50.0)	33 (66.0)	47 (49.5)	29 (76.3)	8 (53.3)	4 (33.3)
Current/ previous smoking, N (%)	55 (50.0)	17 (34.0)	48 (50.6)	9 (23.7)	7 (46.6)	8 (66.6)
Height, cm	167 (160-174)	166 (159-173)	167 (160-174)	166 (157-173)	171 (162-177)	168 (161-173)
Weight, kg	80.6 ± 19.4	82.4 ± 15.5	29.1 ± 4.6	80.8 ± 15.9	88.6 ± 15.3	87.2 ± 13.7
BMI, kg/m^2	29.1 ± 4.7	30.7 ± 4.4	29.1 ± 4.6	29.3±5.1	30.3 ± 4.3	31.2 ± 4.7
Waist circumference, cm	90.0 (82.0-97.0)	94.5 (85.0-104)*	89.0 (80.0-96.0)	90.0 (82.0-100.0)	96.0 (92.0-112.0)	101.5 (98.0-104.5)
Waist to height ratio	0.53 (0.59-0.60)	0.59 (0.56-0.65)	0.53 (0.49-0.54)	0.54 (0.48-0.64)	0.59 (0.56-0.63)	0.59 (0.56-0.66)
Total body fat mass, %	28.6 ± 8.5	31.1 ± 7.9*	28.1 ± 8.7	30.5 ± 7.5	31.9 ± 6.2	33.2 ± 9.4
Total body fat mass, Kg	23.7± 9.3	26.8 ± 10.5*	22.9 ± 9.2	26.0 ± 10.4	28.4 ± 9.1	29.7 ± 10
Lean body mass, %	70.1 ± 8.0	69.6 ± 7.7	70.5 ± 8.3	70.4 ± 7.0	68.1 ± 6.3	67.0 ± 9.4
Lean body mass, Kg	55.9 ± 14.5	52.4 ± 14.2	55.0 ±14.8	51.3 ± 14.1	59.3 ± 13.1	56.6 ± 14.8
Hypertension, N (%)	44 (40.0)	21 (42.0)	31 (32.6)	13 (34.2)	13 (86.7)	8 (66.7)

Dyslipidemia, N (%)	80 (72.7)	31 (62.0)	70 (73.7)	21 (55.3)	10 (66.7)	10 (83.3)
MetS, N (%)	40 (36.4)	20 (40.0)	31 (32.6)	15 (39.5)	9 (60.0)	5 (41.7)
Stroke, N (%)	6 (5.5)	5 (10.0)	5 (5.3)	4 (10.5)	1 (6.7)	1 (8.3)
Family history of T2D, N (%)	36 (32.7)	10 (20.0)	30 (31.6)	7 (18.4)	6 (40.0)	3 (25.0)
Mini mental score	28 (27-30)	27 (25-29)*	28 (27-30)	27 (25-29)	28 (27-30)	28 (25-29)
MedDiet score	29 (26-32)	28 (25-30)	29 (27-33)	28 (25-31)	27 (26-31)	29 (27-30)
ABI-Right lower extremity	1.2 (1.1-1.4)	1.1 (1.0-1.3)	1.2 (1.0-1.3)	1.1 (1.1- 1.3)	1.3 (0.8-1.4)	1.2 (1.0-1.2)
ABI-Left lower extremity	1.1 (1.1-1.3)	1.2 (1.1-1.3)	1.2 (1.0-1.3)	1.2 (1.1-1.3)	1.2 (0.8-1.3)	1.2 (1.0-1.3)
PWV (carotid-femoral), m/s	8.1 (7.3-9.6)	9.4 (8.2-10.6)*	7.8 (7.2-8.7)	8.9 (7.9-9.8)^	8.5 (7.3-9.5)	9.6 (8.4-12.2)$^{\#}$
PWV carotid-femoral ≥ 10 m/s, N (%)	14 (12.7)	9 (18.0)	11 (11.6)	4 (10.5)	3 (20.0)	5 (41.7)

Abbreviations: DSPN; distal symmetrical polyneuropathy**,** BMI; body mass index, MetS; metabolic syndrome, T2D; type 2 diabetes, MedDiet; mediterranean diet, ABI; ankle-brachial index, PWV; pulse wave velocity *$p<0.05$ vs. participants with IFG only, ^$p<0.05$ vs. participants without DSPN and with IFG only, $^{\#}p<0.05$ vs. participants with DSPN and with IFG only

Table 7 Hypolipidemic and antihypertensive medications of study participants at baseline

	Overall	**Without DSPN**	**With DSPN**
	N=160	**N=133**	**N=27**
Hypolipidemic treatment			
No treatment, N (%)	44 (27.5)	41 (30.8)	3 (11.11)
Statins, N (%)	50 (31.3)	40 (30.0)	10 (37.0)
Statins + ezetimibe, N (%)	60 (37.5)	50 (37.6)	10 (37.0)
Ezetimibe, N (%)	0 (0.0)	0 (0.0)	0 (0.0)
PCSK9i, N (%)	0 (0.0)	0 (0.0)	0 (0.0)
Statins + ezetimibe + PCSK9i, N (%)	1 (0.6)	1 (0.8)	0 (0.0)
Fenofibrate, N (%)	1 (0.6)	1 (0.8)	0 (0.0)
Statins + ezetimibe + fenofibrate, N (%)	3 (1.9)	2 (1.5)	1 (3.7)
Statins + fenofibrate, N (%)	1 (0.6)	1 (0.8)	0 (0.0)
Statins			
Rosuvastatin, N (%)	72 (45.0)	59 (44.4)	13 (48.1)
Atorvastatin, N (%)	27 (16.9)	23 (17.3)	4 (14.8)
Simvastatin, N (%)	10 (6.3)	7 (1.8)	3 (11.1)
Pravastatin, N (%)	2 (1.3)	2 (1.5)	0 (0.0)
Fluvastatin, N (%)	1 (0.6)	1 (0.8)	0 (0.0)
Pitavastatin, N (%)	3 (1.9)	2 (1.5)	1 (3.7)
Antihypertensive treatment			
No treatment, N (%)	79 (49.4)	70 (52.6)	9 (33.3)

ACEs, N (%)	1 (0.6)	1 (0.8)	0 (0.0)
ARBs, N (%)	7 (4.4)	5 (3.8)	2 (7.4)
CCBs, N (%)	11 (6.9)	10 (7.5)	1 (3.7)
B-blockers, N (%)	11(6.9)	9 (6.8)	2 (7.4)
Thiazide diuretics, N (%)	2 (1.3)	2 (1.5)	0 (0.0)
MRAs, N (%)	2 (1.3)	1 (0.8)	1 (3.7)
ACEs + CCBs, N (%)	1 (0.6)	1 (0.8)	0 (0.0)
ARBs + CCBs, N (%)	18 (11.3)	14 (10.5)	4 (14.8)
ACEs + b-blockers, N (%)	3 (1.9)	3 (2.6)	0 (0.0)
ARBs + b-blockers, N (%)	1 (0.6)	1 (0.8)	0 (0.0)
ARBs + thiazide diuretics, N (%)	9 (5.6)	6 (1.2)	3 (11.1)
Thiazide diuretics + b-blockers, N (%)	2 (1.3)	2 (1.5)	0 (0.0)
ARBs + CCBs + thiazide diuretics, N (%)	5 (3.1)	3 (2.6)	2 (7.4)
ACEs + CCBs + thiazide diuretics, N (%)	2 (1.3)	0 (0.0)	2 (7.4)
ACEs + CCBs + b-blockers, N (%)	3 (1.9)	2 (1.5)	1 (3.7)
ACEs + CCBs + thiazides + b-blockers, N (%)	1 (0.6)	1 (0.8)	0 (0.0)
ARBs + b-blockers + thiazide diuretics, N (%)	2 (1.3)	2 (1.5)	0 (0.0)

Abbreviations: PCSK9i; proprotein convertase subtilisin/kexin type 9 inhibitor, ACEs; angiotensin-converting enzyme inhibitors, ARBs; angiotensin ii receptor blockers, CCBs;

calcium channel blockers, B-blockers; beta blockers, MRAs; mineralocorticoid receptor antagonists

6.1.2 Neuropathic symptoms and signs according to DSPN prevalence

Neuropathic symptoms and signs in all participants stratified by DSPN are presented in **Table 8**. Muscle cramps (77.8 vs 35.3%, p=0.020) were the most common symptom, followed by fatigue (44.4 vs 22.6%, p=0.042), numbness (37.0 vs 13.5%, p=0.014) and burning sensation (33.3 vs 15.3%, p=0.033) in participants with DSPN vs without, respectively. Regarding neuropathic signs, subjects with DSPN vs without had decreased Achilles reflexes (66.7 vs 17.3%, p=0.031), abnormal pain perception with pinprick (40.7 vs 9.8%, p=0.035) and abnormal thermal discrimination using the Tip-Therm (74.0 vs 30.0%, p=0.022). Furthermore, 10-g monofilament insensitivity was significantly more common in DSPN vs no DSPN individuals (22.2 vs 1.5%, p=0.043).

Table 8 Neuropathic symptoms and signs overall and stratified by DSPN presence at baseline

	Overall	**Without DSPN**	**With DSPN**
	N=160	**N=133**	**N=27**
NSS	4 (0-6)	4 (0-6)	6 (5-8)*
Burning, N (%)	30 (18.8)	21 (15.8)	9 (33.3)*
Numbness, N (%)	28 (17.5)	18 (13.5)	10 (37.0)*
Paresthesia, N (%)	16 (10.0)	11 (8.3)	5 (18.5)
Fatigue, N (%)	42 (26.3)	30 (22.6)	12 (44.4)*
Cramps, N (%)	68 (42.5)	47 (35.3)	21 (77.8)*
Pain, N (%)	19 (11.9)	14 (15.5)	5 (18.5)
NDS	2 (0-3)	1 (0-2)	4 (3-6)*
Achilles tendon reflexes			
Right lower extremity			
Decreased, N (%)	29 (18.1)	17 (12.8)	12 (44.5)*
Absent, N (%)	2 (1.3)	1 (0.8)	1 (3.7)
Left lower extremity			
Decreased, N (%)	41 (26.3)	23 (17.3)	18 (66.7)*
Absent, N (%)	0	0	0
Both lower extremities			
Decreased, N (%)	26 (16.3)	14 (10.5)	12 (44.4) *
Absent, N (%)	0	0	0
Abnormal vibration perception as examined with tuning fork 128 Hz			
Right lower extremity, N (%)	18 (11.3)	5 (3.8)	13 (48.1)*
Left lower extremity, N (%)	20 (12.5)	5 (3.8)	15 (55.6)*
Both lower extremities, N (%)	15 (9.4)	4 (3.0)	11 (40.7)*
Abnormal pain perception as examined with pinprick			
Right lower extremity, N (%)	23 (14.4)	13 (9.8)	10 (37.0)*
Left lower extremity, N (%)	24 (15.0)	13 (9.8)	11 (40.7)*
Both lower extremities, N (%)	21 (13.1)	11 (8.2)	10 (37.0)*
Abnormal temperature discrimination as examined with thermal probe			
Right lower extremity, N (%)	52 (32.5)	35 (26.3)	17 (63.0)*

Left lower extremity, N (%)	60 (37.5)	40 (30.0)	20 (74.0)*
Both lower extremities, N (%)	50 (31.3)	38 (28.6)	12 (44.4)*
Absent 10-g monofilament sensation at least at one site			
Right lower extremity, N (%)	8 (5.0)	2 (1.5)	6 (22.2)*
Left lower extremity, N (%)	8 (5.0)	2 (1.5)	6 (22.2)*
Both lower extremities, N (%)	7 (4.4)	2 (1.5)	5 (18.5)
Abnormal VPT age-adjusted, N (%)	28 (17.5)	16 (12.0)	12 (44.4) *
VPT, V	20 (13-25)	20 (12-22)	25 (20-30)*

Abbreviations: DSPN; distal symmetrical polyneuropathy, NSS; neuropathy symptom score, NDS; neuropathy disability score, VPT; vibration perception threshold *p<0.05 vs participants without DSPN

6.1.3 Laboratory findings

Laboratory tests were similar between the 2 subgroups, except for 2hPG after 75-g oral glucose administration (139 ± 40 vs 121 ± 39 mg/dL, p=0.043) and HOMA-IR (2.8 [2.0-4.1] vs 2.5 [1.5-3.5], p=0.042), which were significantly higher in the DSPN vs no DSPN group (**Table 9**).

Table 9 Laboratory tests of study participants at baseline stratified by DSPN prevalence

	Overall	**Without DSPN**	**With DSPN**
	N=160	**N=133**	**N=27**
FPG, mg/dL	105 (100-111)	105 (100-112)	106 (102-113)
2-h plasma glucose after 75-g oral glucose administration, mg/dL	124 ± 41	121 ± 39	139 ± 40*
Insulin, μIU/ mL	10.2 (6.8-13.3)	10.2 (6.4-13.0)	10.9 (7.6-14.7)
HOMA-IR	2.6 (1.7-3.7)	2.5 (1.5-3.5)	2.8 (2.0-4.1)*

HbA1c, %	5.9 (5.6-6.2)	5.9 (5.6-6.2)	5.9 (5.4-6.1)
TC, mg/dL	164 (141-193)	164 (143-199)	168 (141-193)
LDL-C, mg/dL	98 (70-118)	89 (71-118)	88 (67-125)
TGs, mg/dL	100 (74-137)	98 (75-142)	105 (73-137)
HDL-C, mg/dL	50 (45-58)	50 (44-59)	53 (45-56)
ApoB, mg/dL	72 (60-88)	74 (61-91)	65 (55-84)
Lp(a), mg/dL	10.9 (3.7-29.1)	11.6 (4.1-32.1)	8.5 (2.6-18.3)
eGFR, mL/min/1.73 m^2	81 ± 14	81 ± 14	82 ± 10
UACR, mg/g	9.0 (5.2-20.0)	8.9 (5.3-15.5)	13.6 (6.3-40.0)
UACR > 30 mg/g, N (%)	25 (15.6)	18 (13.5)	7 (25.9)
25 (OH) Vitamin D levels	20.2 ± 10.2	20.9 ± 10.5	17.3 ± 8.1
< 20 ng/mL (%)	53 (33.1)	40 (30.0)	11 (40.7)
< 10 ng/mL (%)	23 (14.4)	16 (12.0)	6 (22.2)

Abbreviations: DSPN; distal symmetrical polyneuropathy**,** FPG; fasting plasma glucose, IFG; impaired fasting glucose, IGT; impaired glucose tolerance, HOMA-IR; homeostasis model assessment of insulin resistance, HbA1c; hemoglobin A1c, TC; total cholesterol, LDL-C; low-density lipoprotein cholesterol, TGs; triglycerides, HDL-C; high-density lipoprotein cholesterol, ApoB; apolipoprotein B, Lp(a); lipoprotein(a), eGFR; estimated glomerular filtration rate, UACR; urine albumin to creatinine ratio

Parametric variables are expressed as mean ± SD, non-parametric variables as median (IQR), and categorical variables as number (percentage). *$p<0.05$ vs. participants without DSPN

6.1.4 Associations of DSPN with cardiometabolic risk factors

Univariate associations of prevalent DSPN with cardiometabolic risk factors and arterial stiffness are shown in **Table 10**.

Multivariate analyses were adjusted for covariates with p<0.10 in univariate analyses, along with clinical important confounders: age, smoking, height, waist circumference, hypertension, 2h PG level after 75-g glucose administration, HOMA- IR, HbA1c, LDL-C, TGs, and PWV. In multivariate analysis, age (odds ratio [OR] per 1 year increase: 1.093, 95% confidence interval [CI]: 1.005-1.188, p=0.041), smoking (OR current/previous vs never-smoking: 1.347, 95% CI: 1.116-1.891, p=0.042), height (OR per 1 cm increase: 1.083, 95% CI: 1.004-1.168, p=0.039), waist circumference (OR per 1 cm increase: 1.123, 95% CI: 1.049-1.202, p<0.001), and HOMA-IR (OR per 0.1 increase: 1.304, 95% CI: 1.133-1.739, p=0.023) were independently associated with prevalent DSPN (**Figure 29**).

Stratified analyses of the associations of DSPN according to age (≤ vs > 62 years [mean age]), gender (female vs male), smoking status (current/former vs never), hypertension (yes vs no), and LDL-C (≤ vs > 98 mg/dL [median LDL-C levels]) are presented in **Figure 30**. No significant interaction was found for any of these associations.

Table 10 Univariate analysis for the association of DSPN with cardiometabolic risk factors and arterial stiffness at baseline

	Univariate (unadjusted)	
	OR (95% CI)	**P -value**
Age, for every 1-year increase	1.058 (1.010-1.108)	0.017
Gender, Female vs male	1.012 (0.414-2.476)	0.978
Smoking, Current/previous vs never	1.026 (0.431-2.446)	0.311
Height, for every 1 cm increase	1.026 (0.986-1.068)	0.208
BMI, for every 1 kg/m^2 increase	1.074 (0.986-1.169)	0.094

Waist circumference, for every 1 cm increase	1.050 (1.018-1.083)	0.002
Total body fat mass, for every 1% increase	1.050 (0.986-1.118)	0.132
Total body fat mass, for every 1 kg increase	1.043 (0.994-1.095)	0.085
Lean body mass, for every 1% increase	0.953 (0.894-1.015)	0.132
Lean body mass, for every 1 kg increase	1.025 (0.984-1.068)	0.237
Hypertension	5.833 (1.861-18.287)	0.002
Systolic blood pressure, for every 1 mmHg increase	1.018 (0.989-1.048)	0.223
2-hour plasma glucose level after 75-g glucose administration, for every 1 mg/dL increase	1.012 (1.001-1.023)	0.040
HOMA-IR, for every 0.1-unit increase	1.244 (1.005-1.539)	0.045
HbA1c, for every 0.1% increase	1.049 (0.971-1.134)	0.225
LDL-C, for every 1 mg/dL increase	1.003 (0.991-1.015)	0.645
TGs, for every 1 mg/dL increase	0.998 (0.992-1.005)	0.615
HDL-C, for every 1 mg/dL increase	1.003 (0.969-1.038)	0.877
PWV (carotid-femoral), for every 1 m/s increase	2.163 (1.172-4.183)	<0.001

Abbreviations: DSPN; distal symmetrical polyneuropathy, BMI; body mass index, HOMA-IR; homeostatic model assessment of insulin resistance, HbA1c; hemoglobin A1C, LDL-C; low-density lipoprotein cholesterol, TGs; triglycerides, HDL-C; high-density lipoprotein cholesterol, PWV; pulse wave velocity, VIF; variance inflation factor

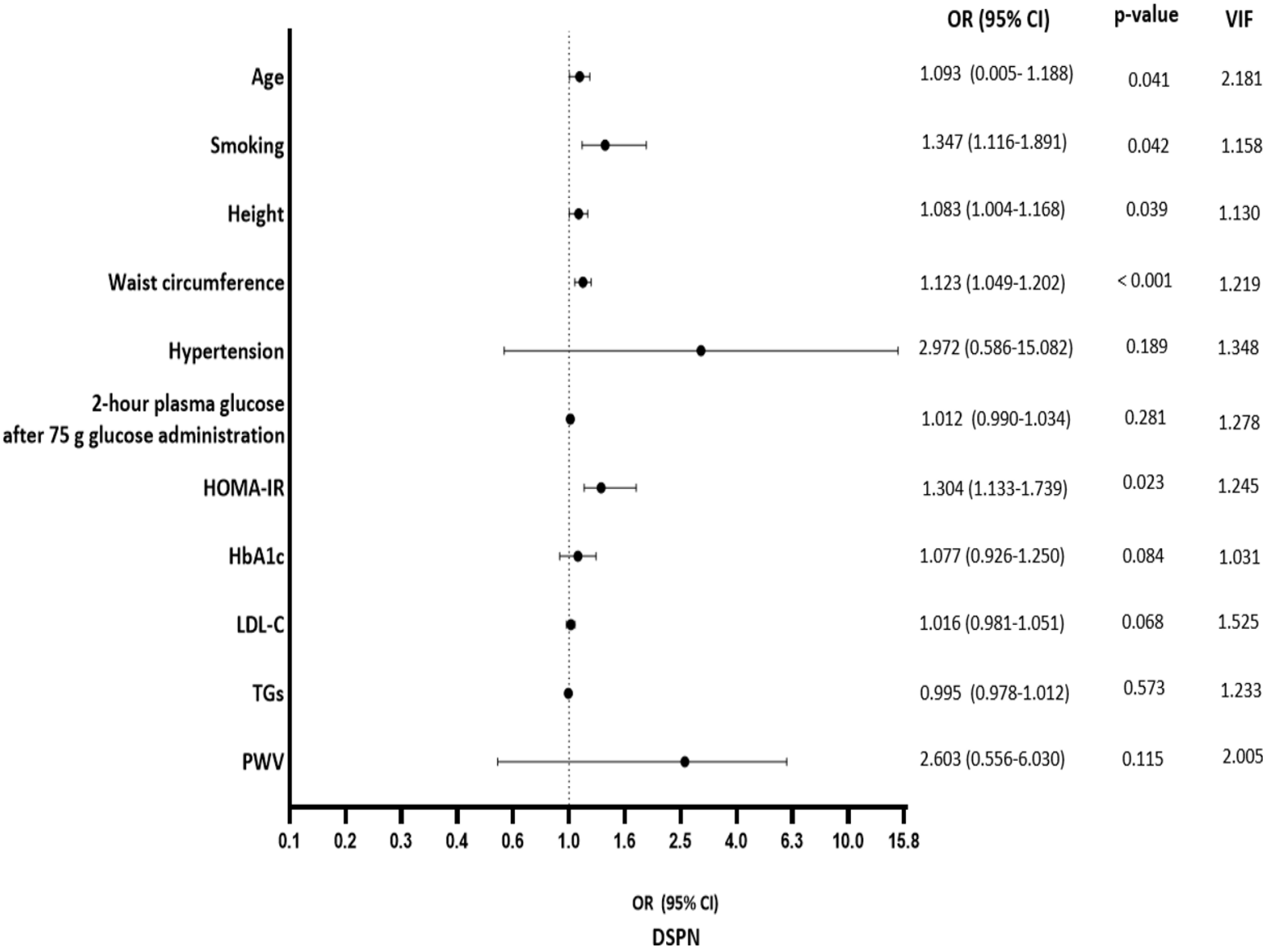


Figure 29 Multivariate associations of DSPN with cardiometabolic risk factors and arterial stiffness

Forest plot presenting OR (odds ratio) and 95% confidence intervals (CIs) from a multivariate binary logistic regression model assessing the associations of DSPN with cardiometabolic and cardiovascular risk factors. Each point represents the OR and error bars the 95% CIs. Multivariate analyses were adjusted for covariates with $p<0.10$ in univariate analyses, along with clinical important confounders: age, smoking, height, waist circumference, hypertension, 2 hour plasma glucose after 75 g glucose administration, HOMA- IR, HbA1c, LDL-C, TGs, and PWV. BMI was excluded from the multivariate analysis due to high collinearity with waist circumference (VIF 23.5 and 21.3, respectively). **Abbreviations:** DSPN; distal symmetrical polyneuropathy, BMI; body mass index, HOMA-IR; homeostatic model assessment of insulin resistance PWV; pulse wave velocity, VIF: variance inflation factor

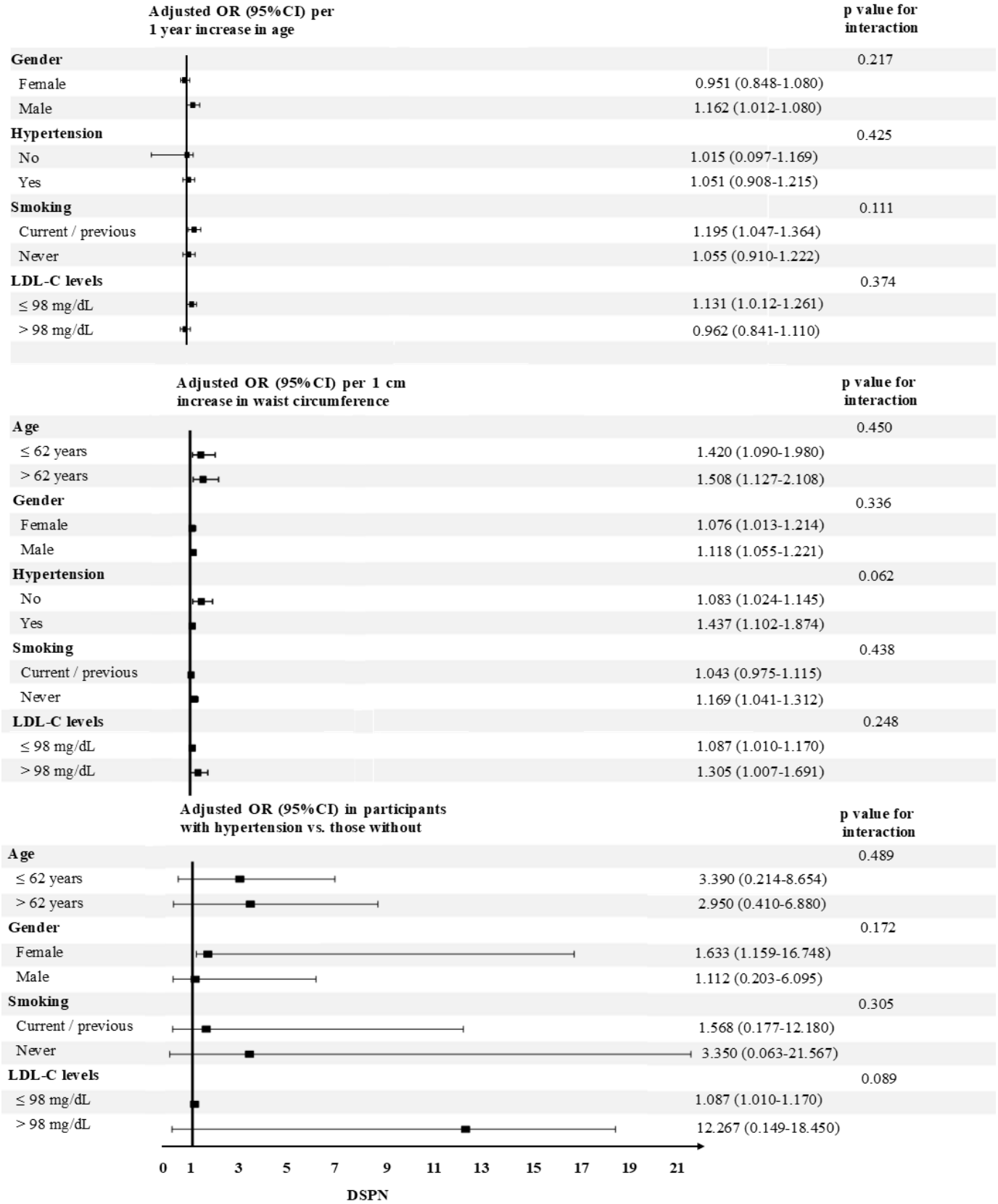


Figure 30 Stratified analyses of the associations between cardiometabolic risk factors, arterial stiffness and DSPN across prespecified subgroups at baseline

Stratified analyses of the associations of DSPN according to age (≤ vs > 62 years [mean age]), gender (female vs male), smoking status (current/former vs never), hypertension (yes vs no), and LDL-C (≤ vs > 98 mg/dL [median LDL-C levels]) **Abbreviations:** DSPN, distal symmetric polyneuropathy; LDL-C, low-density lipoprotein cholesterol; PWV, pulse wave velocity; HOMA-IR, homeostasis model assessment of insulin resistance, VIF; variance inflation factor

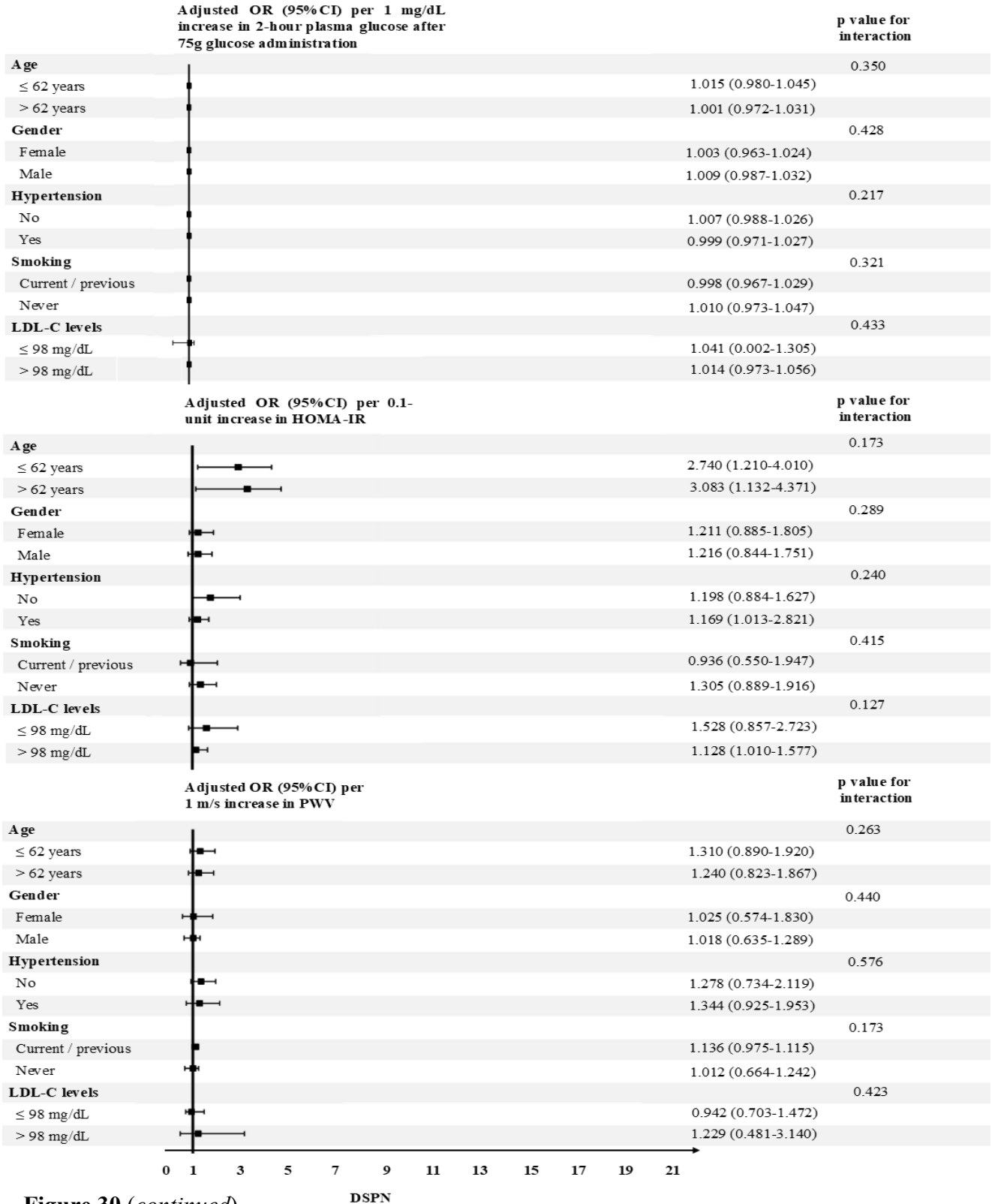


Figure 30 (*continued*)

6.1.5 Associations of NSS, NDS, and VPT with cardiometabolic risk factors

Univariate associations of NSS, NDS, VPT with cardiometabolic risk factors are presented in **Table 11**. In multivariate analysis, NSS was independently associated with higher waist circumference (beta: 0.096, 95% CI: 0.015-0.207, p=0.004) (**Figure 31**). NDS was independently correlated with age (beta: 0.043, 95% CI: 0.015-0.072, p=0.003), waist circumference (beta: 0.027, 95% CI: 0.007-0.046, p=0.008) and total body fat mass (beta: 0.056, 95% CI: 0.014-0.099, p=0.011) (**Figure 32**). Furthermore, VPT was independently associated with age (beta: 0.355, 95% CI: 0.164-0.546, p<0.001), total body fat mass (beta: 0.354, 95% CI: 0.131-0.577, p=0.002) hypertension (beta: 6.885, 95% CI: 2.570-10.800, p=0.002) and HOMA-IR (beta: 1.192, 95% CI: 0.253-2.131, p=0.014) (**Figure 33**).

Table 11 Univariate analysis for association of NSS, NDS and VPT with cardiometabolic risk factors and arterial stiffness at baseline

	Univariate		**Univariate**		**Univariate**	
	NSS		**NDS**		**VPT**	
	Beta (95% CI)	**P - value**	**Beta (95% CI)**	**P - value**	**Beta (95% CI)**	**P - value**
Age, for every 1-year increase	0.067 (0.028-0.105)	<0.001	0.290 (0.007-0.050)	0.009	0.379 (0.292-0.466)	<0.001
Gender, Female vs male	1.100 (-0.015-2.216)	0.053	-0.233 (-0.839-0.372)	0.448	-1.766 (-4.682-1.149)	0.233
Smoking, Current/previous vs never	0.008 (-0.033-0.107)	0.096	0.058 (-0. 061-0.178)	0.284	0.054 (-1.866-1.974)	0.956
Height, for every 1 cm increase	0.001 (-0.018-0.020)	0.884	0.006 (-0.004-0.017)	0.249	-0.028 (-0.079-0.022)	0.269
BMI, for every 1 kg/m^2 increase	0.151 (0.044-0.528)	0.006	0.044 (-0.016-0.104)	0.153	0.185 (-0.106-0.477)	0.211

Waist circumference, for every 1 cm increase	0.051 (0.019-0.084)	0.002	0.028 (0.010-0.046)	0.003	0.097 (0.008-0.185)	0.032
Total body fat mass, for every 1% increase	0.083 (-0.025-0.191)	0.131	0.038 (-0.020-0.096)	0.196	0.334 (0.057-0.611)	0.019
Total body fat mass, for every 1 kg increase	0.063 (-0.039-0.165)	0.224	0.059 (0.006-0.112)	0.029	0.330 (0.080-0.579)	0.011
Lean body mass, for every 1% increase	-0.068 (-0.146-0.010)	0.089	-0.030 (0.073-0.013)	0.170	-0.155 (-0.379-0.069)	0.174
Lean body mass, for every 1 kg increase	0.004 (-0.046-0.054)	0.867	0.013 (-0.13-0.039)	0.329	0.040 (-0.091-0.172)	0.545
SBP, for every 1 mmHg increase	0.026 (-0.001-0.053)	0.110	0.013 (-0.002-0.027)	0.121	0.167 (0.100-0.234)	<0.001
Hypertension	1.811 (0.689-2.934)	0.002	0.939 (0.0316-1.563)	0.003	6.577 (3.508 -9.646)	<0.001
2-hour plasma glucose level after 75-g glucose administration, for every 1 mg/dL increase	0.020 (0.006-0.033)	0.004	0.007 (0.000-0.015)	0.550	0.064 (0.026-0.095)	<0.001
HOMA-IR, for every 0.1 increase	0.274 (-0.022-0.570)	0.069	0.132 (0.031-0.294)	0.112	0.943 (0.184-1.702)	0.015
HbA1c, for every 0.1% increase	0.061 (-0.064-0.186)	0.338	0.046 (-0.023-2.057)	0.189	0.069 (-0.254-0.392)	0.674

LDL-C, for every 1 mg/dL increase	-0.009 (-0.025-0.007)	0.290	0.000 (-0.009-0.008)	0.937	-0.058 (-0.099-0.148)	0.005
TGs, for every 1 mg/dL increase	-0.004 (-0.011-0.003)	0.281	0.000 (-0.004-0.004)	0.932	0.124 (0.036-0.211)	0.039
HDL-C, for every 1 mg/dL increase	0.012 (-0.032-0.056)	0.600	0.005 (-0.019-0.029)	0.685	-0.016 (-0.130-0.098)	0.782
PWV, for every 1 m/s increase	0.256 (0.011-0.561)	0.042	0.193 (0.042-0.345)	0.013	0.193 (0.042-0.345)	0.013

Abbreviations: DSPN; distal symmetrical polyneuropathy, BMI; body mass index, HOMA-IR; homeostatic model assessment of insulin resistance, HbA1c; hemoglobin A1C, LDL-C; low-density lipoprotein cholesterol, TGs; triglycerides, HDL-C; high-density lipoprotein cholesterol, PWV; pulse wave velocity

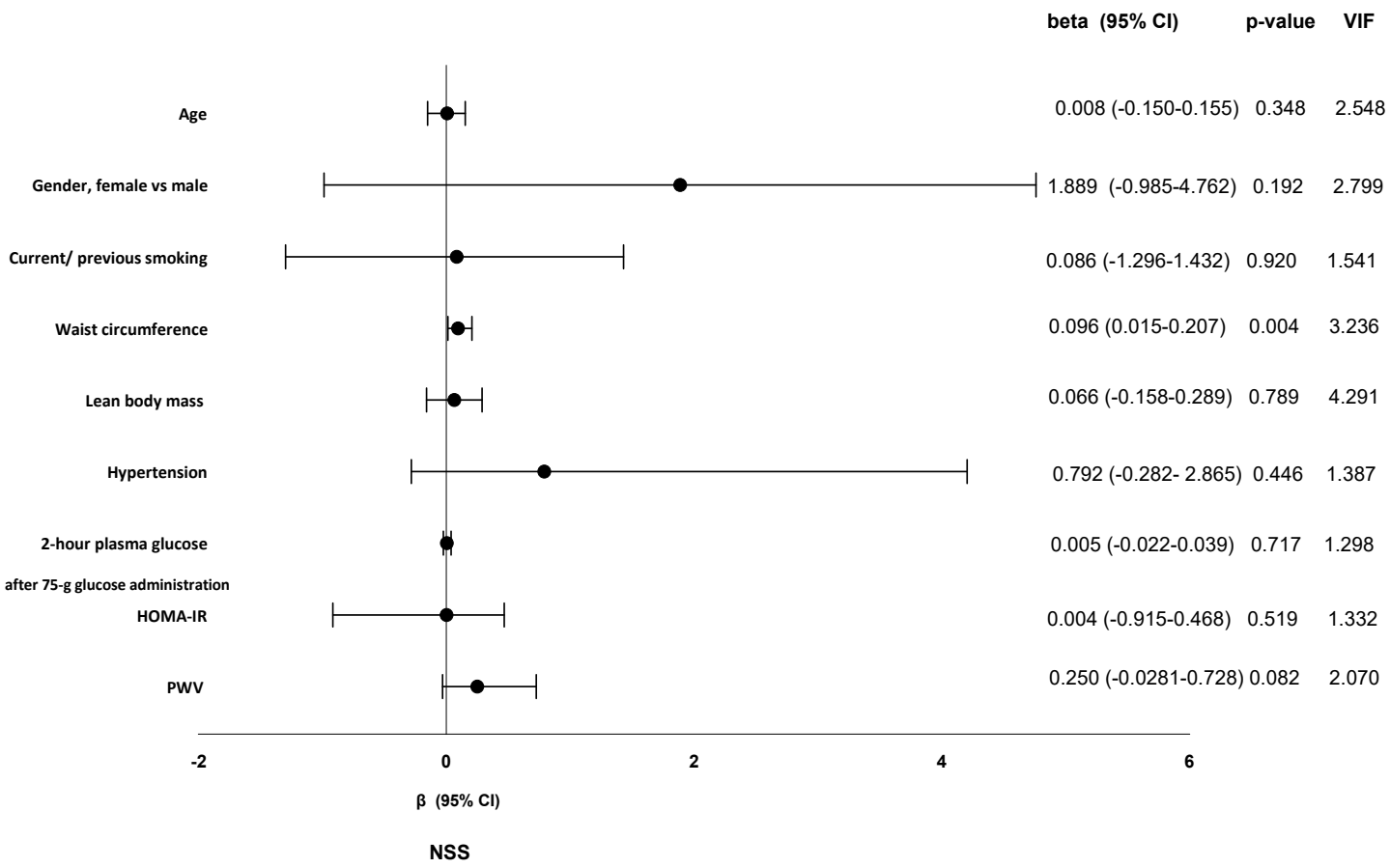


Figure 31 Multivariate associations of NSS with cardiometabolic risk factors and arterial stiffness at baseline

Forest plot presenting beta coefficients (β) and 95% confidence intervals (CIs) from a multivariate linear regression model assessing the associations of NSS with cardiometabolic and cardiovascular risk factors. Each point represents the β coefficient and error bars the 95% CIs. The multivariate analyses were adjusted for all covariates that showed a p-value < 0.1 in the univariate analysis. The multivariate analyses were adjusted for age, gender, smoking status, waist circumference, lean body mass percentage, hypertension, 2-h plasma glucose level after 75-g glucose administration, HOMA-IR and PWV. BMI was excluded from the multivariate analysis due to high collinearity with waist circumference (VIF 29.4 and 26.6, respectively).

Abbreviations: NSS; neuropathy symptom score, BMI; body mass index, HOMA-IR; homeostatic model assessment of insulin resistance, PWV; pulse wave velocity, VIF; variance inflation factor

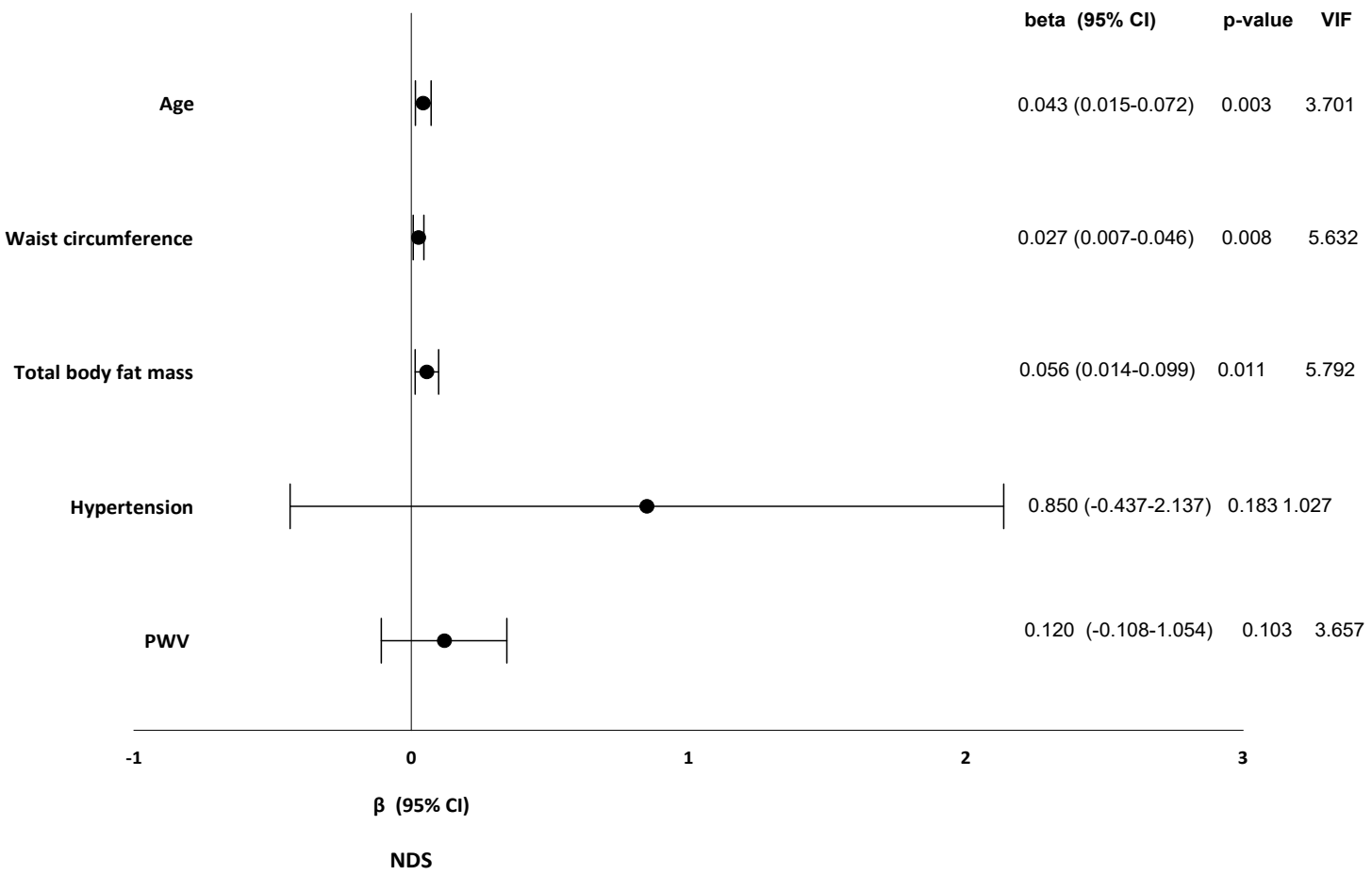


Figure 32 Multivariate associations of NDS with cardiometabolic risk factors and arterial stiffness at baseline

Forest plot presenting beta coefficients (β) and 95% confidence intervals (CIs) from a multivariate linear regression model assessing the associations of NDS with cardiometabolic and cardiovascular risk factors. Each point represents the β coefficient and error bars the 95% CIs. The multivariate analyses were adjusted for all covariates that showed a p-value < 0.1 in the univariate analysis. The multivariate analysis was adjusted for age, waist circumference, total body fat mass, hypertension and PWV.

Abbreviations: NDS; neuropathy disability score, PWV; pulse wave velocity

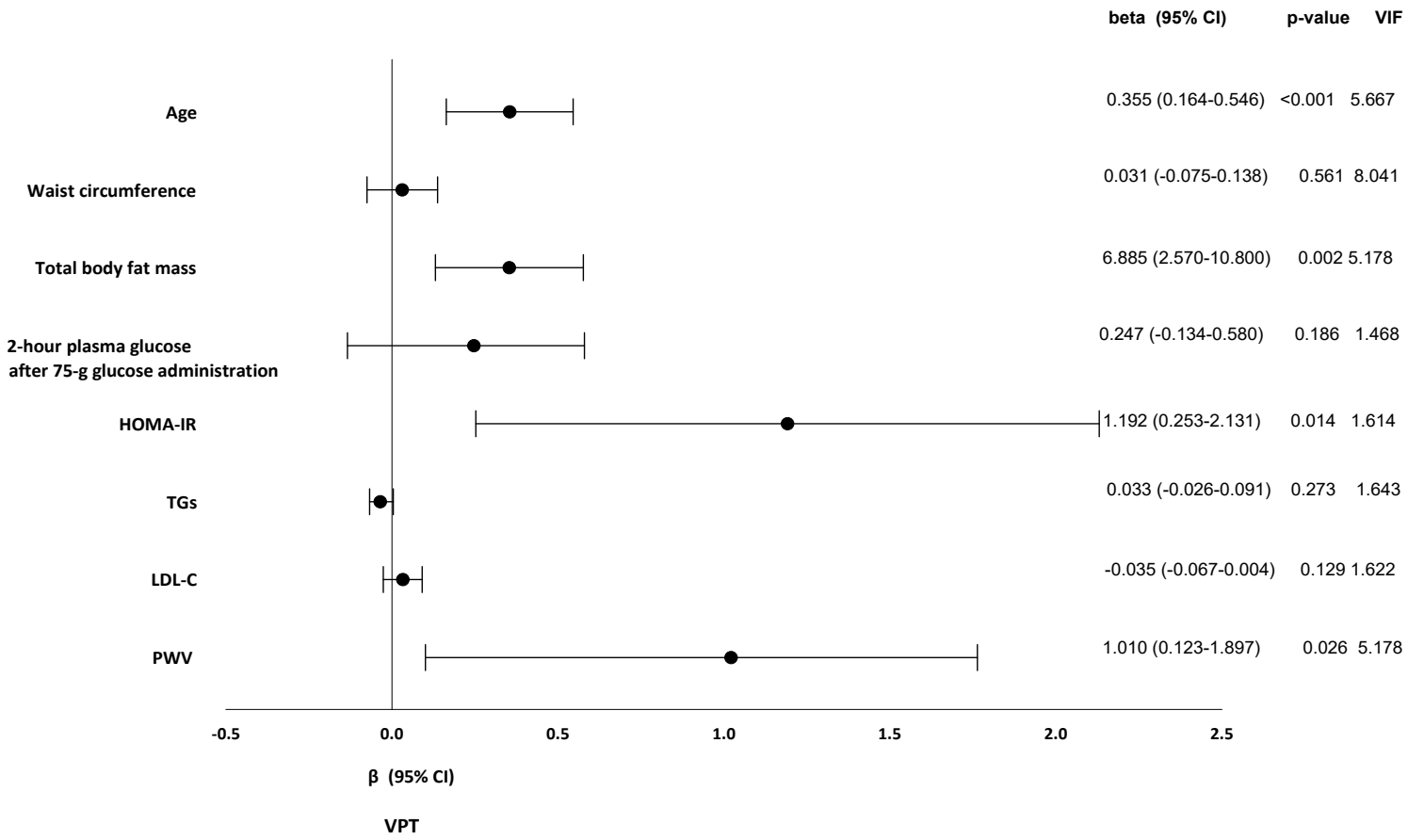


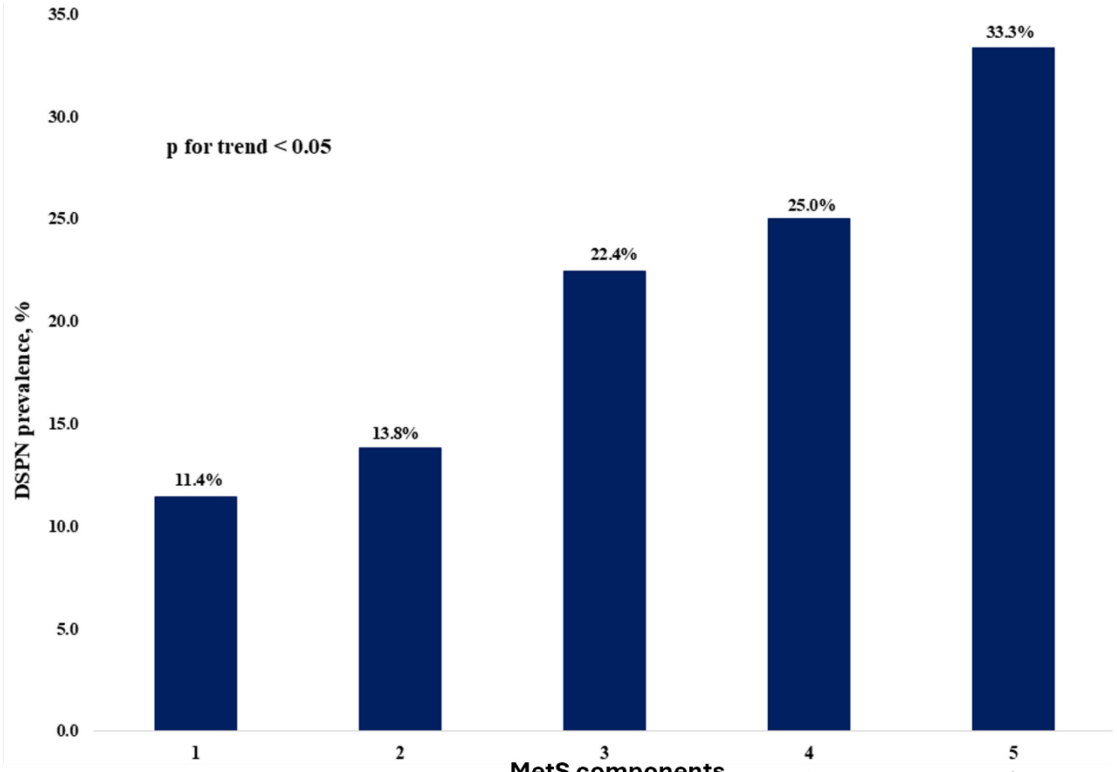
Figure 33 Multivariate associations of VPT with cardiometabolic risk factors and arterial stiffness at baseline

Forest plot presenting beta coefficients (β) and 95% confidence intervals (CIs) from a multivariate linear regression model assessing the associations of VPT with cardiometabolic and cardiovascular risk factors. Each point represents the β coefficient and error bars the 95% CIs. The multivariate analyses were adjusted for all covariates that showed a p-value < 0.1 in the univariate analysis. The multivariate analysis was adjusted for age, waist circumference, total body fat mass, hypertension, 2-h plasma glucose level after 75-g glucose administration, HOMA-IR, LDL-C, TGs and PWV.

Abbreviations: VPT; vibration perception threshold, HOMA-IR; homeostasis model assessment of insulin resistance, TGs; triglycerides, LDL-C; low-density lipoprotein cholesterol, PWV; pulse wave velocity

6.1.6 Association of MetS components and DSPN

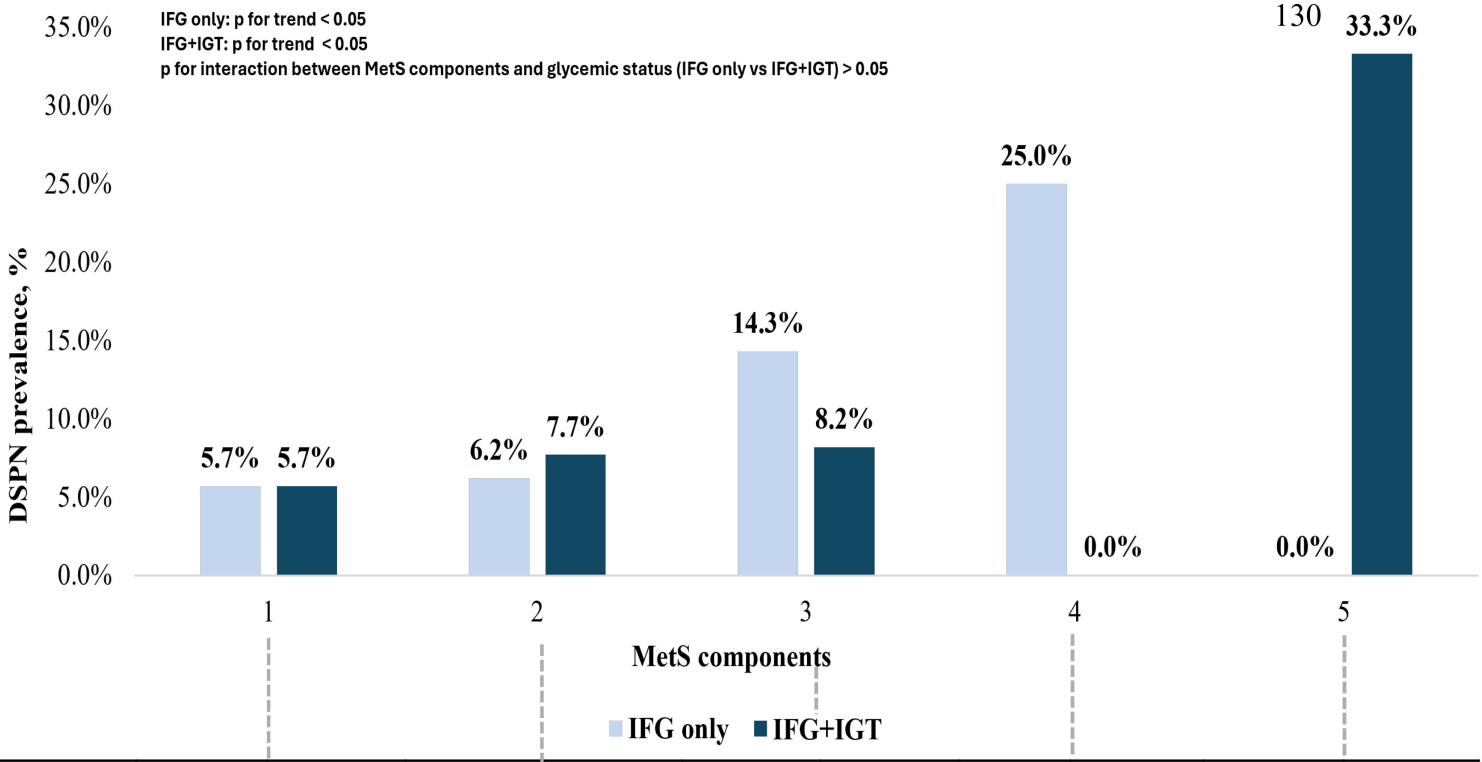
We grouped participants based on the number of MetS components present. The prevalence of DSPN was significantly higher in participants with a greater number of MetS components (p for trend=0.025) (OR per 1 MetS criterion increase: 1.467, 95% CI: 1.167 to 1.767, p=0.018) (**Figure 34**). Stratified analyses by glycemic status showed that the prevalence of DSPN was significantly higher in participants with a higher number of MetS components within both IFG only and IFG+IGT groups (p for trend=0.029 and p for trend=0.040, respectively). No significant interaction was observed between glycemic status and the number of MetS components for DSPN (**Figure 35**).



DSPN, n	4	9	11	2	1
Overall, n	35	65	49	8	3

Figure 34 The prevalence of DSPN with increasing MetS components

DSPN cases/total subjects (%) in each group: 1 MetS component: 4/35 (11.4%), 2 MetS components: 9/65 (13.8%), 3 MetS components: 11/49 (22.4%), 4 MetS components: 2/8 (25.0%), 5 MetS components: 1/3 (33.3%).
Abbreviations: DSPN; distal symmetrical polyneuropathy, MetS; metabolic syndrome
Data are presented as percentages with group sizes indicated below each bar.



	IFG only	IFG+IGT	IFG only	IFG+IGT	IFG only	IFG+IGT	IFG only	IFG+IGT	IFG only	IFG+IGT
DSPN, n	2	2	4	5	7	4	2	0	0	1
Overall, n	35		65		49		8		3	

Figure 35 The prevalence of DSPN with increasing MetS components by glycemic status at baseline

DSPN cases/total subjects (%) in each MetS component group: **IFG only:** 1 MetS component: 2/35 (5.7%), 2 MetS components: 4/65 (6.2%), 3 MetS components: 7/49 (14.3%), 4 MetS components: 2/8 (25.0%), 5 MetS components: 0/3 (0.0%). **IFG+IGT:** 1 MetS component: 2/35 (5.7%), 2 MetS components: 5/65 (7.7%), 3 MetS components: 4/49 (8.2%), 4 MetS components: 0/8 (0.0%), 5 MetS components: 1/3 (33.3%)

Abbreviations: IFG; impaired fasting glucose, IGT; impaired glucose tolerance, DSPN; distal symmetrical polyneuropathy, MetS; metabolic syndrome

6.1.7 Prevalent DSPN and arterial stiffness

A higher percentage of participants with DSPN had carotid-femoral PWV ≥10 m/s vs those without (29.6 vs 11.3%, p=0.029) (**Table 5**). Also, median PWV was significantly greater in the subgroup with vs without DSPN (8.8 vs 8.0 m/s, p=0.031) (**Table 5**). Demographic and clinical characteristics grouped by arterial stiffness are presented in **Table 12**. In the overall study population, those participants with PWV ≥10 m/s were significantly older and had higher prevalence of hypertension than those with PWV <10 m/s (72 vs 60 years, p=0.010 and 82.6 vs 33.6%, p=0.003, respectively). Participants with DSPN and PWV ≥10 m/s were more likely to be previous/current smokers (75.0 vs 43.0%, p=0.015) and have dyslipidemia (75.0 vs 66.7%, p=0.031) than those with DSPN but PWV <10 m/s (**Table 12**). In univariate analyses, PWV was significantly associated with DSPN (OR: 2.163, 95% CI: 1.172-4.183, p<0.001), but this association did not remain significant after adjustment for confounding variables (**Table 10** and **Figure 29**). Stratified analyses of the associations of DSPN with PWV according to age, gender, smoking status, hypertension, and LDL-C are presented in **Figure 30.** No significant interaction was observed. Univariate associations of NSS, NDS, and VPT with PWV are presented in **Table 11**. PWV was independently associated with VPT (beta: 1.010, 95% CI: 0.123-1.897, p=0.026) (**Figure 33**).

Table 12 Demographic and clinical characteristics of study participants grouped by PWV at baseline

	Overall N=160		**Without DSPN N=133**		**With DSPN N=27**	
	PWV <10 m/s (N=137)	**PWV ≥10 m/s (N= 23)**	**PWV <10 m/s (N=118)**	**PWV ≥10 m/s (N= 15)**	**PWV <10 m/s (N=21)**	**PWV ≥10 m/s (N= 8)**
Age, years	60 ± 8	72 ± 8*	60 ± 9	71 ± 10^	63 ± 7	75 ± 5 #
Male gender, N (%)	80 (58.4)	17 (73.9)	69 (58.5)	12 (80.0)	11 (52.4)	5 (23.8)
Never smoking, N (%)	77 (56.2)	11 (47.8)	67 (56.8)	9 (60.0)	10 (47.6)	2 (62.5)
Previous/current smoking, N (%)	60 (43.7)	12 (52.2)	51 (43.2)	6 (40.0)	9 (43.0)	6 (75.0)#
Height, cm	168 (160-174)	167 (163-172)	168 (160-174)	166 (159-168)	170 (158-174)	172 (165-183)
Weight, kg	80.8 ± 19.6	83.8 ± 15.8	80.0 ± 20.1	80.0 ± 14.5	85.1 ± 16.4	95.3 ± 12.2
BMI, kg/m^2	28.0 (26.2-31.7)	30.3 (26.9-34.0)	27.9 (26.1-31.6)	29.7 (26.4-32.6)	29.1 (27.1-33.0)	31.0 (29.8-35.5)
Waist circumference, cm	90.0 (82.0-98.0)	94.0 (84.0-104.0)	89.0 (81.0-98.0)	92.0 (79.0-104.0)	96.5 (91.0-104.5)	102.0 (97.0-114.0)
Waist to height ratio	0.53 (0.49-0.62)	0.56 (0.47-0.65)	0.52 (0.49-0.60)	0.56 (0.46-0.65)	0.59 (0.52-0.65)	0.59 (0.56-0.66)
Total body fat mass, %	29.5 ± 8.9	29.8 ± 10.5	29.0 ± 8.7	27.4 ± 7.2	32.0 ± 7.8	34.6 ± 7.8
Total body fat mass, Kg	24.5 ± 9.9	25.0 ± 12.7	24.0 ± 9.9	21.5 ± 11.9	27.7 ± 9.6	33.4 ± 7.8
Lean body mass, %	70.1 ± 8.2	68.2 ± 9.1	70.6 ± 8.2	69.8 ± 9.9	68.0 ± 7.7	65.4 ± 7.8
Lean body mass, Kg	54.4 ± 12.7	57.6 ± 11.5	54.3 ± 12.4	49.7 ± 9.0	54.7 ± 14.8	67.5 ± 2.9
Glycemic status IFG, N (%) IFG + IGT, N (%)	96 (70.1) 37 (27.0)	14 (60.9) 13 (56.6)	84 (71.2) 32 (27.1)	11 (73.3) 6 (40.0)	12 (57.1) 5 (23.8)	3 (37.5) 7 (87.5)
Hypertension, N (%)	46 (33.6)	19 (82.6)*	32 (27.1)	12 (80.0)^	14 (66.7)	7 (87.5)
Dyslipidemia, N (%)	92 (67.2)	19 (82.6)	78 (66.1)	13 (86.7)	14 (66.7)	6 (75.0)#
MetS, N (%)	49 (35.8)	11 (47.8)	39 (33.1)	7 (46.7)	10 (47.6)	4 (50.0)

Stroke, N (%)	7 (5.1)	4 (17.4)	6 (5.1)	3 (20.0)	1 (4.8)	1 (12.5)
Family history of T2D, N (%)	35 (25.5)	11 (47.8)	29 (24.6)	8 (53.3)	6 (28.6)	3 (37.5)
Mini mental score	28 (26-29)	27 (23-29)*	28 (26-29)	27 (23-28)^	28 (27-30)	27 (24-30)
MedDiet score	29 (27-32)	28 (26-31)	29 (27-32)	27 (25-31)	28 (27-31)	30 (27-31)
ABI-Right lower extremity	1.2 (1.1-1.3)	1.2 (1.0-1.3)	1.1 (1.1-1.3)	1.1 (1.0-1.3)	1.3 (1.1-1.4)	1.1 (1.0- 1.2)
ABI-Left lower extremity	1.2 (1.1-1.3)	1.2 (1.1-1.2)	1.2 (1.0-1.3)	1.2 (1.1-1.3)	1.2 (1.0 -1.3)	1.2 (1.1-1.3)

Abbreviations: DSPN; distal symmetrical polyneuropathy**,** BMI; body mass index, MetS; metabolic syndrome, T2D; type 2 diabetes, MedDiet; mediterranean diet, ABI; ankle-brachial index, PWV; pulse wave velocity *$p<0.05$ vs. participants with PWV < 10 m/s, ^$p<0.05$ vs participants without DSPN and PWV <10 m/s, $^{\#}p<0.05$ vs participants with DSPN and PWV <10 m/s

6.2 Follow-up

6.2.1 Clinical characteristics of study population and incidence of DSPN at follow up

Among 160 participants with prediabetes, 27 (16.9%) had DSPN at baseline. The remaining 133 participants were enrolled in the prospective study, and all completed study visit at 24 months. **Table 13** summarizes demographic and clinical characteristics at baseline and follow-up. Over the median follow-up of 24 months (IQR 20-27), 13 subjects developed DSPN, corresponding to an incidence rate of 9.8%, 17 participants (12.8%) progressed to T2D and 24 (21.8%) regressed to normoglycemia (**Table 13**).

At baseline visit, individuals progressing to incident DSPN vs those who did not were older (68 ± 10 vs 60 ± 14 years, p=0.011), and had higher BMI (28.7 [27.3-32.0] vs 27.3 [25.0-31.5] kg/m^2, p=0.046), total fat mass (29.4 ± 16.4 vs 23.3 ± 12.4 kg, p=0.026) and PWV (8.6 [8.19.3] vs 6.4 [5.6-7.4] m/s, p=0.041) (**Table 13**). Rates of reversion to normoglycemia or progression to T2D during follow-up did not differ between the 2 groups (**Table 13**). In addition, no significant differences in routine treatment were observed between the 2 groups at baseline. In participants who developed DSPN, the use of statin and ezetimibe therapy did not change significantly, while in those who did not, statins increased by 30.8% and ezetimibe by 21.7% (both $p<0.05$) at follow-up (**Table 14**).

Table 13 Baseline and follow-up demographic and clinical characteristics of participants according to DSPN status at follow-up (among those without DSPN at baseline)

	Overall Baseline	**Overall Follow-up**	**No DSPN Baseline**	**No DSPN Follow-up**	**Incident DSPN Baseline**	**Incident DSPN Follow-up**
	N=133	**N=133**	**N=120**	**N=120**	**N=13**	**N=13**
Age, years	61 ± 13	63 ± 16	60 ± 13	62 ± 16	68 ± 14[c]	70 ± 15
Sex, Male, N (%)	81 (60.9)	-	71 (59.2)	-	10 (76.9)[c]	-
Never smoking, N (%)	61 (50.8)	61 (50.8)	58 (48.3)	58 (48.3)	3 (23.1) [c]	3 (23.1)
Previous/ current smoking, N (%)	72 (60.0)	72 (60.0)	62 (51.7)	62 (51.7)	10 (76.9) [c]	10 (76.9)
Height, cm	167 (160-173)	-	166 (159-174)	-	169 (164-176)	-
Weight, kg	79.7 ± 18.7	79.4 ± 17.5	78.8 ± 18.8	78.6 ± 17.9	89.2 ± 15.4[c]	87.0 ± 12.0 [d]
BMI, kg/m^2	28.3 (26.2-31.5)	28.4 (25.8-31.6)	27.3 (25.0-31.5)	27.2 (25.8-31.6)	28.7 (27.3-32.0)[c]	28.5 (26.9-30.7)
Waist circumference, cm	89.0 (81.0-98.0)	90.0 (81.0-97.0)	88.0 (81.0-91.0)	90.0 (80.0-96.0)	92.0 (86.0-98.0)[c]	92.0 (86.0-97.0)

Waist to height ratio	0.53 (0.49-0.60)	0.53 (0.50-0.60)	0.53 (0.48-0.60)	0.54 (0.48-0.57)	0.54 (0.51-0.58)	0.54 (0.51-0.57) [d]
MedDiet score	28 (25-32)	30 (26-32)	29 (25-32)	30 (26-32)	31 (28-33)	31 (26 -33)
SBP, mmHg	128 ± 22	131 ± 20	128± 23	131 ± 20	130 ± 8	134 ± 18
DBP, mmHg	78 ± 12	79 ± 12	78 ± 12	78 ± 13	78 ± 12	81 ± 12
Total body fat mass, %	28.7 ± 8.4	29.2 ± 7.6	28.9 ± 8.6	29.0 ±7.6	27.0 ± 7.0	30.3 ± 8.47 [d]
Total body fat mass, Kg	23.8 ± 9.6	25.4 ± 12.5	23.3 ± 8.8	25.2 ± 12.4[a]	29.4 ± 16.4[c]	28.1 ± 14.3[d]
Lean body mass, %	70.7 ± 7.9	70.6 ± 7.4	71.1 ± 7.4	71.0 ± 7.6	73.0 ± 7.8	69.7 ± 7.1
Lean body mass, Kg	55.9 ± 9.6	54.1 ± 13.8	55.5 ± 13.4	53.4 ± 14.1	59.8 ± 16.1	58.9 ± 5.8
IFG, N (%) **IGT, N (%)** **T2D, N (%)** **Normoglycemia, N (%)**	95 (71.4) 38 (28.6) 0 (0.0) 0 (0.0)	92(69.2) - 17 (12.8) 24 (21.8)	87 (72.5) 33(27.5) 0 (0.0) 0 (0.0)	82 (68.3)[a] - 16 (13.3) 22 (18.3)	8 (61.5) [c] 5 (38.5) [c] 0 (0.0) 0 (0.0)	10 (76.9)[b] - 1 (7.7) 2 (15.4)
ABI-Right lower extremity	1.19 (1.0-1.22)	1.19 (1.07-1.32)	1.19 (1.04-1.29)	1.19 (1.09-1.30)	1.14 (1.08-1.25)	1.27 (1.14-1.35)
ABI-Left lower extremity	1.18 (1.0-1.3)	1.18 (1.08-1.30)	1.17 (1.01-1.28)	1.17 (1.07-1.30)	1.25 (1.17-1.33)	1.26 (1.13-1.35)
PWV (carotid-femoral), m/s	8.0 (7.3-9.1)	8.3 (7.4-9.7)	6.4 (5.6-7.4)	8.2 (7.4-9.6)[a]	8.6 (8.1-9.3) [c]	9.7 (8.2 12.4) [d]

PWV carotid-femoral ≥10 m/s, N (%)	17 (12.8)	20 (15.0)	15 (12.5)	16 (13.3)	2 (15.4)	4 (30.8) [d]

Abbreviations: DSPN; distal symmetrical polyneuropathy, BMI; Body-Mass-Index, SBP; systolic blood pressure, DBP; diastolic blood pressure, IFG; impaired fasting glucose, IGT; impaired glucose tolerance, T2D; Type 2 Diabetes, MetS; Metabolic Syndrome, UACR; urinary albumin-to-creatinine ratio, MedDiet; Mediterranean Diet, ABI; Ankle-Brachial Index, PWV; Pulse Wave Velocity

Parametric variables are expressed as mean ± SD, non-parametric variables as median (IQR), and categorical variables as number (percentage), [a]p-value<0.05 for within-group change from baseline to follow-up in the No DSPN group,[b]p-value<0.05 for within-group change from baseline to follow-up in the incident DSPN group.[c]p-value<0.05 for between-group comparisons across DSPN groups at baseline, [d]p-value<0.05 for between-group comparisons across DSPN groups at follow-up

Table 14 Baseline and follow-up medications of study participants according to DSPN group

	Overall Baseline	Overall Follow-up	No DSPN Baseline	No DSPN Follow-up	Incident DSPN Baseline	Incident DSPN Follow-up
	N=133	**N=133**	**N=120**	**N=120**	**N=13**	**N=13**
Hypolipidemic, N (%)	91 (68.4)	129 (97.0)	81 (67.5)	118 (98.3)[a]	10 (76.9)	11 (84.6)
Statin, N (%)	91 (68.4)	128 (96.2)	81 (67.5)	118 (98.3)[a]	10 (76.9)	10 (76.9)
Ezetimibe, N (%)	53 (39.8)	81 (60.9)	48 (40.0)	74 (61.7)[a]	5 (38.5)	7 (53.8)
Fenofibrate, N (%)	4 (3.0)	0 (0.0)	4 (3.3)	0 (0.0)	0 (0.0)	0 (0.0)
ACEs, N (%)	8 (6.0)	8 (6.0)	8 (6.7)	8 (6.7)	0 (0.0)	0 (0.0)
ARBs, N (%)	31 (23.3)	36 (27.1)	28 (23.3)	31 (25.8)	3 (23.1)	5 (38.5)
CCBs, N (%)	31 (23.3)	31 (23.3)	26 (21.6)	26 (21.6)	5 (38.5)	5 (38.5)
B-blockers, N (%)	20 (15.0)	21 (15.8)	19 (15.8)	20 (16.7)	1 (7.7)	1 (7.7)
Thiazide diuretics, N (%)	16 (12.0)	18 (13.5)	14 (11.6)	16 (13.3)	2 (15.4)	2 (15.4)
MRAs, N (%)	1 (0.8)	1 (0.8)	1 (0.8)	1 (0.8)	0 (0.0)	0 (0.0)

Abbreviations: ACEs; angiotensin-converting enzyme inhibitors, ARBs; angiotensin ii receptor blockers, CCBs; calcium channel blockers, B-blockers; beta blockers, MRAs; mineralocorticoid receptor antagonists

[a] p-value for within-group change from baseline to follow-up in the No DSPN group

6.2.2. Changes in neuropathic symptoms and signs from baseline to follow-up in participants without DSPN at baseline

Baseline and follow-up neuropathic symptoms and signs are presented in **Table 15**. Participants developing incident DSPN vs those who did not had higher baseline NSS (5 [3-8] vs 3 [0-5], p<0.001) (**Table 15**). Regarding neuropathic symptom severity at baseline, severe symptoms (NSS scores: 7-10) were more common (23.1 vs 10.8%, p=0.042) in those who developed incident DSPN vs those who did not (**Table 16**). No significant differences were observed in baseline NDS and neuropathic sign severity between the 2 groups (**Table 15** and **Table 16**). Baseline abnormal vibration perception assessed with a tuning fork was more frequent at baseline (7.7 vs 2.5%, p=0.021) in those who developed incident DSPN (**Table 15**). Also, baseline VPT was higher (25 [21-30] vs 18 [11-21] V, p=0.011) and more frequently abnormal (23.1 vs 10.0%, p=0.017) in those developing incident (**Table 15** and **Table 16**).

Table 15 Baseline and follow-up neuropathic symptoms and signs according to DSPN group during the study

	Overall Baseline	**Overall Follow-up**	**No DSPN Baseline**	**No DSPN Follow-up**	**Incident DSPN Baseline**	**Incident DSPN Follow-up**
	N=133	**N=133**	**N=120**	**N=120**	**N=13**	**N=13**
NSS	4 (0-6)	4 (0-6)	3 (0-5)	3 (0-5)	5 (3-8)[b]	6 (5-6)[c]
Burning, N (%)	21 (15.8)	23 (17.3)	19 (15.8)	16 (13.3)	2 (15.4)	7 (53.8)[c]
Numbness, N (%)	18 (13.5)	23 (17.3)	16 (13.3)	10 (8.3)	2 (15.4)	13 (100.0)[c]
Paresthesia, N (%)	11 (8.3)	10 (7.5)	9 (7.5)	7 (5.8)	2 (15.4)	3 (23.1)[c]

Fatigue, N (%)	30 (22.6)	15 (11.3)	25 (20.8)	13 (10.8)[a]	5 (38.5)	2 (15.4)
Cramps, N (%)	47 (35.3)	42 (31.6)	42 (35.0)	36 (30.0)	5 (38.5)	6 (46.2)
Pain, N (%)	14 (10.5)	10 (7.5)	13 (10.8)	9 (7.5)	1 (7.7)	1 (7.7)
Distal (foot) distribution of symptoms, N (%)	35 (26.3)	39 (29.3)	29 (24.2)	30 (25.0)	6 (46.2)[b]	9 (69.2)[c]
Distal (lower leg) distribution of symptoms, N (%)	40 (30.1)	38 (28.6)	36 (30.0)	34 (28.3)	4 (30.8)	4 (30.8)
Exacerbation at night, N (%)	31 (23.3)	32 (24.1)	26 (21.6)	27 (22.5)	5 (38.5)[b]	5 (38.5)[c]
Awaken at night, N (%)	16 (12.0)	19 (14.3)	14 (11.7)	14 (11.7)	2 (15.4)[b]	5 (38.5)
Symptoms improvement while walking, N (%)	17 (12.8)	17 (12.8)	14 (11.7)	13 (10.8)	3 (23.1)	4 (30.8)[c]
Symptoms improvement while standing, N (%)	8 (6.0)	3 (2.3)	6 (5.0)	2 (1.7)	2 (15.4)	1 (7.7)
Symptoms improvement while sitting, N (%)	100 (75.2)	71 (53.4)	92 (76.7)	64 (53.3)	8 (61.5)	7 (53.8)

NDS	3 (2.3)	4 (3.0)	1 (0-2)	0 (0-2)	2 (0-2)	4 (3-5)[c]
Achilles tendon reflexes						
Right lower extremity						
Decreased, N (%)	17 (12.8)	26 (19.5)	16 (13.3)	18 (15.0)	1 (7.7)	8 (61.5)[c]
Absent, N(%)	1 (0.8)	2 (1.5)	1 (1.0)	2 (1.7)	0 (0.0)	0 (0.0)
Left lower extremity						
Decreased, N (%)	23 (17.3)	23 (17.3)	22 (18.3)	15 (12.5)	1 (7.7)	8 (61.5)[c]
Absent, N (%)	0 (0.0)	2 (1.5)	0 (0.0)	2 (1.7)	0 (0.0)	0 (0.0)
Both lower extremities						
Decreased, N (%)	14 (10.5)	20 (15.0)	14 (11.7)	15 (12.5)	0 (0.0)	5 (38.5)
Absent, N (%)	0 (0.0)	2 (1.5)	0 (0.0)	2 (1.7)	0 (0.0)	0 (0.0)
Abnormal vibration perception as examined with tuning fork						
Right lower extremity, N (%)	5 (3.8)	10 (7.5)	3 (2.5)	4 (3.3)	2 (15.4)	6 (46.2)[c]
Left lower extremity, N (%)	5 (3.8)	11 (8.3)	3 (2.5)	5 (4.2)	2 (15.4)	6 (46.2)[c]
Both lower extremities, N (%)	4 (3.0)	4 (3.0)	3 (2.5)	0 (0.0)	1 (7.7)	4 (30.8)[c]
Abnormal pain perception as examined with pinprick						
Right lower extremity, N (%)	13 (9.8)	22 (16.5)	11 (9.2)	16 (13.3)	2 (15.4)	6 (46.2)[c]

Left lower extremity, N (%)	13 (9.8)	17 (12.8)	11 (9.2)	12 (10.0)	2 (15.4)	5 (38.5)
Both lower extremities, N (%)	13 (9.8)	14 (10.5)	11 (9.2)	10 (8.3)	2 (15.4)	4 (30.8)
Abnormal temperature discrimination as examined with thermal probe						
Right lower extremity, N (%)	35 (26.3)	35 (26.3)	30 (25.0)	26 (21.7)	5 (38.5)	9 (69.2)[c]
Left lower extremity, N (%)	40 (30.1)	33 (24.8)	37 (30.8)	23 (19.2)	3 (23.1)	10 (76.9)[c]
Both lower extremities, N (%)	32 (24.1)	29 (21.8)	30 (25.0)	22 (18.3)	2 (15.4)	7 (53.8)[c]
Absent 10-g monofilament sensation at least at one site						
Right lower extremity, N (%)	2 (1.5)	5 (3.8)	2 (1.7)	2 (1.7)	0 (0.0)	3 (23.1)[c]
Left lower extremity, N (%)	2 (1.5)	5 (3.8)	2 (1.7)	2 (1.7)	0 (0.0)	3 (23.1)[c]
Both lower extremities, N (%)	2 (1.5)	4 (3.0)	2 (1.7)	2 (1.7)	0 (0.0)	2 (15.4)
VPT, V	20 (15-25)	20 (15-27)	18 (11-21)	18 (12-23)	25 (21-30)[b]	28 (20-31)[c]

Abbreviations: NSS; neuropathic symptom score, NDS; neuropathy disability score, VPT; vibration perception threshold

Parametric variables are expressed as mean ± SD, non-parametric variables as median (IQR), and categorical variables as number (percentage) [a]p-value for within-group change from baseline to follow-up in the No DSPN group, [b]p-value for between-group comparisons across DSPN groups at baseline, [c]p-value for between-group comparisons across DSPN groups at follow-up

Table 16 Severity categories of NSS, NDS, and VPT at baseline and follow-up according to DSPN group

	Overall Baseline	**Overall Follow-up**	**No DSPN Baseline**	**No DSPN Follow-up**	**Incident DSPN Baseline**	**Incident DSPN Follow-up**
	N=133	**N=133**	**N=120**	**N=120**	**N=13**	**N=13**
Mild (NSS: 3-4), N (%)	31 (23.3)	30 (22.6)	27 (22.5)	29 (24.2)	4 (30.8)	1 (7.7)
Moderate (NSS: 5-6), N (%)	31 (23.3)	34 (25.6)	27 (22.5)	25 (20.8)	4 (30.8)[a]	9 (69.2)[c]
Severe (NSS: 7-10), N (%)	16 (12.0)	19 (14.3)	13 (10.8)	16 (13.3)	3 (23.1)[b]	3 (23.1)
Mild (NDS: 3-5), N (%)	14 (10.5)	26 (19.5)	13 (10.8)	16 (13.3)	1 (7.7)[a]	10 (76.9)[c]
Moderate (NDS: 6-8), N (%)	0 (0.0)	3 (2.3)	0 (0.0)	0 (0.0)	0 (0.0)	3 (23.1)[c]
Severe (NDS: 9-10), N (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Abnormal VPT age-specific N (%)	15 (11.3)	19 (14.3)	12 (10.0)	15 (12.5)	3 (23.1)[b]	4 (30.8)[c]

Abbreviations: NSS; neuropathic symptom score, NDS; neuropathy disability score, VPT; vibration perception threshold

Severity categories for NSS and NDS were defined using pragmatic score ranges commonly applied.[a] p-value for within-group change from baseline to follow-up in the incident DSPN group, [b]p-value for between group comparisons across DSPN groups at baseline, [c]p-value for between-group comparisons across DSPN groups at follow-up

6.2.3 Laboratory findings from baseline to follow-up in participants without DSPN at baseline

Baseline and follow-up laboratory parameters for those without DSPN at baseline are presented in **Table 17**. Baseline laboratory parameters were similar between individuals who developed incident DSPN vs those who did not, except for higher baseline HOMA-IR (2.9 [1.4-4.6] vs 2.5 [1.5-3.4], p=0.045), and lower eGFR (71 ± 11 vs 82 ± 14 mL/min/1.73 m², p=0.037) and Lp(a) (3.0 [2.6-8.1] vs 13.2 [5.6-35.9] mg/dL, p=0.004) in the incident DSPN group (**Table 17**). LDL-C increased in those progressing to incident DSPN (+5.1%), but it decreased in those who did not (-14.6%) over the median 24-month follow-up (**Table 17**). No additional laboratory parameter showed significant between-group differences over time (**Table 17**).

Table 17 Baseline and follow-up laboratory profile in those without DSPN at baseline

	Overall Baseline	**Overall Follow-up**	**No DSPN Baseline**	**No DSPN Follow-up**	**Incident DSPN Baseline**	**Incident DSPN Follow-up**
	N=133	**N=133**	**N=120**	**N=120**	**N=13**	**N=13**
FPG, mg/dL	105 (100-111)	102 (97-110)[a]	104 (100- 111)	102 (97-110)	108 (102-112)	107 (100-114)
2-h plasma glucose after 75 g glucose administration, mg/dL	121 ± 40	-	121 ± 40	-	124 ± 39	-
Insulin, µIU/mL	10.1 (6.3-12.8)	8.3 (5.1-12.1)	10.1 (6.3-12.6)	8.3 (5.1-11.9)	11.1 (7.2-13.9)	11.3 (5.0-14.0)[d]
HOMA-IR	2.6 (1.5-3.5)	2.2 (1.2-3.1)	2.5 (1.5-3.4)	2.0 (1.2-3.1)	2.9 (1.4-4.6)[c]	3.2 (1.2-3.7)[d]
HbA1c, %	5.9 (5.6-6.2)	5.9 (5.7-6.2)	5.9 (5.7-6.2)	5.9 (5.7-6.1)	6.0 (5.5 -6.0)	6.0 (5.6-6.4)

eGFR, mL/min/1.73 m^2	81 ± 14	80± 15	82± 14	80 ± 16	71 ± 11[c]	75 ±13
UACR ≥ 30 mg/g, N (%)	20 (15.0)	23	18 (15.0)	21 (17.5)[b]	2 (15.4)	2 (15.4)
TC, mg/dL	162 (140-198)	155 (137-178)[a]	164 (140-204)	155 (134-178)[b]	151 (144-171)	162 (155-177)
LDL-C, mg/dL	87 (70-118)	77 (63-96)[a]	86 (71-119)	76 (63-97)[b]	80 (68-97)	83 (78-90)
TGs, mg/dL	98 (75-137)	98 (80-130)	97 (75-133)	98 (80-130)	104 (75-149)	112 (83-130)
HDL-C, mg/dL	50 (45-58)	51 (44-62)	50 (43-60)	50 (44-62)	53 (46-57)	55 (49-62)
ApoB, mg/dL	73 (61-90)	66 (57-76)	74 (61-91)	66 (56-76)	69 (59-74)	74 (62-78)
Lp(a), mg/dL	12.6 (3.8-32.1)	10.0 (4.0-32.0)	13.2 (5.6-35.9)	13.0 (4.0-39.2)	3.0 (2.6-8.1)[c]	3.2 (3.6-8.5)
25 (OH) Vitamin D **< 20 ng/mL (%)** **< 10 ng/mL (%)**	 42 (31.6) 17 (12.8)	 27 (20.3)[a] 4 (3.0)[a]	 38 (32.5) 14 (11.7)	 23 (19.2)[b] 1 (0.8)[b]	 4 (30.8) 3 (23.1)	 4 (30.8)[d] 3 (23.1)
25 (OH) Vitamin D levels, ng/mL	20.9±10.5	26.5 ± 9.2[a]	21.3 ± 10.7	26.9 ± 9.3[b]	17.6 ± 8.4	23.8 ± 8.2
UACR, mg/g	8.4 (5.1-15.0)	8.1 (5.2-19.4)	8.9 (5.1-16.0)	8.1 (5.2-19.4)	7.8 (5.8-12.4)	7.8 (6.3 -17.2)

Abbreviations: FPG; fasting plasma glucose, HOMA-IR; homeostasis model assessment of insulin resistance, HbA1c; glycated hemoglobin, Cre; serum creatinine, eGFR; estimated glomerular filtration rate, TC; total cholesterol, LDL-C; low-density lipoprotein cholesterol, TGs; triglycerides, HDL-C; high-density lipoprotein cholesterol, ApoB; apolipoprotein B, Lp(a); lipoprotein(a), 25(OH)D; 25-hydroxy vitamin D, UACR; urine albumin-creatinine ratio.
Parametric variables are expressed as mean ± SD, non-parametric variables as median (IQR), and categorical variables as number (percentage) [a]p-value<0.05 for within-group change from

baseline to follow-up in the overall cohort, [b]p-value<0.05 for within-group change from baseline to follow-up in the No DSPN group, [c]p-value<0.05 for between-group comparisons across DSPN groups at baseline, [d]p-value<0.05 for between-group comparisons across DSPN groups at follow-up

6.2.4 Predictors of DSPN onset

Univariate associations of incident DSPN with risk factors and their longitudinal 24-month changes (Δ) are shown in **Table 18**.

Multivariate analyses were adjusted for covariates and their 24-month changes with p<0.10 in univariate analyses, along with clinical important confounders: age, sex, smoking, waist circumference, total body fat mass (kgs) and ΔTotal body fat mass, HOMA-IR and ΔHOMA-IR, LDL-C and ΔLDL-C, TGS, PWV, VPT, and statin and/or ezetimibe initiation during follow-up (**Table 18** and **Figure 36**). Age (OR for every 1-year increase 1.112, 95% CI 1.023-1.241, p=0.021), sex (OR male vs female 1.072, 95% CI 1.012-3.276, p=0.032), smoking (OR current/previous vs never 1.173, 95% CI 1.072-3.025, p=0.024), waist circumference (OR for every 1 cm increase 1.039, 95% CI 1.018-1.280, p=0.019), HOMA-IR (OR for every 0.1 increase 1.829, 95% CI 1.025-2.460, p=0.026) and ΔHOMA-IR (OR for every 0.1 increase 1.456, 95% CI 1.072-2.870, p=0.029) were independently associated with incident DSPN (**Table 18** and **Figure 36**).

Table 18 Adjusted ORs for incident DPSN per change in clinically significant covariates

	Univariate (unadjusted)		**[a]Multivariate adjusted model**		
	OR (95% CI)	**p-value**	**OR (95% CI)**	**p-value**	**VIF**
Age, for every 1-year increase	**1.080 (1.013-1.154)**	**0.019**	**1.112 (1.023-1.241)**	**0.021**	3.717
Sex, Male vs female	**1.035 (1.002-1.317)**	**0.046**	**1.072 (1.012-3.276)**	**0.032**	1.668
Smoking, Current/previous vs never	**1.340 (1.042-1.632)**	**0.017**	**1.173 (1.072-3.025)**	**0.024**	2.376
BMI, for every 1 kg/m^2 increase	1.066 (0.940-1.198)	0.199	(-)	(-)	(-)
ΔBMI, for every 1 kg/m^2 increase	1.113 (0.771-1.605)	0.568	(-)	(-)	(-)
Waist circumference, for every 1 cm increase	**1.028 (1.004-1.079)**	**0.033**	**1.039 (1.018-1.280)**	**0.019**	3.962
ΔWaist circumference, for every 1 cm increase	1.092 (0.912-1.179)	0.851	(-)	(-)	(-)
Total body fat mass, for every 1% increase	0.974 (0.893-1.067)	0.557	(-)	(-)	(-)
ΔTotal body fat mass, for every 1% increase	0.935 (0.770-1.272)	0.935	(-)	(-)	(-)
Total body fat mass, for every 1 kg increase	**1.058 (1.006-1.129)**	**0.029**	1.027 (0.869-1.116)	0.506	4.221
ΔTotal body fat mass, for every 1 kg increase	**1.192 (1.057-1.310)**	**0.044**	0.992 (0.900-1.256)	0.322	2.584
Lean body mass, for every 1% increase	1.045 (0.9451.151)	0.372	(-)	(-)	(-)
ΔLean body mass, for every 1% increase	1.038 (0.960-1.123)	0.344	(-)	(-)	(-)

Lean body mass, for every 1 kg increase	1.006 (0.942-1.075)	0.412	(-)	(-)	(-)
ΔLean body mass, for every 1 kg increase	1.009 (0.987-1.246)	0.202	(-)	(-)	(-)
Systolic blood pressure, for every 1 mmHg increase	1.006 (0.971-1.041)	0.748	(-)	(-)	(-)
ΔSystolic blood pressure, for every 1 mmHg increase	1.089 (0.005-2.229)	0.975	(-)	(-)	(-)
2-hour plasma glucose level after 75-g glucose administration, for every 1 mg/dL increase	1.002 (0.988-1.017)	0.758	(-)	(-)	(-)
HOMA-IR, for every 0.1-unit increase	**1.267 (1.011-1.694)**	**0.027**	**1.829 (1.025-2.460)**	**0.026**	1.919
ΔHOMA-IR, for every 0.1-unit increase	**1.188 (0.834-1.692)**	**0.070**	**1.456 (1.072-2.870)**	**0.029**	2.052
HbA1c, for every 0.1% increase	1.132 (0.039-3.856)	0.655	(-)	(-)	(-)
ΔHbA1c, for every 0.1% increase	1.008 (0.220-6.507)	0.835	(-)	(-)	(-)
LDL-C, for every 1 mg/dL increase	1.166 (0.978-1.324)	0.120	1.000 (0.954-1.042)	0.473	2.204
ΔLDL-C, for every 1 mg/dL increase	**1.025 (1.002-1.051)**	**0.041**	1.049 (1.000-1.100)	0.051	2.048
TGs, for every 1 mg/dL increase	1.000 (0.992-1.007)	0.975	0.988 (0.962-1.016)	0.394	1.996
ΔTGs, for every 1 mg/dL increase	1.033 (0.912-1.219)	0.715	(-)	(-)	(-)

HDL-C, for every 1 mg/dL increase	0.987 (0.936-1.040)	0.615	(-)	(-)	(-)
ΔHDL-C, for every 1 mg/dL increase	1.061 (0.975-1.155)	0.171	(-)	(-)	(-)
PWV (carotid-femoral), for every 1 m/s increase	**1.197 (1.022-1.487)**	**0.028**	1.300 (0.688-2.454)	0.419	3.919
ΔPWV (carotid-femoral), for every 1 m/s increase	1.061 (0.743-1.516)	0.743	(-)	(-)	(-)
UACR, for every 1 mg/g increase	1.040 (0.925-2.625)	0.380	(-)	(-)	(-)
ΔUACR, for every 1 mg/g increase	0.999 (0.983-1.015)	0.861	(-)	(-)	(-)
VPT age specified, abnormal vs normal	2.312 (0.644-5.307)	0.076	1.774 (0.463-7.667)	0.479	1.089
Statin and/or ezetimibe initiation during follow-up, vs no change	1.222 (0.043-3.516)	0.098	0.668 (0.010-7.145)	0.432	1.493

Abbreviations: DSPN; distal symmetrical polyneuropathy, BMI; body mass index, HOMA-IR; homeostatic model assessment of insulin resistance, HbA1c; hemoglobin A1C, LDL-C; low-density lipoprotein cholesterol, TGs; triglycerides, HDL-C; high-density lipoprotein cholesterol, PWV; pulse wave velocity, UACR; urine albumin-creatinine ratio, VPT; vibration perception threshold, VIF: variance inflation factor

Values are presented as odds ratios (ORs) with 95% confidence intervals (CIs). Δ indicates change between baseline and follow-up (follow-up - baseline). BMI was excluded from the multivariate analysis due to high collinearity with waist circumference. [a] The multivariate model was adjusted for baseline variables and their 24-month changes with $p<0.10$ in univariate analyses and clinical important confounders: age, sex, smoking, waist circumference, total body fat mass (kgs) and ΔTotal body fat mass (kgs), HOMA-IR and ΔHOMA-IR, LDL-C and ΔLDL-C, TGS, PWV, VPT, statin and/or ezetimibe initiation during follow-up

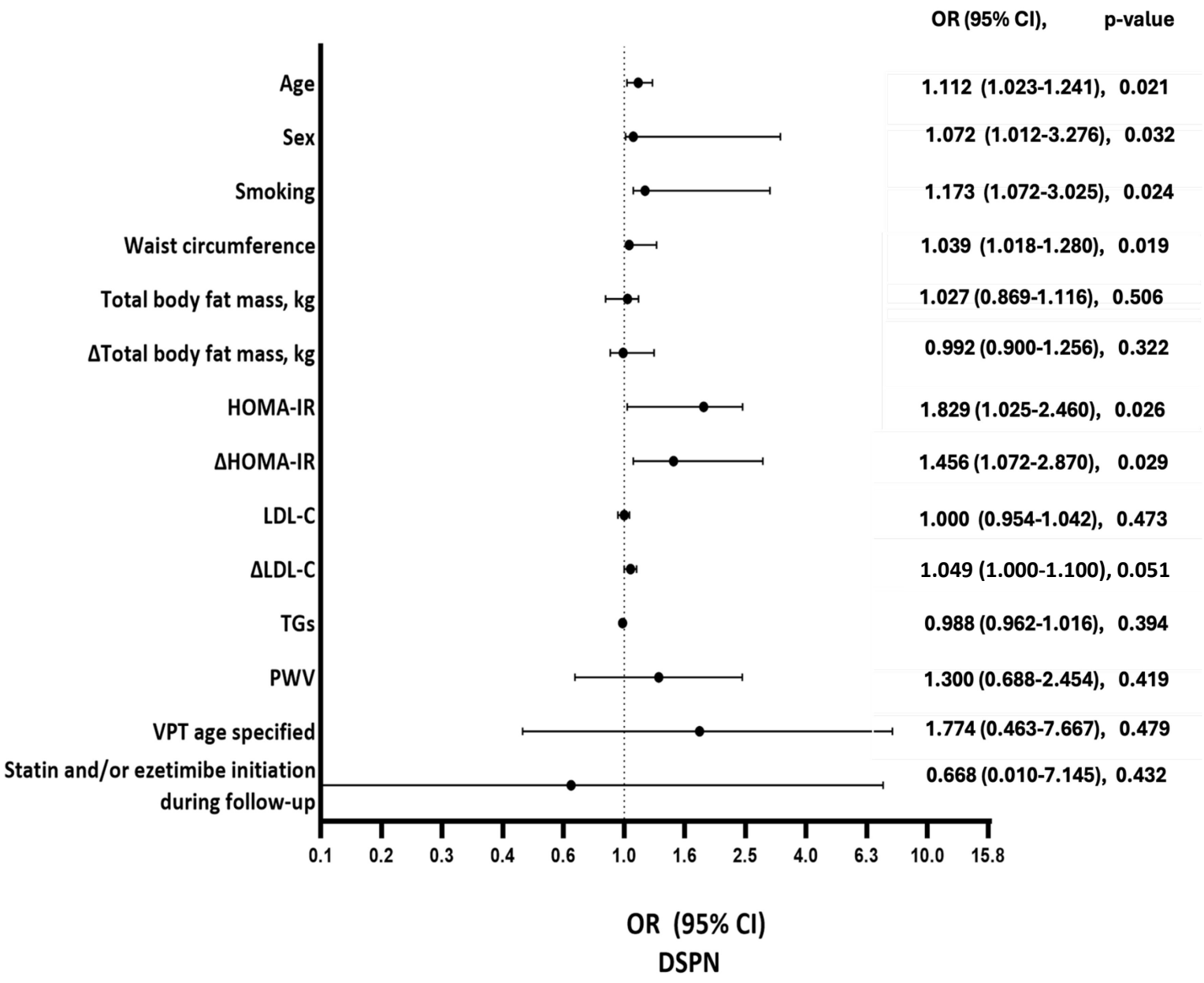


Figure 36 Adjusted ORs for incident DPSN per change in clinically significant covariates

Abbreviations: DSPN; distal symmetrical polyneuropathy, BMI; body mass index, HOMA-IR; homeostatic model assessment of insulin resistance, HbA1c; hemoglobin A1C, LDL-C; low-density lipoprotein cholesterol, TGs; triglycerides, HDL-C; high-density lipoprotein cholesterol, PWV; pulse wave velocity, UACR; urine albumin-creatinine ratio, VPT; vibration perception threshold

Values are presented as odds ratios (ORs) with 95% confidence intervals (CIs). Δ indicates change between baseline and follow-up (follow-up - baseline). BMI was excluded from the multivariate analysis due to high collinearity with waist circumference. [a]The multivariate model was adjusted for baseline variables and their 24-month changes with $p<0.10$ in

univariate analyses and clinical important confounders: age, sex, smoking, waist circumference, total body fat mass (kgs) and ΔTotal body fat mass (kgs), HOMA-IR and ΔHOMA-IR, LDL-C and ΔLDL-C, TGS, PWV, VPT, statin and/or ezetimibe initiation during follow-up

7. DISCUSSION

We have shown that DSPN was prevalent in 16.9% of adults with prediabetes. In this setting, DSPN was significantly and independently associated with height, smoking, central obesity and insulin resistance. In the prospective study, we observed a 9.8% incidence of DSPN over a median follow-up of 24 months. Age, male sex, smoking, central obesity as well as baseline and changes in insulin resistance over time were independently associated with incident DSPN.

7.1 DSPN prevalence in prediabetes

A growing body of literature reports on the presence of DSPN in prediabetes [226, 227]. In a large systematic review of 29 studies (9351 participants with prediabetes) the prevalence estimates varied widely, with most studies reporting a prevalence of ≥10% [226]. Similarly, we demonstrated that 16.9% of individuals with prediabetes had DSPN in real-world clinical practice in a Mediterranean population. In our study, DSPN was more frequent among individuals with both IFG+IGT vs IFG. Large population-based studies indicated that the prevalence of DSPN in prediabetes varies from 4.2 to 11.3% in IFG and from 1.5 to 26.0% in IFG/IGT. The KORA F4 study showed that DSPN prevalence of 14.8% in the IGT vs 23.9% in the IFG + IGT group, similarly to our findings [231].

It should be noted that not only participants with DSPN, but also those without an established diagnosis of DSPN exhibited neuropathic symptoms up to 35.3%, neuropathic signs in up to 30.0% and increased age-adjusted VPT in 12.0%. Prior studies have

reported neuropathic pain from 8.7% among participants with IGT (n=48) up to 14.8% among those with IGT (n=61) [330, 331]. Moreover, the PROMISE study (n=467) showed that participants with IGT had higher mean VPT (7.6 vs 6.5 V, p= 0.024) than those with normoglycemia prior to DSPN diagnosis [143]. These findings suggest that neuropathic alterations may develop early during prediabetes.

7.2 DSPN incidence in prediabetes

In the prospective cohort, 9.8% of individuals with prediabetes developed new DSPN over a median 24-month follow-up, equating an annualized incidence of 4.9%. Prospective data evaluating DSPN incidence in prediabetes remain limited. In the KORA F4/FF4 study, based on our calculation from published data, the incidence of DSPN was 26% in prediabetes (n=215) over a mean follow-up of 6.5 years, corresponding to an annual incidence of 4.0-4.5%, which is very similar to ours [332]. In KORA F4/FF4, DSPN diagnosis was based on the physical examination component of the MNSI without inclusion of symptom-based questionnaire, corresponding to “possible” DSPN per Toronto Diabetic Neuropathy Expert Group criteria [332].To the best of our knowledge, KORA F4/FF4 and ours represent the only available prospective studies on DSPN incidence in individuals with prediabetes.

The global burden of prediabetes is rising, with an estimated 635 million people affected by IGT and 488 million by IFG worldwide in 2024 as per the International Diabetes Federation [17]. This growing burden fuels T2D epidemic and represents a major public health challenge, closely linked with metabolic syndrome, cardiovascular disease and microvascular complications, including DSPN [312, 333]. DSPN is particularly concerning, as it is associated with the risk of disability, reduced quality of life,

neuropathic pain, impaired mobility, and increased morbidity as well as substantial socioeconomic burden on healthcare systems [313, 334]. Therefore, prediabetes rising is expected to further amplify both the individual and healthcare system burden of DSPN and its consequences.

7.3 Determinants of prevalent DSPN

Individuals with DSPN at baseline examination were older, more likely to be current/former smokers, and had higher BMI, waist circumference, and prevalence of MetS. Likewise, in a large cross-sectional study of 2035 participants, including 1043 with prediabetes, age was significantly higher in participants with DSPN both among individuals with MetS (70.5 vs 61.7 years, $p < 0.05$) and among those without MetS (76.6 vs 60.5 years, $p < 0.05$) [335]. Beyond cardiometabolic risk factors, height and current/former smoking were also independently associated with prevalent DSPN [314]. This observation is consistent with previous population-based studies identifying height as risk factor for DSPN, possibly reflecting increased vulnerability of longer peripheral nerves to metabolic or microvascular injury [267]. In our study, the prevalence of DSPN increased as the number of MetS criteria increased. Likewise, in a cross-sectional study (n=2382 adults, 29.9% with prediabetes), DSPN was prevalent in 5.6% among those individuals with prediabetes without additional MetS components, increasing to 10.6% with 4 additional MetS components [336].

HOMA-IR, an established marker of insulin resistance, was significantly higher in the DSPN vs no DSPN group. In agreement with our results, a large cross-sectional study showed that HOMA-IR was significantly associated with DSPN (OR: 1.2, 95% CI: 1.1-1.4, $p<0.001$) in 1043 participants with prediabetes [335]. We have also demonstrated significant independent correlations of obesity and HOMA-IR with neuropathic

symptoms, signs and VPT. Evidence about the association between cardiometabolic factors and neuropathic symptoms and signs and quantitative sensory function, indicative of early nerve damage in prediabetes, remains scarce. In the Maastricht study, higher FPG and HbA1c levels as well as current smoking (OR 2.13 [1.38, 3.29]) were significantly associated with increased neuropathic pain, while the same covariates along with increased age and waist circumference ($\beta = 0.08$ [0.04, 0.13]) were correlated with higher VPT [127].

Prediabetes affects the structure of both unmyelinated and myelinated nerve fibers; hyperglycemia, even acute and transient (postprandial) induce mitochondrial dysfunction and oxidative stress, leading to impaired neuronal metabolism [89]. This results in microvascular damage and inflammation contributing to nerve injury [89]. Furthermore, insulin resistance downregulates phosphatidylinositol 3-kinase (PI3K)/Akt signaling, resulting in mitochondrial dysfunction, increased oxidative stress and neurodegeneration [337].

The increased risk of DSPN in individuals with combined IFG + IGT vs IFG is likely due to the distinct mechanisms of insulin resistance in these conditions. In isolated IFG, insulin resistance is primarily hepatic, while in IGT mainly muscular [224]. Reduced skeletal muscle sensitivity increases glucose uptake by adipose tissue, triggering lipolysis and the release of free fatty acids in the circulation [224]. This enhances oxidative stress, and subsequently nerve damage. Furthermore, studies suggest that dietary fatty acids may influence this process [263] and oxidized LDLs accelerate ROS accumulation through receptor-mediated pathways enhancing the process of nerve injury [338].

Arterial stiffness refers to reduced flexibility and elasticity of the arteries in response to pulsatile pressure changes during the cardiac cycle [339]. PWV, particularly carotid-femoral, is the gold standard non-invasive method for assessing arterial stiffness [339].

Increased arterial stiffness, a natural result of aging, is further exacerbated by pathological conditions and is independently associated with cardiovascular risk [298, 339]. PWV was higher in participants with IFG+IGT than in those with IFG, irrespective of DSPN. Similarly, a population-based study (n=747; 278 with normal glucose metabolism, 168 with IFG or IGT and 301 with T2D) has shown that prediabetes is associated with increased arterial stiffness (beta; -0.06, 95% CI: -0.23-0.10 for carotid distensibility) compared with normoglycemia, with hyperglycemia or hyperinsulinemia linked with 30% of the arterial changes [340]. In our study, although significantly higher PWV was observed in participants with prediabetes and DSPN vs those without DSPN, PWV was not independently related to DSPN. Notably, PWV was independently associated with neuropathic symptoms and VPT, suggesting a potential link between macrovascular and early microvascular complications. Likewise, in case-control study of 240 participants with diabetes and 110 non-diabetic controls, PWV was independently associated with VPT (beta = 0.14, p = 0.025) [341]. These findings indicate that increased arterial stiffness may be a surrogate marker of the individual effects of cardiometabolic risk factors on peripheral nerve dysfunction, rather than DSPN per se.

In our study, participants with increased PWV and DSPN were more likely to be current/former smokers and have dyslipidemia, two well-recognized cardiovascular risk factors associated with arterial stiffening and DSPN in diabetes [236, 342, 343]. A study of individuals with T2D (n=107) showed elevated PWV in participants with DSPN compared to those without (11.7 vs 10.1 m/s, $p < 0.001$, respectively) [344]. In a recent meta-analysis of 26 studies, arterial stiffness, as measured by PWV or pulse pressure, was significantly higher in subjects with T2D and DSPN (mean difference: 1.22 m/s, 95% CI 0.87 to 1.58, $p<0.00001$) compared to those without [345]. However, given the predominantly cross-sectional nature of these studies, the temporality of the association

between arterial stiffness and DSPN remains unclear. Increased arterial stiffness augments pulsatile hemodynamic stress, reducing the damping capacity of the large elastic arteries [346]. As a result, increased pulsatile energy is transmitted into the microcirculation of low-resistance organs, including the vasa nervorum of the peripheral nerves, leading to microvascular damage and ischemia [346].

An alternative explanation could be that the association between arterial stiffness and DSPN is not causal, but they share a common underlying pathway. Previous studies in T2D show that hyperglycemia can promote the development of advanced glycation end products (AGEs), which may reduce arterial elasticity [347, 348] and induce neural inflammation, progressing to DSPN [349].

7.4 Predictors of incident DSPN

In multivariate analysis, increasing age, male sex, current or former smoking, waist circumference as well as baseline and increases in HOMA-IR over time were independently associated with incident DSPN.

Similarly, the KORAF4/FF4 study showed that subjects with incident DSPN, including 215 individuals with prediabetes were significantly older (70.2 vs 67.9 years, $p<0.001$) [332]. As discussed above, individuals with prediabetes and prevalent DSPN were older (67 vs 61 years, $p=0.017$) compared to without DSPN in our cross-sectional study [314]. Smoking emerged as risk factor for incident DSPN in our cohort. Likewise, the KORAF4/FF4 study showed that subjects with incident DSPN were more likely to be current smokers (11.3 vs 5.8%, $p=0.003$) [332]. As discussed above, in our baseline cross-sectional analysis, individuals with prediabetes and prevalent DSPN had a higher prevalence of current/previous smoking (55.5 vs 42.9%, $p=0.028$) compared to those

without DSPN [314]. Also, a recent meta-analysis of cross-sectional and case-control (n = 228,699 participants with diabetes) reported that smoking is linked with increased risk of DSPN (OR 1.29, 95% CI 1.17-1.41 and OR: 1.48, 95% CI: 1.23-1.72, respectively) [268].

Our study adds to the growing literature of central obesity as a risk factor for DSPN. In KORAF4/FF4, subjects with incident DSPN had significantly higher BMI (29.1 vs 27.6 kg/m^2, p<0.001) and waist circumference (99.8 vs 94.7 cm, p<0.001) [332]. As reported previously, in the cross-sectional analyses from this study, waist circumference was an independent risk factor for prevalent DSPN (OR 1.092, 95% CI 1.037-1.148, p<0.001) in prediabetes, and individuals with prevalent DSPN had higher BMI (30.3 vs 28.2 kg/m^2, p=0.011) compared to without DSPN [314]. Consistently, cross-sectional studies including participants with prediabetes have shown an independent relationship between waist circumference and DSPN [350, 351]. In T2D, the ADDITION-Denmark study further reported a significant association between waist circumference and incident DSPN (HR 1.14 [95% CI 1.05; 1.24]) [71].

HOMA-IR, an established marker of insulin resistance, was independently and robustly associated with incident DSPN. In a large cross-sectional study (n=1043 participants with prediabetes), HOMA-IR was significantly linked with prevalent DSPN (OR 1.2, 95% CI 1.1-1.4, p<0.001) [335]. Similarly, in a community-based, cross-sectional study of 2035 participants, including 1043 with prediabetes, HOMA-IR was significantly associated with higher risk of DSPN (OR 1.1, 95% CI 1.1-1.4, p<0.001) [335]. In our baseline cross-sectional analysis of this study, HOMA-IR (OR 1.247, 95% CI: 1.095-1.425, p=0.004) was also independently associated with prevalent DSPN [7].

The present study extends this association by demonstrating that worsening of insulin resistance over time is independently linked to incident DSPN. Insulin resistance and

related metabolic disturbances promote mitochondrial dysfunction, oxidative stress, and inflammation contributing to neuronal and Schwann cell injury [89, 95, 151]. Furthermore, insulin resistance downregulates PI3K/Akt signaling, resulting in impaired axonal maintenance, reduced neuronal survival and axonal degeneration [95, 151, 337].

It should be noted that participants who developed DSPN already had greater neuropathic symptom burden (NSS), and higher VPT at baseline. These findings suggest the presence of subclinical or early nerve dysfunction preceding overt DSPN. Similar findings have been observed in prospective studies in diabetes. In a prospective study (n=175 with diabetes), baseline VPT was significantly higher in those who subsequently developed DSPN (17.5 vs. 14.8 V) over a follow-up of 4.1 years [352].

LDL-C levels decreased in those who did not develop DSPN and increased in those who did, potentially reflecting the greater use of statin and/or ezetimibe therapy in those without incident DSPN. However, LDL-C levels and statins and/or ezetimibe were not independently associated with DSPN incidence. A meta-analysis of 38 clinical trials including 32,668 individuals with diabetes reported that higher LDL-C was associated with increased the risk of DSPN (OR 1.10, 95% CI 1.02-1.19; I^2=17.8%) [131]. On the contrary, in the Maastricht Study (n=2401; 59.3% with normoglycemia, 25.3% with diabetes, 15.4% with prediabetes), LDL-C was not independently associated with DSPN [127].

In our prospective cohort, PWV was higher in individuals with prediabetes who developed incident DSPN; however, PWV was not independently associated with incident DSPN. As previously mentioned, we have shown cross-sectionally that PWV was independently associated with neuropathic symptoms and VPT, supporting a link between arterial stiffness and early nerve dysfunction [314].

7.5 Study strengths and limitations

The main strengths of this study include the enrollment of participants from a real-world outpatient setting and its prospective design. However, several limitations should be considered. First, generalizability of our findings to broader and more diverse populations may be substantially limited due to single center design. Secondly, DSPN diagnosis was established using clinical examination combined with quantitative sensory testing. These diagnostic methods mainly evaluate large, myelinated nerve fibers, although certain components also provide insights into small-fiber function. However, specialized techniques for the direct assessment of small-fiber neuropathy, such as skin biopsy for intraepidermal nerve fiber density or comprehensive electrophysiological studies, were not undertaken, potentially resulting in an underestimation of DSPN incidence. Thirdly, the small sample size with limited number of new DSPN cases and the relatively short follow-up may reduce the power of the multivariable regression analyses. Moreover, prediabetes duration prior to study enrollment was unknown.

7.6 Clinical implications and future perspectives

This study addresses a gap in literature by providing both cross-sectional and prospective evidence on the prevalence and incidence of DSPN in prediabetes. Among nonmodifiable factors, height was independently associated with prevalent DSPN, whereas age and male sex independently predicted the development of DSPN. Smoking, central obesity as well as insulin resistance and its deterioration over time were consistently associated with the presence and predicted the development of DSPN over time. These findings suggest the potential significance of early stratification in prediabetes for screening of population at

higher risk. Preventive strategies focusing on weight management, smoking cessation, and insulin resistance amelioration may mitigate the risk.

8. CONCLUSIONS

The prevalence of DSPN in prediabetes is 16.9%, a finding largely consistent with existing literature. The 24-month cumulative incidence of DSPN in prediabetes was 9.8% in our study, representing novel prospective evidence. Prevalent DSPN in prediabetes is independently associated with height, central obesity and insulin resistance. In addition, age, male sex, smoking, central obesity as well as insulin resistance and its deterioration over time independently predicted incident DSPN. These findings may have clinical implications for screening and risk mitigation strategies for DSPN in prediabetes if confirmed in other studies.

9. ABSTRACT

Background: Distal Symmetrical polyneuropathy **(**DSPN) is a prevalent complication of diabetes, with emerging evidence of onset in earlier stages of dysglycemia, such as prediabetes. Prediabetes is characterized by insulin resistance, central adiposity, and dyslipidemia. These cardiometabolic factors may contribute to early peripheral nerve injury.

Aim: To investigate the prevalence and incidence of DSPN in individuals with prediabetes, and identify cardiometabolic risk factors associated with prevalent DSPN as well as predictors of incident DSPN.

Methods: Consecutive adults with prediabetes attending the Outpatient Lipid and Obesity Clinic at the University Hospital of Ioannina, Greece were enrolled and followed for a median of 24 months. The study included: 1) a cross-sectional analysis of baseline characteristics and prevalence of DSPN and 2) a prospective analysis of DSPN incidence and predictors at follow-up, after excluding patients diagnosed with DSPN at baseline. Participants with secondary causes of neuropathy were excluded. DSPN was diagnosed using the Neuropathy Symptom Score (NSS), the Neuropathy Disability Score (NDS) and the Vibration Perception Threshold (VPT). Arterial stiffness was assessed with Pulse Wave Velocity (PWV). Risk factors of prevalent DSPN and predictors of incident DSPN were examined using multivariate regression analysis.

Results: We studied 160 consecutive adults with prediabetes, of whom 27 (16.9%) were diagnosed with DSPN at baseline. In multivariate analysis, age (odds ratio [OR] per 1 year increase: 1.093, 95% confidence interval [CI]: 1.005-1.188, p=0.041), smoking (OR current/previous vs never-smoking: 1.347, 95% CI: 1.116-1.891, p=0.042), height (OR per 1 cm increase: 1.083, 95% CI: 1.004-1.168, p=0.039), waist circumference (OR per 1 cm increase: 1.123, 95% CI: 1.049-1.202, p<0.001), and HOMA-IR (OR per 0.1

increase: 1.304, 95% CI: 1.133-1.739, p=0.023) were independently associated with prevalent DSPN.

Subjects with DSPN had significantly higher median PWV (8.8 vs 8.0 m/s, p=0.031) and prevalence of abnormal PWV (≥10 m/s) (29.6% vs 11.3%, p=0.029) compared with no DSPN. PWV was independently associated with VPT (beta: 1.010, 95% CI:0.123-1.897, p=0.026).

Among the n=133 participants who were eligible for and included in the prospective study, n=13 developed DSPN over a median follow-up of 24 months (IQR 20-27) for an incidence rate of 9.8%. In multivariable analysis, age (OR for every 1-year increase 1.112, 95% CI 1.023-1.241), sex (OR for male vs female 1.072, 95% CI 1.012-3.276), smoking (OR for current/previous vs never-smoking 1.173, 95% CI 1.072-3.025), waist circumference (OR for every 1 cm increase 1.039, 95% CI 1.018-1.280), HOMA-IR (OR for every 0.1 increase 1.829, 95% CI 1.025-2.460) as well as ΔHOMA-IR (OR for every 0.1 increase 1.456, 95% CI 1.072-2.460) were independently associated with incident DSPN (all $p<0.05$).

Conclusions: DSPN was prevalent in 16.9% of subjects with prediabetes in our study and independently associated with central adiposity and insulin resistance. We observed a 9.8% 24-month incidence of DSPN. Age, male sex, smoking, central obesity, as well as insulin resistance at baseline and change over time were independent predictors of incident DSPN. These findings may have screening and preventive implications if confirmed by larger studies.

10. ΠΕΡΙΛΗΨΗ

Εισαγωγή: Η περιφερική συμμετρική πολυνευροπάθεια (DSPN) συνιστά μία από τις συχνότερες και κλινικά σημαντικές επιπλοκές του διαβήτη. Νεότερα δεδομένα υποδεικνύουν ότι η εμφάνιση και εξέλιξη της DSPN ξεκινά σε πρωιμότερα στάδια δυσγλυκαιμίας, όπως ο προδιαβήτης.

Σκοπός: Η εκτίμηση του επιπολασμού και της επίπτωσης της DSPN σε άτομα με προδιαβήτη, καθώς και η διερεύνηση πιθανών καρδιομεταβολικών παραγόντων κινδύνου που σχετίζονται με τον επιπολασμό και την επίπτωση της DSPN.

Μέθοδοι: Διαδοχικοί ενήλικες με προδιαβήτη που προσήλθαν στο Εξωτερικό Ιατρείο Λιπιδίων και Παχυσαρκίας του Πανεπιστημιακού Νοσοκομείου Ιωαννίνων εντάχθηκαν στη μελέτη. Οι ασθενείς με δευτεροπαθή αίτια νευροπάθειας αποκλείστηκαν. Η μελέτη περιελάμβανε: 1) διατμηματική ανάλυση του επιπολασμού της DSPN και των χαρακτηριστικών των ασθενών κατά την ένταξη στη μελέτη, και 2) προοπτική ανάλυση της επίπτωσης της DSPN κατά την παρακολούθηση 2 ετών, κατόπιν αποκλεισμού των ασθενών με DSPN κατά την ένταξη.

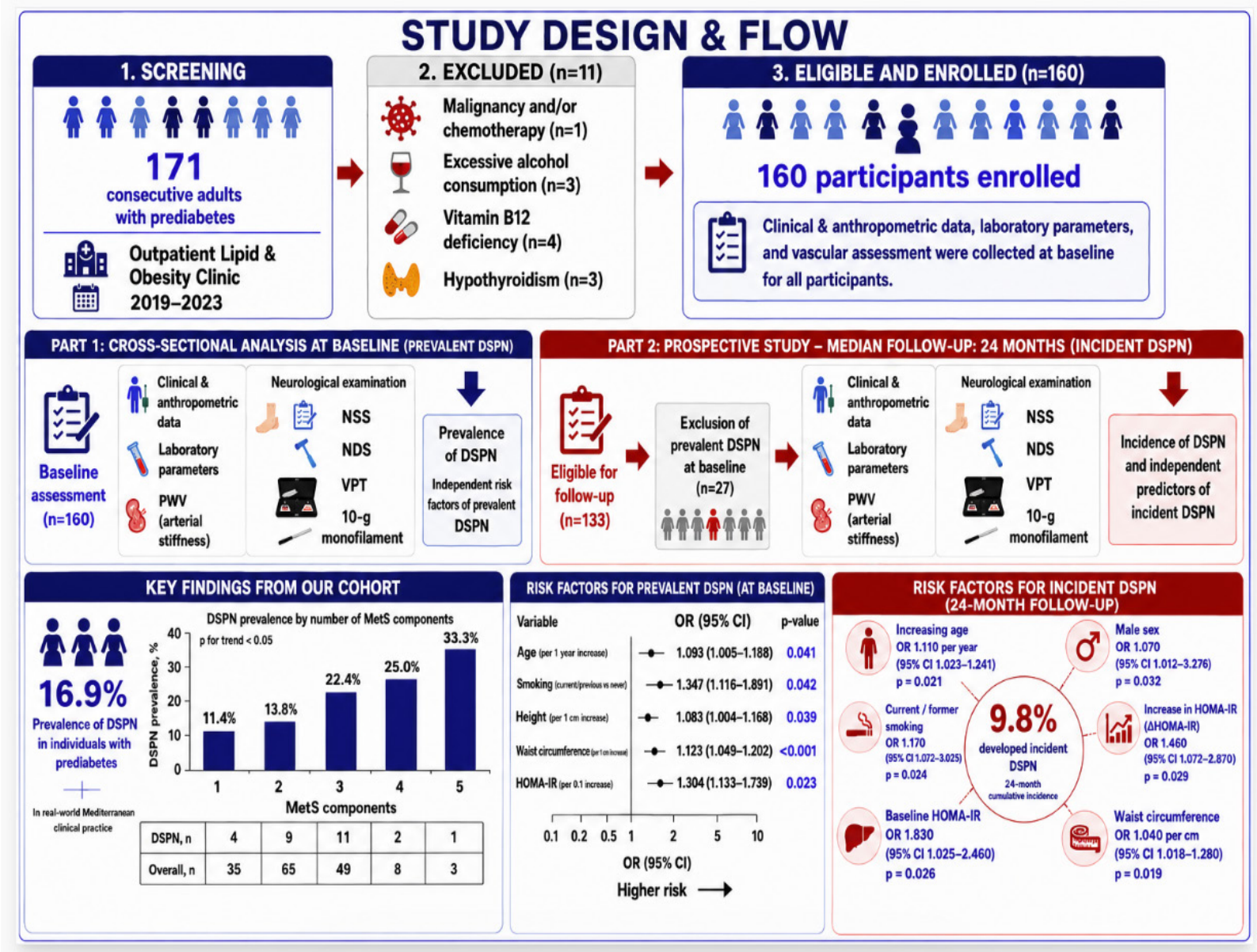
Η διάγνωση της DSPN βασίστηκε στο δείκτη νευροπαθητικών συμπτωμάτων (NSS), το δείκτη νευροπαθητικής ανικανότητας (NDS) και την ουδό αντίληψης των δονήσεων (VPT) όπως αυτή μετρήθηκε με το βιοθεσιόμετρο. Η αρτηριακή σκληρία εκτιμήθηκε με την ταχύτητα σφυγμικού κύματος (PWV). Οι παράγοντες κινδύνου για την παρουσία της DSPN, καθώς και οι προγνωστικοί δείκτες για την εμφάνισή της διερευνήθηκαν με πολυπαραγοντική ανάλυση παλινδρόμησης.

Αποτελέσματα: Μελετήθηκαν 160 διαδοχικοί ενήλικες με προδιαβήτη, εκ των οποίων 27 (16,9%) διαγνώστηκαν με DSPN κατά την ένταξη στη μελέτη. Στην πολυπαραγοντική ανάλυση, η ηλικία (odds ratio [OR] ανά 1 έτος αύξηση: 1,093, 95% confidence interval [CI]: 1,005-1,188, p=0,041), το κάπνισμα (OR ενεργό/ατομικό ιστορικό έναντι μη καπνιστών: 1,347, 95% CI: 1,116-1,891, p=0,042), το ύψος (OR ανά 1 cm αύξηση: 1,083, 95% CI: 1,004-1,168, p=0,039), η περίμετρος μέσης (OR ανά 1 cm αύξηση: 1,123, 95%

CI: 1,049-1,202, p<0,001) και ο δείκτης Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) (OR ανά 0,1 αύξηση: 1,304, 95% CI: 1,133-1,739, p=0,023) συσχετίστηκαν ανεξάρτητα με την παρουσία DSPN. Κατά την ένταξη στη μελέτη, τα άτομα με DSPN εμφάνιζαν σημαντικά υψηλότερο διάμεσο PWV (8,8 έναντι 8,0 m/s, p=0,031), καθώς και μεγαλύτερο ποσοστό παθολογικού PWV (≥10 m/s) (29,6% έναντι 11,3%, p=0,029) συγκριτικά με όσους δεν είχαν DSPN. Το PWV συσχετίστηκε ανεξάρτητα με το VPT (beta: 1,010, 95% CI: 0,123-1,897, p=0,026) κατά την ένταξη. Μεταξύ των 133 συμμετεχόντων που εντάχθηκαν στην προοπτική μελέτη παρακολούθησης, 13 εμφάνισαν DSPN κατά τη διάρκεια μίας διάμεσης παρακολούθησης 24 μηνών (IQR 20-27), αντιστοιχώντας σε επίπτωση 9,8%. Στην πολυπαραγοντική ανάλυση, η ηλικία (OR ανά 1 έτος αύξηση: 1,112, 95% CI: 1,023-1,241), το φύλο (άνδρες έναντι γυναικών: OR: 1,072, 95% CI: 1,012-3,276), το κάπνισμα (ενεργό/ατομικό ιστορικό έναντι μη καπνιστών: OR: 1,173, 95% CI: 1,072-3,025), η περίμετρος μέσης (OR ανά 1 cm αύξηση: 1,039, 95% CI: 1,018-1,280), ο δείκτης HOMA-IR (OR ανά 0,1 αύξηση: 1,829, 95% CI: 1,025-2,460), καθώς και η μεταβολή του HOMA-IR (ΔHOMA-IR) (OR ανά 0,1 αύξηση: 1,456, 95% CI: 1,072-2,460) σχετίστηκαν ανεξάρτητα με την εμφάνιση DSPN (p<0,05).

Συμπεράσματα: Ο επιπολασμός της DSPN ήταν 16.9% στον προδιαβήτη και σχετίστηκε ανεξάρτητα με την κεντρική παχυσαρκία και την ινσουλινοαντίσταση. Η επίπτωση της DSPN σε άτομα με προδιαβήτη ήταν 9,8% σε διάστημα 24 μηνών. Η ηλικία, το άρρεν φύλο, το κάπνισμα, η κεντρική παχυσαρκία, καθώς και η ινσουλινοαντίσταση κατά την ένταξη στη μελέτη, καθώς και η μεταβολή της στο χρόνο, αναδείχθηκαν ως ανεξάρτητοι προγνωστικοί παράγοντες για την εμφάνιση DSPN. Τα ευρήματα αυτά είναι χρήσιμα για το σχεδιασμό προσυμπτωματικού ελέγχου και την εφαρμογή προληπτικών στρατηγικών, εφόσον επιβεβαιωθούν από μεγαλύτερες μελέτες.

11. GRAPHICAL ABSTRACT



12. PUBLICATIONS AND SCIENTIFIC ACTIVITY RELATED TO THE PRESENT THESIS

Peer-Reviewed Publications Derived from the Present Doctorate Thesis

A. Published Articles

1. Anastasiou G, Papanas N, Barkas F, Tentolouris N, Liamis G, Michalis LK, Bechlioulis A, Kalaitzidis R, Liberopoulos E. Distal symmetrical polyneuropathy in prediabetes is associated with abdominal obesity and insulin resistance. *Diabetes Res Clin Pract*. 2026 Mar;233:113140. doi: 10.1016/j.diabres.2026.113140. Epub 2026 Feb 4. PMID: 41651186.
2. Anastasiou G, Maggio V, Rizzo M, Liberopoulos E. Lipoprotein-associated phospholipase A2 and diabetic peripheral neuropathy. *J Diabetes Complications*. 2025 Jul;39(7):109020. doi: 10.1016/j.jdiacomp.2025.109020. Epub 2025 Mar 26. PMID: 40157891.
3. Anastasiou G, Liberopoulos E, Tentolouris N, Papanas N. Diabetic Sensorimotor Polyneuropathy: An Overview on Epidemiology, Risk Factors, Classification, Diagnosis, and Treatment. *Int J Low Extrem Wounds*. 2024 Mar 27:15347346241240513. doi: 10.1177/15347346241240513. Epub ahead of print. PMID: 38533581.
4. Kalampoki A, Ntzani EE, Asimakopoulos AI, Liberopoulos E, Tentolouris N, Anastasiou G, Adamidis PS, Kotsa K, Rizos EC. The Effect of Activity Tracking Apps on Physical Activity and Glycemic Control in People with Prediabetes Compared to Normoglycemic Individuals: A Pilot Study. *Nutrients*. 2024 Dec 31;17(1):135. doi: 10.3390/nu17010135. PMID: 39796569

5. Anastasiou G, Papanas N, Tentolouris N, Liberopoulos E. Diabetic peripheral neuropathy:epidemiology, risk factors, current therapy. *Journal of the Greek Society of the Risk Factors for Vascular Diseases Study (EMPAKAN)*. 2023

B. Articles Submitted-Under Review

1. Anastasiou G, Papanas N, Barkas F, Tentolouris N, Liamis G, Michalis LK, Bechlioulis A, Kalaitzidis R, Mantzoros CS, Liberopoulos E. Incidence and predictors of distal symmetrical polyneuropathy in prediabetes: A 24-month prospective cohort study. Submitted to *Diabetes Res Clin Pract;* under review.

Published Abstracts

1. Anastasiou G, et al. Arterial Stiffness and Microvascular Complications: The Association of PWV with Cardiovascular Risk Factors, Neuropathy and Albuminuria in Individuals with Impaired Fasting Glucose. Abstract #1065. 23rd European Congress of Internal Medicine (ECIM) Abstract book, 2025
2. Anastasiou G, et al. Incidence of distal sensory peripheral neuropathy in individuals with prediabetes and its association with cardiometabolic risk factors. Atherosclerosis, Volume 395, S1, 2024, 118111, ISSN0021-9150, https://doi.org/10.1016 /j.atherosclerosis. 2024.118111.
3. Anastasiou G, et al. Peripheral Neuropathy Unveiled: Exploring its Incidence in Prediabetes and Beyond. Data from Northwest Greece. Annual Congress of the Central Europe Diabetes Association (CEDA) Abstract book, 2024
4. Anastasiou G, et al. Prevalence of microvascular complications in individuals with prediabetes. Atherosclerosis 2023, 379, s159-s16

Accepted Abstracts (to be presented and published in Atherosclerosis Supplement journal)

1. Anastasiou G, et al. FIB-4 as a marker of sensory nerve impairment in prediabetes. Abstract#684. EAS Congress 2026
2. Anastasiou G, et al. Arterial stiffness is independently associated with FIB-4 index in adults with prediabetes. Abstract#674. EAS Congress 2026
3. Anastasiou G, et al. Arterial stiffness and cardiometabolic factors are independently associated with neuropathic symptoms and signs in prediabetes. Abstract#694. EAS Congress 2026
4. Insulin resistance and arterial stiffness are independently associated with DSPN in prediabetes. Absract#1125. EAS Congress 2026
5. Anastasiou G, et al. Clinical characteristics of DSPN in prediabetes. Abstract#1126. EAS Congress 2026

Submitted abstracts (under review)

1. Anastasiou G et al. Incidence and cardiometabolic predictors of distal symmetrical polyneuropathy in prediabetes: a 24-month prospective cohort study. Abstract#533. Submitted to EASD Annual Meeting 2026

Conference Abstracts related to the present Doctorate Thesis

1. Anastasiou G, et al. Predictors of incident Distal Symmetrical Neuropathy in prediabetes: A prospective cohort study. Oral presentation. 24th Panhellenic congress of Hellenic Diabetes Association, 2026

2. Anastasiou G, et al. Insulin resistance and arterial stiffness are independently associated with Sensorimotor Polyneuropathy in Prediabetes. Poster with short presentation. 11th Panhellenic Congress of the Working Groups, Hellenic Atherosclerosis Society, 2025
3. Anastasiou G, et al. Clinical characteristics of Sensorimotor Polyneuropathy in Prediabetes. Poster with short presentation. 11th Panhellenic Congress of the Working Groups, Hellenic Atherosclerosis Society, 2025
4. Anastasiou G, et al. Independent association of neuropathic symptoms and signs with arterial stiffness and cardiometabolic risk factors in prediabetes. Poster with short presentation. 11th Panhellenic Congress of the Working Groups, Hellenic Atherosclerosis Society, 2025
5. Anastasiou G, et al. Prevalence of Diabetic Peripheral Neuropathy in Individuals with Impaired Fasting Glucose and Its Association with Cardiometabolic Parameters: Updated data. Poster with short presentation. 23rd panhellenic congress of Hellenic Diabetes Association, 2025
6. Anastasiou G, et al. Pulse Wave Velocity as a Predictive Marker of Cardiovascular Risk and Microvascular Complications in Patients with Impaired Glucose Tolerance. Poster with short presentation. 23rd panhellenic congress of Hellenic Diabetes Association, 2025
7. Anastasiou G, et al. Arterial stiffness and microvascular complications: Association of Pulse Wave Velocity with cardiovascular risk factors and the prevalence of Neuropathy and albuminuria in individuals with prediabetes. Poster with short presentation. 11th Panhellenic Congress of the Hellenic Atherosclerosis Society, 2024

8. Anastasiou G, et al. The Impact of activity tracking apps on metabolic parameters in individuals with prediabetes compared to normoglycemic individuals: A Randomized Controlled Trial. Poster with short presentation. 11th Panhellenic Congress of the Hellenic Atherosclerosis Society, 2024
9. Anastasiou G, et al. Prediabetes and diabetic sensorimotor neuropathy: a prospective analysis of the correlation with cardiometabolic risk factors. Poster with short presentation. 22nd Panhellenic Congress of Hellenic Diabetes Society, 2024
10. Anastasiou G, et al. Incidence of distal peripheral neuropathy in individuals with prediabetes and its association with cardiometabolic risk factors. Poster with short presentation. 10th Panhellenic Congress of the work groups of Hellenic Atherosclerosis Society, 2023
11. Anastasiou G, et al. Association of PWV with cardiovascular risk factors and diabetic neuropathy or albuminuria in individuals with prediabetes. Oral presentation. 13th Panhellenic Congress of study, research & training institute on diabetes mellitus and metabolic diseases, 2023
12. Anastasiou G, et al. Prevalence of microvascular complications in individuals with prediabetes. Oral presentation. 13th Panhellenic Congress of study, research & training institute on diabetes mellitus and metabolic diseases, 2023
13. Anastasiou G, et al. Prevalence of diabetic peripheral neuropathy and albuminuria in individuals with prediabetes. Oral presentation. 21st panhellenic congress of Hellenic Diabetes Association, Athens, 2023
14. Anastasiou G, et al. Association of PWV with classic cardiovascular risk factors and microvascular complications in patients with prediabetes. Invited oral

presentation. Panhellenic Seminars of work groups of Hellenic Cardiology Society, 2023

15. Anastasiou G, et al. The incidence of diabetic peripheral neuropathy in individuals with prediabetes and the associated risk factors: preliminary data. Oral presentation.10th Panhellenic Congress of the Hellenic Atherosclerosis Society, 2022
16. Anastasiou G, et al. The prevalence of diabetic peripheral neuropathy in individuals with prediabetes and the associated risk factors: preliminary data. Poster with short presentation. 10th Panhellenic Congress of the Hellenic Atherosclerosis Society, 2022
17. Anastasiou G, et al. Prevalence of distal sensory neuropathy in individuals with prediabetes and correlation of neuropathic symptoms and signs with clinical and laboratory parameters: preliminary data. Poster with short presentation. 9th Panhellenic Congress of Working Groups, Hellenic Atherosclerosis Society, 2021
18. Anastasiou G, et al. Prevalence of peripheral neuropathy in individuals with prediabetes and association of vibration perception threshold with clinical and laboratory parameters. Oral presentation. 9th Panhellenic Congress of the Hellenic Atherosclerosis Society, 2020
19. Anastasiou G, et al. Staphylococcus-associated glomerulonephritis with IgA deposition secondary to osteomyelitis in a diabetic patient. 7th Panhellenic Conference of the Diabetic Foot Disease Study Society (E.ME.DI.P), 2020

Invited Lectures and Scientific Presentations Related to the Present Doctorate Thesis

1. Prediabetes. Complications and treatment. Learning session. 3rd Panhellenic Congress, 3rd Postgraduate School of the Hellenic Society of Metabolic Medicine, Hellenic Society of Metabolic Medicine (HESMM), 2025
2. Prediabetes. Complications and treatment. 2nd International Conference 'Modern Challenges in the Diagnosis and Treatment of Metabolic Diseases', Hellenic Society of Metabolic Medicine (HESMM), 2025
3. Prediabetes and microvascular complications. Diagnostic Algorithms and Contemporary Therapeutic Approaches in the Management of Common and Rare Diseases. Medical Society of Ioannina, 2025
4. Association of PWV with classic cardiovascular risk factors and microvascular complications in patients with prediabetes. Invited oral presentation. Panhellenic Seminars of work groups of Hellenic Cardiology Society, 2023
5. Peripheral Neuropathy in Prediabetes, 10th National Congress of the Hellenic Atherosclerosis Society, 2022
6. Neuropathy in Prediabetes. 21st Congress of Medical Chemistry, 2021
7. Diabetic neuropathy of diabetic foot: Clinical case presentation" in the Round table "Diabetic Foot: Causes, clinical presentation, Treatment. Scientific congress about diabetic foot organized by Diabetic Center, Endocrinology Department and Vascular surgery Department of University Hospital of Ioannina, 2020
8. Neuropathy in prediabetes. 24th Scientific Congress of Hellenic Diabetic Foot Research Society (E.ME.DI.P), 2019

Awards and Distinctions Related to the Present Doctorate Thesis

1. Scholarship award, Hellenic Diabetes Association, supporting participation, study and presentation of research related to Distal Symmetric Neuropathy in prediabetes
2. 1st award of posters with short presentation. Prevalence of Diabetic Peripheral Neuropathy in Individuals with Impaired Fasting Glucose and Its Association with Cardiometabolic Parameters: Updated data. 23rd Panhellenic Diabetes Congress, Hellenic Diabetes Association, 2025
3. Scholarships awards by the Hellenic Atherosclerosis Society, the Hellenic Diabetes Association, and the Greek Holy Synod, supporting a research fellowship at Professor Christos S. Mantzoros Lab, Beth Isreal Deaconess Medical Center, Harvard Medical School, Boston, USA in the field of metabolic diseases including prediabetes and obesity, their related complications and management, 2024
4. Award of posters. The Impact of activity tracking apps on metabolic parameters in individuals with prediabetes compared to normoglycemic individuals: A Randomized Controlled Trial. Poster with short presentation. 11th Panhellenic Congress of the Hellenic Atherosclerosis Society, 2024
5. 1st award of posters. Incidence of distal peripheral neuropathy in individuals with prediabetes and its association with cardiometabolic risk factors. Hellenic Atherosclerosis Society. 2023
6. 2nd award of oral presentations. Association of PWV with cardiovascular risk factors and diabetic neuropathy or albuminuria in individuals with prediabetes. 13th Panhellenic Congress of study, research & training institute on diabetes mellitus and metabolic diseases, Ioannina, Greece. 2023

7. 1st award of posters. Incidence of distal peripheral neuropathy in individuals with prediabetes and its association with cardiometabolic risk factors. 10th Panhellenic Congress of the work groups of Hellenic Atherosclerosis Society, Athens, Greece. 2022

13. REFERENCES:

[1] Segers P, Rietzschel ER, Chirinos JA. How to Measure Arterial Stiffness in Humans. Arterioscler Thromb Vasc Biol. 2020;40:1034–43. 10.1161/ATVBAHA.119.313132

[2] Herzog MJ, Müller P, Lechner K, et al. Arterial stiffness and vascular aging: mechanisms, prevention, and therapy. Signal Transduction and Targeted Therapy. 2025;10:282. 10.1038/s41392-025-02346-0

[3] Baranowska-Jurkun A, Matuszewski W, Bandurska-Stankiewicz E. Chronic Microvascular Complications in Prediabetic States-An Overview. J Clin Med. 2020;910.3390/jcm9103289

[4] Committee ADAPP. 2. Diagnosis and Classification of Diabetes: Standards of Care in Diabetes—2025. Diabetes Care. 2024;48:S27–S49. 10.2337/dc25-S002

[5] Chatterton H, Younger T, Fischer A, Khunti K. Risk identification and interventions to prevent type 2 diabetes in adults at high risk: summary of NICE guidance. Bmj. 2012;345:e4624. 10.1136/bmj.e4624

[6] World Health Organization. Diabetes: Fact sheet. Geneva: World Health Organization; 2024. Available from: https://www.who.int/news-room/fact-sheets/detail/diabetes

[7] DeFronzo RA, Abdul-Ghani MA. Preservation of β-cell function: the key to diabetes prevention. J Clin Endocrinol Metab. 2011;96:2354–66. 10.1210/jc.2011-0246

[8] Cosentino F, Grant PJ, Aboyans V, et al. 2019 ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD. Eur Heart J. 2020;41:255–323. 10.1093/eurheartj/ehz486

[9] Marx N, Federici M, Schütt K, et al. 2023 ESC Guidelines for the management of cardiovascular disease in patients with diabetes. Eur Heart J. 2023;44:4043–140. 10.1093/eurheartj/ehad192

[10] Punthakee Z, Goldenberg R, Katz P. Definition, Classification and Diagnosis of Diabetes, Prediabetes and Metabolic Syndrome. Can J Diabetes. 2018;42 Suppl 1:S10–s5. 10.1016/j.jcjd.2017.10.003

[11] Diabetes* ADAPPCf. 2. Diagnosis and Classification of Diabetes: Standards of Care in Diabetes—2026. Diabetes Care. 2025;49:S27–S49. 10.2337/dc26-S002

[12] Bergman M, Manco M, Satman I, et al. International Diabetes Federation Position Statement on the 1-hour post-load plasma glucose for the diagnosis of intermediate hyperglycaemia and type 2 diabetes. Diabetes Res Clin Pract. 2024;209:111589. 10.1016/j.diabres.2024.111589

[13] Unnikrishnan R, Shaw JE, Chan JCN, et al. Prediabetes. Nature Reviews Disease Primers. 2025;11:49. 10.1038/s41572-025-00635-0

[14] Abdul-Ghani MA, Jenkinson CP, Richardson DK, Tripathy D, DeFronzo RA. Insulin secretion and action in subjects with impaired fasting glucose and impaired glucose tolerance: results from the Veterans Administration Genetic Epidemiology Study. Diabetes. 2006;55:1430–5. 10.2337/db05-1200

[15] Rooney MR, He JH, Salpea P, et al. Global and Regional Prediabetes Prevalence: Updates for 2024 and Projections for 2050. Diabetes Care. 2025;48:e142–e4. 10.2337/dc25-1640

[16] Makrilakis K, Kalpourtzi N, Ioannidis I, et al. Prevalence of diabetes and pre-diabetes in Greece. Results of the First National Survey of Morbidity and Risk Factors (EMENO) study. Diabetes Res Clin Pract. 2021;172:108646. 10.1016/j.diabres.2020.108646

[17] International Diabetes Federation. IDF Diabetes Atlas. 11th ed. Brussels, Belgium: International Diabetes Federation; 2025. Available from: https://diabetesatlas.org

[18] Davoodian N, Lotfaliany M, Huxley RR, et al. Prediabetes transitions to normoglycaemia or type 2 diabetes and associated risk factors in the Obesity, Diabetes and Cardiovascular Disease Collaboration: an individual-level pooled analysis of 19 prospective cohort studies. Lancet Glob Health. 2025;13:e1533–e42. 10.1016/s2214-109x(25)00237-2

[19] Kanat M, Norton L, Winnier D, Jenkinson C, DeFronzo RA, Abdul-Ghani MA. Impaired early- but not late-phase insulin secretion in subjects with impaired fasting glucose. Acta Diabetol. 2011;48:209–17. 10.1007/s00592-011-0285-x

[20] Tabák AG, Herder C, Rathmann W, Brunner EJ, Kivimäki M. Prediabetes: a high-risk state for diabetes development. The Lancet. 2012;379:2279–90. 10.1016/S0140-6736(12)60283-9

[21] de Vegt F, Dekker JM, Stehouwer CD, Nijpels G, Bouter LM, Heine RJ. The 1997 American Diabetes Association criteria versus the 1985 World Health Organization criteria for the diagnosis of abnormal glucose tolerance: poor agreement in the Hoorn Study. Diabetes Care. 1998;21:1686–90. 10.2337/diacare.21.10.1686

[22] Dunstan DW, Zimmet PZ, Welborn TA, et al. The rising prevalence of diabetes and impaired glucose tolerance: the Australian Diabetes, Obesity and Lifestyle Study. Diabetes Care. 2002;25:829–34. 10.2337/diacare.25.5.829

[23] Ko GT, Chan JC, Woo J, Cockram CS. Use of the 1997 American Diabetes Association diagnostic criteria for diabetes in a Hong Kong Chinese population. Diabetes Care. 1998;21:2094–7. 10.2337/diacare.21.12.2094

[24] Cowie CC, Rust KF, Byrd-Holt DD, et al. Prevalence of diabetes and impaired fasting glucose in adults in the U.S. population: National Health And Nutrition Examination Survey 1999-2002. Diabetes Care. 2006;29:1263–8. 10.2337/dc06-0062

[25] Shaw JE, Zimmet PZ, Hodge AM, et al. Impaired fasting glucose: how low should it go? Diabetes Care. 2000;23:34–9. 10.2337/diacare.23.1.34

[26] Gabir MM, Hanson RL, Dabelea D, et al. Plasma glucose and prediction of microvascular disease and mortality: evaluation of 1997 American Diabetes Association and 1999 World Health Organization criteria for diagnosis of diabetes. Diabetes Care. 2000;23:1113–8. 10.2337/diacare.23.8.1113

[27] Choi KM, Lee J, Kim DR, et al. Comparison of ADA and WHO criteria for the diagnosis of diabetes in elderly Koreans. Diabet Med. 2002;19:853–7. 10.1046/j.1464-5491.2002.00783.x

[28] Harris MI, Eastman RC, Cowie CC, Flegal KM, Eberhardt MS. Comparison of diabetes diagnostic categories in the U.S. population according to the 1997 American Diabetes Association and 1980-1985 World Health Organization diagnostic criteria. Diabetes Care. 1997;20:1859–62. 10.2337/diacare.20.12.1859

[29] Glucose tolerance and mortality: comparison of WHO and American Diabetes Association diagnostic criteria. The DECODE study group. European Diabetes Epidemiology Group. Diabetes Epidemiology: Collaborative analysis Of Diagnostic criteria in Europe. Lancet. 1999;354:617–21.

[30] Qiao Q, Nakagami T, Tuomilehto J, et al. Comparison of the fasting and the 2-h glucose criteria for diabetes in different Asian cohorts. Diabetologia. 2000;43:1470–5. 10.1007/s001250051557

[31] Nakagami T, Qiao Q, Tuomilehto J, et al. The fasting plasma glucose cut-point predicting a diabetic 2-h OGTT glucose level depends on the phenotype. Diabetes Res Clin Pract. 2002;55:35–43. 10.1016/s0168-8227(01)00270-4

[32] Shaw JE, Zimmet PZ, de Courten M, et al. Impaired fasting glucose or impaired glucose tolerance. What best predicts future diabetes in Mauritius? Diabetes Care. 1999;22:399–402. 10.2337/diacare.22.3.399

[33] Gabir MM, Hanson RL, Dabelea D, et al. The 1997 American Diabetes Association and 1999 World Health Organization criteria for hyperglycemia in the diagnosis and prediction of diabetes. Diabetes Care. 2000;23:1108–12. 10.2337/diacare.23.8.1108

[34] Stern MP, Williams K, Haffner SM. Identification of persons at high risk for type 2 diabetes mellitus: do we need the oral glucose tolerance test? Ann Intern Med. 2002;136:575–81. 10.7326/0003-4819-136-8-200204160-00006

[35] Yang J, Liu D, Du Q, et al. Construction of a 3-year risk prediction model for developing diabetes in patients with pre-diabetes. Front Endocrinol (Lausanne). 2024;15:1410502. 10.3389/fendo.2024.1410502

[36] Kahn SE. The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of Type 2 diabetes. Diabetologia. 2003;46:3–19. 10.1007/s00125-002-1009-0

[37] Liu Y, Li J, Wu Y, et al. Evidence From a Systematic Review and Meta-Analysis: Classical Impaired Glucose Tolerance Should Be Divided Into Subgroups of Isolated Impaired Glucose Tolerance and Impaired Glucose Tolerance Combined With Impaired Fasting Glucose, According to the Risk of Progression to Diabetes. Front Endocrinol (Lausanne). 2022;13:835460. 10.3389/fendo.2022.835460

[38] Weyer C, Tataranni PA, Bogardus C, Pratley RE. Insulin Resistance and Insulin Secretory Dysfunction Are Independent Predictors of Worsening of Glucose Tolerance

During Each Stage of Type 2 Diabetes Development. Diabetes Care. 2001;24:89–94. 10.2337/diacare.24.1.89

[39] Coutinho M, Gerstein HC, Wang Y, Yusuf S. The relationship between glucose and incident cardiovascular events. A metaregression analysis of published data from 20 studies of 95,783 individuals followed for 12.4 years. Diabetes Care. 1999;22:233–40. 10.2337/diacare.22.2.233

[40] Glucose tolerance and cardiovascular mortality: comparison of fasting and 2-hour diagnostic criteria. Arch Intern Med. 2001;161:397–405. 10.1001/archinte.161.3.397

[41] Is the current definition for diabetes relevant to mortality risk from all causes and cardiovascular and noncardiovascular diseases? Diabetes Care. 2003;26:688–96. 10.2337/diacare.26.3.688

[42] Barzilay JI, Spiekerman CF, Wahl PW, et al. Cardiovascular disease in older adults with glucose disorders: comparison of American Diabetes Association criteria for diabetes mellitus with WHO criteria. Lancet. 1999;354:622–5. 10.1016/s0140-6736(98)12030-5

[43] Saydah SH, Miret M, Sung J, Varas C, Gause D, Brancati FL. Postchallenge hyperglycemia and mortality in a national sample of U.S. adults. Diabetes Care. 2001;24:1397–402. 10.2337/diacare.24.8.1397

[44] Meigs JB, Nathan DM, D'Agostino RB, Sr., Wilson PW. Fasting and postchallenge glycemia and cardiovascular disease risk: the Framingham Offspring Study. Diabetes Care. 2002;25:1845–50. 10.2337/diacare.25.10.1845

[45] Weyer C, Bogardus C, Pratley RE. Metabolic characteristics of individuals with impaired fasting glucose and/or impaired glucose tolerance. Diabetes. 1999;48:2197–203. 10.2337/diabetes.48.11.2197

[46] Davies MJ, Raymond NT, Day JL, Hales CN, Burden AC. Impaired glucose tolerance and fasting hyperglycaemia have different characteristics. Diabet Med. 2000;17:433–40. 10.1046/j.1464-5491.2000.00246.x

[47] Heldgaard PE, Olivarius Nde F, Hindsberger C, Henriksen JE. Impaired fasting glycaemia resembles impaired glucose tolerance with regard to cardiovascular risk factors: population-based, cross-sectional study of risk factors for cardiovascular disease. Diabet Med. 2004;21:363–70. 10.1111/j.1464-5491.2004.01231.x

[48] Levitan EB, Song Y, Ford ES, Liu S. Is nondiabetic hyperglycemia a risk factor for cardiovascular disease? A meta-analysis of prospective studies. Arch Intern Med. 2004;164:2147–55. 10.1001/archinte.164.19.2147

[49] Hanefeld M, Koehler C, Fuecker K, Henkel E, Schaper F, Temelkova-Kurktschiev T. Insulin secretion and insulin sensitivity pattern is different in isolated impaired glucose tolerance and impaired fasting glucose: the risk factor in Impaired Glucose Tolerance for Atherosclerosis and Diabetes study. Diabetes Care. 2003;26:868–74. 10.2337/diacare.26.3.868

[50] Abdul-Ghani MA, Tripathy D, DeFronzo RA. Contributions of beta-cell dysfunction and insulin resistance to the pathogenesis of impaired glucose tolerance and impaired fasting glucose. Diabetes Care. 2006;29:1130–9. 10.2337/diacare.2951130

[51] Festa A, D'Agostino R, Jr., Hanley AJ, Karter AJ, Saad MF, Haffner SM. Differences in insulin resistance in nondiabetic subjects with isolated impaired glucose tolerance or isolated impaired fasting glucose. Diabetes. 2004;53:1549–55. 10.2337/diabetes.53.6.1549

[52] Abdul-Ghani MA, Tripathy D, DeFronzo RA. Contributions of β-Cell Dysfunction and Insulin Resistance to the Pathogenesis of Impaired Glucose Tolerance and Impaired Fasting Glucose. Diabetes Care. 2006;29:1130–9. 10.2337/dc05-2179

[53] Bock G, Chittilapilly E, Basu R, et al. Contribution of Hepatic and Extrahepatic Insulin Resistance to the Pathogenesis of Impaired Fasting Glucose: Role of Increased Rates of Gluconeogenesis. Diabetes. 2007;56:1703–11. 10.2337/db06-1776

[54] Aoyama-Sasabe S, Fukushima M, Xin X, et al. Insulin Secretory Defect and Insulin Resistance in Isolated Impaired Fasting Glucose and Isolated Impaired Glucose Tolerance. J Diabetes Res. 2016;2016:1298601. 10.1155/2016/1298601

[55] Li Y, Feng D, Esangbedo IC, et al. Insulin resistance, beta-cell function, adipokine profiles and cardiometabolic risk factors among Chinese youth with isolated impaired fasting glucose versus impaired glucose tolerance: the BCAMS study. BMJ Open Diabetes Research & Care. 2020;8:e000724. 10.1136/bmjdrc-2019-000724

[56] ter Horst KW, Gilijamse PW, Ackermans MT, et al. Impaired insulin action in the liver, but not in adipose tissue or muscle, is a distinct metabolic feature of impaired fasting glucose in obese humans. Metabolism. 2016;65:757–63. https://doi.org/10.1016/j.metabol.2016.02.010

[57] Abdul-Ghani MA, DeFronzo RA. Pathophysiology of prediabetes. Curr Diab Rep. 2009;9:193–9. 10.1007/s11892-009-0032-7

[58] Pop-Busui R, Boulton AJ, Feldman EL, et al. Diabetic Neuropathy: A Position Statement by the American Diabetes Association. Diabetes Care. 2017;40:136–54. 10.2337/dc16-2042

[59] Galer BS, Gianas A, Jensen MP. Painful diabetic polyneuropathy: epidemiology, pain description, and quality of life. Diabetes Res Clin Pract. 2000;47:123–8. 10.1016/s0168-8227(99)00112-6

[60] Hicks CW, Wang D, Matsushita K, Windham BG, Selvin E. Peripheral Neuropathy and All-Cause and Cardiovascular Mortality in U.S. Adults : A Prospective Cohort Study. Ann Intern Med. 2021;174:167–74. 10.7326/M20-1340

[61] Armstrong DG, Tan TW, Boulton AJM, Bus SA. Diabetic Foot Ulcers: A Review. JAMA. 2023;330:62–75. 10.1001/jama.2023.10578

[62] Ziegler D, Papanas N, Vinik AI, Shaw JE. Epidemiology of polyneuropathy in diabetes and prediabetes. Handb Clin Neurol. 2014;126:3–22. 10.1016/B978-0-444-53480-4.00001-1

[63] Tesfaye S, Brill S, Eerdekens M, et al. Diagnosis, management and impact of painful diabetic peripheral neuropathy: A patient survey in four European countries. J Diabetes Complications. 2023;37:108417. 10.1016/j.jdiacomp.2023.108417

[64] Iqbal Z, Azmi S, Yadav R, et al. Diabetic Peripheral Neuropathy: Epidemiology, Diagnosis, and Pharmacotherapy. Clin Ther. 2018;40:828–49. 10.1016/j.clinthera.2018.04.001

[65] Abbott CA, Malik RA, van Ross ER, Kulkarni J, Boulton AJ. Prevalence and characteristics of painful diabetic neuropathy in a large community-based diabetic population in the U.K. Diabetes Care. 2011;34:2220–4. 10.2337/dc11-1108

[66] Young MJ, Boulton AJ, MacLeod AF, Williams DR, Sonksen PH. A multicentre study of the prevalence of diabetic peripheral neuropathy in the United Kingdom hospital clinic population. Diabetologia. 1993;36:150–4. 10.1007/BF00400697

[67] Ismail-Beigi F, Craven T, Banerji MA, et al. Effect of intensive treatment of hyperglycaemia on microvascular outcomes in type 2 diabetes: an analysis of the ACCORD randomised trial. The Lancet. 2010;376:419–30. 10.1016/s0140-6736(10)60576-4

[68] Dyck PJ, Davies JL, Wilson DM, Service FJ, Melton LJ, 3rd, O'Brien PC. Risk factors for severity of diabetic polyneuropathy: intensive longitudinal assessment of the Rochester Diabetic Neuropathy Study cohort. Diabetes Care. 1999;22:1479–86. 10.2337/diacare.22.9.1479

[69] Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). The Lancet. 1998;352:837–53. 10.1016/s0140-6736(98)07019-6

[70] Tesfaye S, Chaturvedi N, Eaton SE, et al. Vascular risk factors and diabetic neuropathy. N Engl J Med. 2005;352:341–50. 10.1056/NEJMoa032782

[71] Andersen ST, Witte DR, Dalsgaard EM, et al. Risk Factors for Incident Diabetic Polyneuropathy in a Cohort With Screen-Detected Type 2 Diabetes Followed for 13 Years: ADDITION-Denmark. Diabetes Care. 2018;41:1068–75. 10.2337/dc17-2062

[72] Zochodne DW. Diabetes mellitus and the peripheral nervous system: manifestations and mechanisms. Muscle Nerve. 2007;36:144–66. 10.1002/mus.20785

[73] Zochodne DW. Mechanisms of diabetic neuron damage: Molecular pathways. Handb Clin Neurol. 2014;126:379–99. 10.1016/b978-0-444-53480-4.00028-x

[74] Zochodne DW. Sensory Neurodegeneration in Diabetes: Beyond Glucotoxicity. Int Rev Neurobiol. 2016;127:151–80. 10.1016/bs.irn.2016.03.007

[75] Zochodne DW, Sun H-S, Cheng C, Eyer J. Accelerated diabetic neuropathy in axons without neurofilaments. Brain. 2004;127:2193–200. 10.1093/brain/awh251

[76] Zochodne DW, Verge VM, Cheng C, Sun H, Johnston J. Does diabetes target ganglion neurones? Progressive sensory neurone involvement in long-term experimental diabetes. Brain. 2001;124:2319–34. 10.1093/brain/124.11.2319

[77] Kennedy JM, Zochodne DW. Experimental diabetic neuropathy with spontaneous recovery: is there irreparable damage? Diabetes. 2005;54:830–7. 10.2337/diabetes.54.3.830

[78] Cheng C, Guo GF, Martinez JA, Singh V, Zochodne DW. Dynamic plasticity of axons within a cutaneous milieu. J Neurosci. 2010;30:14735–44. 10.1523/jneurosci.2919-10.2010

[79] Scott JN, Clark AW, Zochodne DW. Neurofilament and tubulin gene expression in progressive experimental diabetes: failure of synthesis and export by sensory neurons. Brain. 1999;122 (Pt 11):2109–18. 10.1093/brain/122.11.2109

[80] Mauermann ML, Staff NP. Peripheral Neuropathy: A Review. Jama. 2026;335:255–66. 10.1001/jama.2025.19400

[81] Arvidson B. Distribution of intravenously injected protein tracers in peripheral ganglia of adult mice. Experimental Neurology. 1979;63:388–410. https://doi.org/10.1016/0014-4886(79)90134-1

[82] Greenbaum D, Richardson PC, Salmon MV, Urich H. PATHOLOGICAL OBSERVATIONS ON SIX CASES OF DIABETIC NEUROPATHY. Brain. 1964;87:201–14. 10.1093/brain/87.2.201

[83] Schmidt RE, Beaudet LN, Plurad SB, Dorsey DA. Axonal cytoskeletal pathology in aged and diabetic human sympathetic autonomic ganglia. Brain Research. 1997;769:375–83. https://doi.org/10.1016/S0006-8993(97)00806-8

[84] Li XQ, Zochodne DW. Microvacuolar neuronopathy: a postmortem artifact of sensory neurons. J Neurocytol. 2003;32:393–8. 10.1023/B:NEUR.0000011333.06638.b6

[85] Funakoshi H, Frisén J, Barbany G, et al. Differential expression of mRNAs for neurotrophins and their receptors after axotomy of the sciatic nerve. J Cell Biol. 1993;123:455–65. 10.1083/jcb.123.2.455

[86] Lieberman AR. The axon reaction: a review of the principal features of perikaryal responses to axon injury. Int Rev Neurobiol. 1971;14:49–124. 10.1016/s0074-7742(08)60183-x

[87] Richardson PM, Verge VMK. The induction of a regenerative propensity in sensory neurons following peripheral axonal injury. Journal of Neurocytology. 1986;15:585–94. 10.1007/BF01611859

[88] Feldman EL, Callaghan BC, Pop-Busui R, et al. Diabetic neuropathy. Nat Rev Dis Primers. 2019;5:42. 10.1038/s41572-019-0097-9

[89] Feldman EL, Nave KA, Jensen TS, Bennett DLH. New Horizons in Diabetic Neuropathy: Mechanisms, Bioenergetics, and Pain. Neuron. 2017;93:1296–313. 10.1016/j.neuron.2017.02.005

[90] Zochodne DW, Ho LT, Allison JA. Dorsal root ganglia microenvironment of female BB Wistar diabetic rats with mild neuropathy. J Neurol Sci. 1994;127:36–42. 10.1016/0022-510x(94)90132-5

[91] Zochodne DW, Ho LT. The influence of sulindac on experimental streptozotocin-induced diabetic neuropathy. Can J Neurol Sci. 1994;21:194–202. 10.1017/s0317167100041160

[92] Zochodne DW, Cheng C. Diabetic peripheral nerves are susceptible to multifocal ischemic damage from endothelin. Brain Res. 1999;838:11–7. 10.1016/s0006-8993(99)01670-4

[93] Zochodne DW, Ho LT. Diabetes mellitus prevents capsaicin from inducing hyperaemia in the rat sciatic nerve. Diabetologia. 1993;36:493–6. 10.1007/bf02743263

[94] Xu QG, Cheng C, Sun H, Thomsen K, Zochodne DW. Local sensory ganglion ischemia induced by endothelin vasoconstriction: vulnerability of diabetic neurons and microvessels. Neuroscience. 2003;122:897–905. 10.1016/s0306-4522(03)00520-7

[95] Zhu J, Hu Z, Luo Y, et al. Diabetic peripheral neuropathy: pathogenetic mechanisms and treatment. Frontiers in Endocrinology. 2024;Volume 14 - 202310.3389/fendo.2023.1265372

[96] Toth C, Brussee V, Cheng C, Zochodne DW. Diabetes mellitus and the sensory neuron. J Neuropathol Exp Neurol. 2004;63:561–73. 10.1093/jnen/63.6.561

[97] Cheng C, Kobayashi M, Martinez JA, et al. Evidence for Epigenetic Regulation of Gene Expression and Function in Chronic Experimental Diabetic Neuropathy. J Neuropathol Exp Neurol. 2015;74:804–17. 10.1097/nen.0000000000000219

[98] Caselli A, Rich J, Hanane T, Uccioli L, Veves A. Role of C-nociceptive fibers in the nerve axon reflex-related vasodilation in diabetes. Neurology. 2003;60:297–300. 10.1212/01.wnl.0000040250.31755.f9

[99] Kobayashi M, Chandrasekhar A, Cheng C, et al. Diabetic polyneuropathy, sensory neurons, nuclear structure and spliceosome alterations: a role for CWC22. Dis Model Mech. 2017;10:215–24. 10.1242/dmm.028225

[100] Cheng Y, Liu J, Luan Y, et al. Sarm1 Gene Deficiency Attenuates Diabetic Peripheral Neuropathy in Mice. Diabetes. 2019;68:2120–30. 10.2337/db18-1233

[101] Gerdts J, Summers DW, Milbrandt J, DiAntonio A. Axon Self-Destruction: New Links among SARM1, MAPKs, and NAD+ Metabolism. Neuron. 2016;89:449–60. 10.1016/j.neuron.2015.12.023

[102] Woo V, Cheng C, Duraikannu A, et al. Caspase-6 is a Dispensable Enabler of Adult Mammalian Axonal Degeneration. Neuroscience. 2018;371:242–53. https://doi.org/10.1016/j.neuroscience.2017.11.052

[103] Düll MM, Riegel K, Tappenbeck J, et al. Methylglyoxal causes pain and hyperalgesia in human through C-fiber activation. Pain. 2019;160:2497–507. 10.1097/j.pain.0000000000001644

[104] Zenker J, Poirot O, de Preux Charles AS, et al. Altered distribution of juxtaparanodal kv1.2 subunits mediates peripheral nerve hyperexcitability in type 2 diabetes mellitus. J Neurosci. 2012;32:7493–8. 10.1523/jneurosci.0719-12.2012

[105] Tsantoulas C, Laínez S, Wong S, Mehta I, Vilar B, McNaughton PA. Hyperpolarization-activated cyclic nucleotide-gated 2 (HCN2) ion channels drive pain in

mouse models of diabetic neuropathy. Sci Transl Med. 2017;9:eaam6072. 10.1126/scitranslmed.aam6072

[106] Feldman EL, Callaghan BC, Pop-Busui R, et al. Diabetic neuropathy. Nature Reviews Disease Primers. 2019;5:41. 10.1038/s41572-019-0092-1

[107] Selvarajah D, Wilkinson ID, Davies J, Gandhi R, Tesfaye S. Central nervous system involvement in diabetic neuropathy. Curr Diab Rep. 2011;11:310–22. 10.1007/s11892-011-0205-z

[108] Tesfaye S, Boulton AJ, Dickenson AH. Mechanisms and management of diabetic painful distal symmetrical polyneuropathy. Diabetes Care. 2013;36:2456–65. 10.2337/dc12-1964

[109] Dyck PJ, Albers JW, Andersen H, et al. Diabetic polyneuropathies: update on research definition, diagnostic criteria and estimation of severity. Diabetes Metab Res Rev. 2011;27:620–8. 10.1002/dmrr.1226

[110] Devigili G, Tugnoli V, Penza P, et al. The diagnostic criteria for small fibre neuropathy: from symptoms to neuropathology. Brain. 2008;131:1912–25. 10.1093/brain/awn093

[111] Lauria G, Hsieh ST, Johansson O, et al. European Federation of Neurological Societies/Peripheral Nerve Society Guideline on the use of skin biopsy in the diagnosis of small fiber neuropathy. Report of a joint task force of the European Federation of Neurological Societies and the Peripheral Nerve Society. Eur J Neurol. 2010;17:903–12, e44–9. 10.1111/j.1468-1331.2010.03023.x

[112] Selvarajah D, Wilkinson ID, Maxwell M, et al. Magnetic resonance neuroimaging study of brain structural differences in diabetic peripheral neuropathy. Diabetes Care. 2014;37:1681–8. 10.2337/dc13-2610

[113] Malik RA, Veves A, Tesfaye S, et al. Small fibre neuropathy: role in the diagnosis of diabetic sensorimotor polyneuropathy. Diabetes Metab Res Rev. 2011;27:678–84. 10.1002/dmrr.1222

[114] Themistocleous AC, Backonja MM. Pathophysiology of Neuropathic Pain. In: Tesfaye S, Gibbons CH, Malik RA, Veves A, editors. Diabetic Neuropathy: Advances in Pathophysiology and Clinical Management. Cham: Springer International Publishing; 2023. p. 415–25.

[115] Tesfaye S, Gibbons CH, Malik RA, Veves A. Diabetic Neuropathy: Advances in Pathophysiology and Clinical Management: Springer International Publishing; 2023.

[116] Marshall AG, Marshall A, Calcutt NA. Chapter 7 - Spinal cord involvement in diabetic neuropathy and neuropathic pain. In: Tavakoli M, editor. Diabetic Neuropathy: Elsevier; 2022. p. 103–22.

[117] Jensen TS, Finnerup NB. Allodynia and hyperalgesia in neuropathic pain: clinical manifestations and mechanisms. Lancet Neurol. 2014;13:924–35. 10.1016/s1474-4422(14)70102-4

[118] Bansal V, Kalita J, Misra UK. Diabetic neuropathy. Postgrad Med J. 2006;82:95–100. 10.1136/pgmj.2005.036137

[119] Dyck PJ, Kratz KM, Karnes JL, et al. The prevalence by staged severity of various types of diabetic neuropathy, retinopathy, and nephropathy in a population-based cohort. The Rochester Diabetic Neuropathy Study. 1993;43:817–. 10.1212/wnl.43.4.817

[120] Pop-Busui R, Herman WH, Feldman EL, et al. DCCT and EDIC studies in type 1 diabetes: lessons for diabetic neuropathy regarding metabolic memory and natural history. Curr Diab Rep. 2010;10:276–82. 10.1007/s11892-010-0120-8

[121] Azad N, Emanuele NV, Abraira C, et al. The effects of intensive glycemic control on neuropathy in the VA cooperative study on type II diabetes mellitus (VA CSDM). J Diabetes Complications. 1999;13:307–13. 10.1016/s1056-8727(99)00062-8

[122] Gaede P, Vedel P, Larsen N, Jensen GV, Parving HH, Pedersen O. Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes. N Engl J Med. 2003;348:383–93. 10.1056/NEJMoa021778

[123] Young MJ, Breddy JL, Veves A, Boulton AJ. The prediction of diabetic neuropathic foot ulceration using vibration perception thresholds. A prospective study. Diabetes Care. 1994;17:557–60. 10.2337/diacare.17.6.557

[124] Hu YM, Zhao LH, Zhang XL, et al. Association of glycaemic variability evaluated by continuous glucose monitoring with diabetic peripheral neuropathy in type 2 diabetic patients. Endocrine. 2018;60:292–300. 10.1007/s12020-018-1546-z

[125] Ponirakis G, Petropoulos IN, Alam U, et al. Hypertension Contributes to Neuropathy in Patients With Type 1 Diabetes. Am J Hypertens. 2019;32:796–803. 10.1093/ajh/hpz058

[126] Cheng Y, Cao W, Zhang J, et al. Determinants of Diabetic Peripheral Neuropathy and Their Clinical Significance: A Retrospective Cohort Study. Front Endocrinol (Lausanne). 2022;13:934020. 10.3389/fendo.2022.934020

[127] van der Velde J, Koster A, Strotmeyer ES, et al. Cardiometabolic risk factors as determinants of peripheral nerve function: the Maastricht Study. Diabetologia. 2020;63:1648–58. 10.1007/s00125-020-05194-5

[128] Gregory JA, Jolivalt CG, Goor J, Mizisin AP, Calcutt NA. Hypertension-induced peripheral neuropathy and the combined effects of hypertension and diabetes on nerve structure and function in rats. Acta Neuropathol. 2012;124:561–73. 10.1007/s00401-012-1012-6

[129] Ozaki K, Matsuura T. Superimposition of hypertension on diabetic peripheral neuropathy affects small unmyelinated sensory nerves in the skin and myelinated tibial and sural nerves in rats with alloxan-induced type 1 diabetes. J Toxicol Pathol. 2020;33:161–9. 10.1293/tox.2020-0003

[130] Elliott J, Tesfaye S, Chaturvedi N, et al. Large-fiber dysfunction in diabetic peripheral neuropathy is predicted by cardiovascular risk factors. Diabetes Care. 2009;32:1896–900. 10.2337/dc09-0554

[131] Cai Z, Yang Y, Zhang J. A systematic review and meta-analysis of the serum lipid profile in prediction of diabetic neuropathy. Sci Rep. 2021;11:499. 10.1038/s41598-020-79276-0

[132] Afshinnia F, Reynolds EL, Rajendiran TM, et al. Serum lipidomic determinants of human diabetic neuropathy in type 2 diabetes. Ann Clin Transl Neurol. 2022;9:1392–404. 10.1002/acn3.51639

[133] Smith AG, Singleton JR. Obesity and hyperlipidemia are risk factors for early diabetic neuropathy. J Diabetes Complications. 2013;27:436–42. 10.1016/j.jdiacomp.2013.04.003

[134] Rajamani K, Colman PG, Li LP, et al. Effect of fenofibrate on amputation events in people with type 2 diabetes mellitus (FIELD study): a prespecified analysis of a randomised controlled trial. Lancet. 2009;373:1780–8. 10.1016/s0140-6736(09)60698-x

[135] Davis TM, Yeap BB, Davis WA, Bruce DG. Lipid-lowering therapy and peripheral sensory neuropathy in type 2 diabetes: the Fremantle Diabetes Study. Diabetologia. 2008;51:562–6. 10.1007/s00125-007-0919-2

[136] Marinho LL, Everett BM, Aday AW, et al. Effect of Pemafibrate on Diabetic Foot Ulceration and Gangrene: An Exploratory Analysis From PROMINENT. J Am Coll Cardiol. 2024;84:408–10. 10.1016/j.jacc.2024.05.028

[137] Schlesinger S, Herder C, Kannenberg JM, et al. General and Abdominal Obesity and Incident Distal Sensorimotor Polyneuropathy: Insights Into Inflammatory Biomarkers as Potential Mediators in the KORA F4/FF4 Cohort. Diabetes Care. 2019;42:240–7. 10.2337/dc18-1842

[138] Zhou R, Li F, Chen G, Fu Q, Gu S, Wu X. Associations between general and abdominal obesity and incident diabetic neuropathy in participants with type 2 diabetes mellitus. J Diabetes. 2021;13:33–42. 10.1111/1753-0407.13075

[139] Adam S, Azmi S, Ho JH, et al. Improvements in Diabetic Neuropathy and Nephropathy After Bariatric Surgery: a Prospective Cohort Study. Obes Surg. 2021;31:554–63. 10.1007/s11695-020-05052-8

[140] Ylitalo KR, Sowers M, Heeringa S. Peripheral vascular disease and peripheral neuropathy in individuals with cardiometabolic clustering and obesity: National Health and Nutrition Examination Survey 2001-2004. Diabetes Care. 2011;34:1642–7. 10.2337/dc10-2150

[141] Cho YN, Lee KO, Jeong J, et al. The role of insulin resistance in diabetic neuropathy in Koreans with type 2 diabetes mellitus: a 6-year follow-up study. Yonsei Med J. 2014;55:700–8. 10.3349/ymj.2014.55.3.700

[142] Callaghan BC, Xia R, Banerjee M, et al. Metabolic Syndrome Components Are Associated With Symptomatic Polyneuropathy Independent of Glycemic Status. Diabetes Care. 2016;39:801–7. 10.2337/dc16-0081

[143] Lee CC, Perkins BA, Kayaniyil S, et al. Peripheral Neuropathy and Nerve Dysfunction in Individuals at High Risk for Type 2 Diabetes: The PROMISE Cohort. Diabetes Care. 2015;38:793–800. 10.2337/dc14-2585

[144] Ziegler D, Rathmann W, Dickhaus T, Meisinger C, Mielck A, Group KS. Prevalence of polyneuropathy in pre-diabetes and diabetes is associated with abdominal

obesity and macroangiopathy: the MONICA/KORA Augsburg Surveys S2 and S3. Diabetes Care. 2008;31:464–9. 10.2337/dc07-1796

[145] Franklin GM, Kahn LB, Baxter J, Marshall JA, Hamman RF. Sensory neuropathy in non-insulin-dependent diabetes mellitus. The San Luis Valley Diabetes Study. Am J Epidemiol. 1990;131:633–43. 10.1093/oxfordjournals.aje.a115547

[146] Partanen J, Niskanen L, Lehtinen J, Mervaala E, Siitonen O, Uusitupa M. Natural history of peripheral neuropathy in patients with non-insulin-dependent diabetes mellitus. N Engl J Med. 1995;333:89–94. 10.1056/nejm199507133330203

[147] Yokoyama H, Tsuji T, Hayashi S, Kabata D, Shintani A. Factors associated with diabetic polyneuropathy-related sensory symptoms and signs in patients with polyneuropathy: A cross-sectional Japanese study (JDDM 52) using a non-linear model. J Diabetes Investig. 2020;11:450–7. 10.1111/jdi.13117

[148] Dinh Le T, Phi Thi Nguyen N, Thanh Thi Tran H, et al. Diabetic Peripheral Neuropathy Associated with Cardiovascular Risk Factors and Glucagon-Like Peptide-1 Concentrations Among Newly Diagnosed Patients with Type 2 Diabetes Mellitus. Diabetes Metab Syndr Obes. 2022;15:35–44. 10.2147/DMSO.S344532

[149] Szwarcbard N, Villani M, Earnest A, et al. The association of smoking status with glycemic control, metabolic profile and diabetic complications- Results of the Australian National Diabetes Audit (ANDA). J Diabetes Complications. 2020;34:107626. 10.1016/j.jdiacomp.2020.107626

[150] Callaghan BC, Cheng HT, Stables CL, Smith AL, Feldman EL. Diabetic neuropathy: clinical manifestations and current treatments. Lancet Neurol. 2012;11:521–34. 10.1016/s1474-4422(12)70065-0

[151] Yang Y, Zhao B, Wang Y, et al. Diabetic neuropathy: cutting-edge research and future directions. Signal Transduct Target Ther. 2025;10:132. 10.1038/s41392-025-02175-1

[152] Elafros MA, Andersen H, Bennett DL, et al. Towards prevention of diabetic peripheral neuropathy: clinical presentation, pathogenesis, and new treatments. The Lancet Neurology. 2022;21:922–36. https://doi.org/10.1016/S1474-4422(22)00188-0

[153] Peltier A, Goutman SA, Callaghan BC. Painful diabetic neuropathy. Bmj. 2014;348:g1799. 10.1136/bmj.g1799

[154] Staehelin Jensen T. The pathogenesis of painful diabetic neuropathy and clinical presentation. Diabetes Res Clin Pract. 2023;206 Suppl 1:110753. 10.1016/j.diabres.2023.110753

[155] Terkelsen AJ, Karlsson P, Lauria G, Freeman R, Finnerup NB, Jensen TS. The diagnostic challenge of small fibre neuropathy: clinical presentations, evaluations, and causes. The Lancet Neurology. 2017;16:934–44. 10.1016/s1474-4422(17)30329-0

[156] Chang MC, Yang S. Diabetic peripheral neuropathy essentials: a narrative review. Ann Palliat Med. 2023;12:390–8. 10.21037/apm-22-693

[157] Truini A, Barbanti P, Pozzilli C, Cruccu G. A mechanism-based classification of pain in multiple sclerosis. J Neurol. 2013;260:351–67. 10.1007/s00415-012-6579-2

[158] Colloca L, Ludman T, Bouhassira D, et al. Neuropathic pain. Nat Rev Dis Primers. 2017;3:17002. 10.1038/nrdp.2017.2

[159] Tsapas A, Liakos A, Paschos P, et al. A simple plaster for screening for diabetic neuropathy: a diagnostic test accuracy systematic review and meta-analysis. Metabolism. 2014;63:584–92. 10.1016/j.metabol.2013.11.019

[160] McIllhatton A, Lanting S, Lambkin D, Leigh L, Casey S, Chuter V. Reliability of recommended non-invasive chairside screening tests for diabetes-related peripheral

neuropathy: a systematic review with meta-analyses. BMJ Open Diabetes Res Care. 2021;910.1136/bmjdrc-2021-002528

[161] Feldman EL, Stevens MJ, Thomas PK, Brown MB, Canal N, Greene DA. A practical two-step quantitative clinical and electrophysiological assessment for the diagnosis and staging of diabetic neuropathy. Diabetes Care. 1994;17:1281–9. 10.2337/diacare.17.11.1281

[162] Ziegler D, Hanefeld M, Ruhnau KJ, et al. Treatment of symptomatic diabetic peripheral neuropathy with the anti-oxidant alpha-lipoic acid. A 3-week multicentre randomized controlled trial (ALADIN Study). Diabetologia. 1995;38:1425–33. 10.1007/bf00400603

[163] Tentolouris N, Achtsidis V, Marinou K, Katsilambros N. Evaluation of the self-administered indicator plaster neuropad for the diagnosis of neuropathy in diabetes. Diabetes Care. 2008;31:236–7. 10.2337/dc07-1942

[164] Zografou I, Iliadis F, Sambanis C, Didangelos T. Validation of Neuropad in the Assessment of Peripheral Diabetic Neuropathy in Patients with Diabetes Mellitus Versus the Michigan Neuropathy Screening Instrument, 10g Monofilament Application and Biothesiometer Measurement. Curr Vasc Pharmacol. 2020;18:517–22. 10.2174/1570161117666190723155324

[165] Papanas N, Papatheodorou K, Papazoglou D, Monastiriotis C, Christakidis D, Maltezos E. A comparison of the new indicator test for sudomotor function (Neuropad) with the vibration perception threshold and the clinical examination in the diagnosis of peripheral neuropathy in subjects with type 2 diabetes. Exp Clin Endocrinol Diabetes. 2008;116:135–8. 10.1055/s-2007-984455

[166] Gwathmey KG, Pearson KT. Diagnosis and management of sensory polyneuropathy. BMJ. 2019;365:l1108. 10.1136/bmj.l1108

[167] Davalos L, Stino A, Smith AG. Diagnostic Techniques for Diabetic Peripheral Neuropathy. In: Tesfaye S, Gibbons CH, Malik RA, Veves A, editors. Diabetic Neuropathy: Advances in Pathophysiology and Clinical Management. Cham: Springer International Publishing; 2023. p. 93–112.

[168] Dy SM, Bennett WL, Sharma R, Zhang A, Waldfogel JM, Nesbit SA, et al. Preventing complications and treating symptoms of diabetic peripheral neuropathy. Comparative Effectiveness Review No. 187. Rockville (MD): Agency for Healthcare Research and Quality; 2017. doi:10.23970/AHRQEPCCER187.

[169] Aladdin Y, Jeerakathil T, Siddiqi ZA. Insulin Neuritis and Effect of Pregabalin. J Clin Neuromuscul Dis. 2017;19:1–4. 10.1097/cnd.0000000000000120

[170] Finnerup NB, Attal N, Haroutounian S, et al. Pharmacotherapy for neuropathic pain in adults: a systematic review and meta-analysis. The Lancet Neurology. 2015;14:162–73. 10.1016/s1474-4422(14)70251-0

[171] ElSayed NA, Aleppo G, Aroda VR, et al. 12. Retinopathy, Neuropathy, and Foot Care: Standards of Care in Diabetes-2023. Diabetes Care. 2023;46:S203–S15. 10.2337/dc23-S012

[172] Price R, Smith D, Franklin G, et al. Oral and Topical Treatment of Painful Diabetic Polyneuropathy: Practice Guideline Update Summary: Report of the AAN Guideline Subcommittee. Neurology. 2022;98:31–43. 10.1212/WNL.0000000000013038

[173] Tesfaye S, Sloan G, Petrie J, et al. Comparison of amitriptyline supplemented with pregabalin, pregabalin supplemented with amitriptyline, and duloxetine supplemented with pregabalin for the treatment of diabetic peripheral neuropathic pain (OPTION-DM): a multicentre, double-blind, randomised crossover trial. Lancet. 2022;400:680–90. 10.1016/S0140-6736(22)01472-6

[174] Alam U, Sloan G, Tesfaye S. Treating Pain in Diabetic Neuropathy: Current and Developmental Drugs. Drugs. 2020;80:363–84. 10.1007/s40265-020-01259-2

[175] Rosenstock J, Tuchman M, LaMoreaux L, Sharma U. Pregabalin for the treatment of painful diabetic peripheral neuropathy: a double-blind, placebo-controlled trial. Pain. 2004;110:628–38. 10.1016/j.pain.2004.05.001

[176] Pop-Busui R, Ang L, Boulton AJM, et al. Diagnosis and treatment of painful diabetic peripheral neuropathy. ADA Clinical Compendia Series. American Diabetes Association; 2022.

[177] Freeman R, Raskin P, Hewitt DJ, et al. Randomized study of tramadol/acetaminophen versus placebo in painful diabetic peripheral neuropathy. Curr Med Res Opin. 2007;23:147–61. 10.1185/030079906X162674

[178] Buckley PF. Association Between Opioid Prescribing Patterns and Opioid Overdose-Related Deaths. Yearbook of Psychiatry and Applied Mental Health. 2012;2012:128–30. 10.1016/j.ypsy.2011.07.071

[179] Tesfaye S, Wilhelm S, Lledo A, et al. Duloxetine and pregabalin: high-dose monotherapy or their combination? The "COMBO-DN study"--a multinational, randomized, double-blind, parallel-group study in patients with diabetic peripheral neuropathic pain. Pain. 2013;154:2616–25. 10.1016/j.pain.2013.05.043

[180] The effect of intensive diabetes therapy on the development and progression of neuropathy. The Diabetes Control and Complications Trial Research Group. Ann Intern Med. 1995;122:561–8. 10.7326/0003-4819-122-8-199504150-00001

[181] Kennedy WR, Navarro X, Goetz FC, Sutherland DER, Najarian JS. Effects of pancreatic transplantation on diabetic neuropathy. *N Engl J Med*. 1990;322(15):1031-1037.

[182] Azmi S, Jeziorska M, Ferdousi M, et al. Early nerve fibre regeneration in individuals with type 1 diabetes after simultaneous pancreas and kidney transplantation. Diabetologia. 2019;62:1478–87. 10.1007/s00125-019-4897-y

[183] Ishibashi F, Taniguchi M, Kosaka A, Uetake H, Tavakoli M. Improvement in Neuropathy Outcomes With Normalizing HbA1c in Patients With Type 2 Diabetes. Diabetes Care. 2019;42:110–8. 10.2337/dc18-1560

[184] Song Y, Zhang H, Sun J, et al. Glycemic Variability and the Risk of Diabetic Peripheral Neuropathy: A Meta-Analysis. Horm Metab Res. 202310.1055/a-2165-3579

[185] Xu F, Zhao LH, Su JB, et al. The relationship between glycemic variability and diabetic peripheral neuropathy in type 2 diabetes with well-controlled HbA1c. Diabetol Metab Syndr. 2014;6:139. 10.1186/1758-5996-6-139

[186] Kuroda N, Kusunoki Y, Osugi K, et al. Relationships between time in range, glycemic variability including hypoglycemia and types of diabetes therapy in Japanese patients with type 2 diabetes mellitus: Hyogo Diabetes Hypoglycemia Cognition Complications study. J Diabetes Investig. 2021;12:244–53. 10.1111/jdi.13336

[187] Li F, Zhang Y, Li H, et al. TIR generated by continuous glucose monitoring is associated with peripheral nerve function in type 2 diabetes. Diabetes Res Clin Pract. 2020;166:108289. 10.1016/j.diabres.2020.108289

[188] Mayeda L, Katz R, Ahmad I, et al. Glucose time in range and peripheral neuropathy in type 2 diabetes mellitus and chronic kidney disease. BMJ Open Diabetes Res Care. 2020;810.1136/bmjdrc-2019-000991

[189] Gibbons CH, Freeman R. Treatment-induced neuropathy of diabetes: an acute, iatrogenic complication of diabetes. Brain. 2015;138:43–52. 10.1093/brain/awu307

[190] Kluding PM, Pasnoor M, Singh R, et al. The effect of exercise on neuropathic symptoms, nerve function, and cutaneous innervation in people with diabetic peripheral neuropathy. J Diabetes Complications. 2012;26:424–9. 10.1016/j.jdiacomp.2012.05.007

[191] Look ARG. Effects of a long-term lifestyle modification programme on peripheral neuropathy in overweight or obese adults with type 2 diabetes: the Look AHEAD study. Diabetologia. 2017;60:980–8. 10.1007/s00125-017-4253-z

[192] Perreault L, Pan Q, Schroeder EB, et al. Regression From Prediabetes to Normal Glucose Regulation and Prevalence of Microvascular Disease in the Diabetes Prevention Program Outcomes Study (DPPOS). Diabetes Care. 2019;42:1809–15. 10.2337/dc19-0244

[193] Oesterle A, Laufs U, Liao JK. Pleiotropic Effects of Statins on the Cardiovascular System. Circ Res. 2017;120:229–43. 10.1161/CIRCRESAHA.116.308537

[194] Daliri M, Johnston TP, Sahebkar A. Statins and peripheral neuropathy in diabetic and non-diabetic cases: a systematic review. J Pharm Pharmacol. 2023;75:593–611. 10.1093/jpp/rgac104

[195] Kang EY, Chen TH, Garg SJ, et al. Association of Statin Therapy With Prevention of Vision-Threatening Diabetic Retinopathy. JAMA Ophthalmol. 2019;137:363–71. 10.1001/jamaophthalmol.2018.6399

[196] Kristensen FP, Christensen DH, Callaghan BC, et al. Statin Therapy and Risk of Polyneuropathy in Type 2 Diabetes: A Danish Cohort Study. Diabetes Care. 2020;43:2945–52. 10.2337/dc20-1004

[197] Svendsen TK, Norregaard Hansen P, Garcia Rodriguez LA, et al. Statins and polyneuropathy revisited: case-control study in Denmark, 1999-2013. Br J Clin Pharmacol. 2017;83:2087–95. 10.1111/bcp.13298

[198] Warendorf JK, Vrancken AFJE, van Eijk RPA, Visser NA, van den Berg LH, Notermans NC. Statins do not increase risk of polyneuropathy. Neurology. 201910.1212/wnl.0000000000007148

[199] Andreão F, Ribeiro F, Mancilha M, et al. The Impact of Statin Use on the Risk of Diabetic Polyneuropathy in Patients with Type 2 Diabetes and Hypercholesterolemia: A Multicenter Global Retrospective Cohort Study (P6-11.011). Neurology. 2025;104:5729. doi:10.1212/WNL.0000000000212556

[200] Elliott WJ. Effects of Manidipine and Delapril in Hypertensive Patients With Type 2 Diabetes Mellitus: The Delapril and Manidipine for Nephroprotection in Diabetes (DEMAND) Randomized Clinical Trial. Yearbook of Cardiology. 2012;2012:99–101. 10.1016/j.ycar.2012.02.011

[201] Bonhof GJ, Herder C, Strom A, Papanas N, Roden M, Ziegler D. Emerging Biomarkers, Tools, and Treatments for Diabetic Polyneuropathy. Endocr Rev. 2019;40:153–92. 10.1210/er.2018-00107

[202] El-Nahas MR, Elkannishy G, Abdelhafez H, Elkhamisy ET, El-Sehrawy AA. Oral Alpha Lipoic Acid Treatment for Symptomatic Diabetic Peripheral Neuropathy: A Randomized Double-Blinded Placebo-Controlled Study. Endocr Metab Immune Disord Drug Targets. 2020;20:1531–4. 10.2174/1871530320666200506081407

[203] Papanas N, Ziegler D. Efficacy of α-lipoic acid in diabetic neuropathy. Expert Opin Pharmacother. 2014;15:2721–31. 10.1517/14656566.2014.972935

[204] Ziegler D, Low PA, Litchy WJ, Boulton AJ, Vinik AI, Freeman R, Samigullin R, Tritschler H, Munzel U, Maus J, Schütte K, Dyck PJ. Efficacy and safety of antioxidant treatment with α-lipoic acid over 4 years in diabetic polyneuropathy: the NATHAN 1 trial. Diabetes Care. 2011 Sep;34(9):2054-60.

[205] Ziegler D, Keller J, Maier C, Pannek J. Diabetic Neuropathy. Exp Clin Endocrinol Diabetes. 2021;129:S70–S81. 10.1055/a-1284-6245

[206] Stracke H, Gaus W, Achenbach U, Federlin K, Bretzel RG. Benfotiamine in diabetic polyneuropathy (BENDIP): results of a randomised, double blind, placebo-controlled clinical study. Exp Clin Endocrinol Diabetes. 2008;116:600–5. 10.1055/s-2008-1065351

[207] Ziegler D, Papanas N, Schnell O, et al. Current concepts in the management of diabetic polyneuropathy. J Diabetes Investig. 2021;12:464–75. 10.1111/jdi.13401

[208] Ziegler D, Movsesyan L, Mankovsky B, Gurieva I, Abylaiuly Z, Strokov I. Treatment of symptomatic polyneuropathy with actovegin in type 2 diabetic patients. Diabetes Care. 2009;32:1479–84. 10.2337/dc09-0545

[209] Ziegler D, Edmundson S, Gurieva I, Mankovsky B, Papanas N, Strokov I. Predictors of response to treatment with actovegin for 6 months in patients with type 2 diabetes and symptomatic polyneuropathy. J Diabetes Complications. 2017;31:1181–7. 10.1016/j.jdiacomp.2017.03.012

[210] Fonseca VA, Lavery LA, Thethi TK, et al. Metanx in type 2 diabetes with peripheral neuropathy: a randomized trial. Am J Med. 2013;126:141–9. 10.1016/j.amjmed.2012.06.022

[211] Trippe BS, Barrentine LW, Curole MV, Tipa E. Nutritional management of patients with diabetic peripheral neuropathy with L-methylfolate-methylcobalamin-pyridoxal-5-phosphate: results of a real-world patient experience trial. Curr Med Res Opin. 2016;32:219–27. 10.1185/03007995.2015.1103215

[212] Didangelos T, Karlafti E, Kotzakioulafi E, et al. Vitamin B12 Supplementation in Diabetic Neuropathy: A 1-Year, Randomized, Double-Blind, Placebo-Controlled Trial. Nutrients. 2021;1310.3390/nu13020395

[213] Karakousis ND, Pyrgioti EE, Georgakopoulos PN, Papanas N. Vitamin B(12) and Diabetic Foot: A Mini-Review. Int J Low Extrem Wounds. 2025;24:299–302. 10.1177/15347346221143018

[214] Lv WS, Zhao WJ, Gong SL, et al. Serum 25-hydroxyvitamin D levels and peripheral neuropathy in patients with type 2 diabetes: a systematic review and meta-analysis. J Endocrinol Invest. 2015;38:513–8. 10.1007/s40618-014-0210-6

[215] Karonova T, Stepanova A, Bystrova A, Jude EB. High-Dose Vitamin D Supplementation Improves Microcirculation and Reduces Inflammation in Diabetic Neuropathy Patients. Nutrients. 2020;1210.3390/nu12092518

[216] Chen T, Xing X, Huang L, et al. Efficacy and safety of high-dose intramuscular vitamin D(2) injection in type 2 diabetes mellitus with distal symmetric polyneuropathy combined with vitamin D insufficiency: study protocol for a multicenter, randomized, double-blinded, and placebo-controlled trial. Front Endocrinol (Lausanne). 2023;14:1202917. 10.3389/fendo.2023.1202917

[217] Hor CP, Fung WY, Ang HA, et al. Efficacy of Oral Mixed Tocotrienols in Diabetic Peripheral Neuropathy: A Randomized Clinical Trial. JAMA Neurol. 2018;75:444–52. 10.1001/jamaneurol.2017.4609

[218] Apergi K, Papanas N. Dietary and Nutritional Supplementation for Painful Diabetic Neuropathy: A Narrative Review. Exp Clin Endocrinol Diabetes. 2023;131:646–55. 10.1055/a-2188-1745

[219] Papanas N. Diabetic Neuropathy Collection: Progress in Diagnosis and Screening. Diabetes Ther. 2020;11:761–4. 10.1007/s13300-020-00776-3

[220] Piaggio D, Castaldo R, Garibizzo G, Iadanza E, Pecchia L. A smartphone-based tool for screening diabetic neuropathies: A mHealth and 3D printing approach.

Biomedical Signal Processing and Control. 2024;89:105807. https://doi.org/10.1016/j.bspc.2023.105807

[221] Sami A, Javed A, Ozsahin DU, Ozsahin I, Muhammad K, Waheed Y. Genetics of diabetes and its complications: a comprehensive review. Diabetol Metab Syndr. 2025;17:185. 10.1186/s13098-025-01748-y

[222] Veluchamy A, Smith BH, Bennett DL. The Genomics of Diabetic Neuropathy. In: Tesfaye S, Gibbons CH, Malik RA, Veves A, editors. Diabetic Neuropathy: Advances in Pathophysiology and Clinical Management. Cham: Springer International Publishing; 2023. p. 239–51.

[223] Singleton JR, Smith AG, Russell J, Feldman EL. Polyneuropathy with Impaired Glucose Tolerance: Implications for Diagnosis and Therapy. Curr Treat Options Neurol. 2005;7:33–42. 10.1007/s11940-005-0004-4

[224] Stino AM, Smith AG. Peripheral neuropathy in prediabetes and the metabolic syndrome. J Diabetes Investig. 2017;8:646–55. 10.1111/jdi.12650

[225] Smith AG, Singleton JR. Impaired glucose tolerance and neuropathy. Neurologist. 2008;14:23–9. 10.1097/NRL.0b013e31815a3956

[226] Kirthi V, Perumbalath A, Brown E, et al. Prevalence of peripheral neuropathy in pre-diabetes: a systematic review. BMJ Open Diabetes Res Care. 2021;910.1136/bmjdrc-2020-002040

[227] Ziegler D, Herder C, Papanas N. Neuropathy in prediabetes. Diabetes Metab Res Rev. 2023;39:e3693. 10.1002/dmrr.3693

[228] Tesfaye S, Boulton AJ, Dyck PJ, et al. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. Diabetes Care. 2010;33:2285–93. 10.2337/dc10-1303

[229] Kurisu S, Sasaki H, Kishimoto S, et al. Clinical polyneuropathy does not increase with prediabetes or metabolic syndrome in the Japanese general population. J Diabetes Investig. 2019;10:1565–75. 10.1111/jdi.13058

[230] Faerch K, Borch-Johnsen K, Holst JJ, Vaag A. Pathophysiology and aetiology of impaired fasting glycaemia and impaired glucose tolerance: does it matter for prevention and treatment of type 2 diabetes? Diabetologia. 2009;52:1714–23. 10.1007/s00125-009-1443-3

[231] Bongaerts BW, Rathmann W, Heier M, et al. Older subjects with diabetes and prediabetes are frequently unaware of having distal sensorimotor polyneuropathy: the KORA F4 study. Diabetes Care. 2013;36:1141–6. 10.2337/dc12-0744

[232] Singleton JR, Smith AG, Russell JW, Feldman EL. Microvascular complications of impaired glucose tolerance. Diabetes. 2003;52:2867–73. 10.2337/diabetes.52.12.2867

[233] Eid SA, Noureldein M, Savelieff MG, Feldman EL. Mechanisms of Nerve Injury in Diabetes: Dyslipidemia, Bioenergetics, and Oxidative Damage. In: Tesfaye S, Gibbons CH, Malik RA, Veves A, editors. Diabetic Neuropathy: Advances in Pathophysiology and Clinical Management. Cham: Springer International Publishing; 2023. p. 279–306.

[234] Obrosova IG, Ilnytska O, Lyzogubov VV, et al. High-fat diet induced neuropathy of pre-diabetes and obesity: effects of "healthy" diet and aldose reductase inhibition. Diabetes. 2007;56:2598–608. 10.2337/db06-1176

[235] Eid SA, Feldman EL. Advances in diet-induced rodent models of metabolically acquired peripheral neuropathy. Dis Model Mech. 2021;1410.1242/dmm.049337

[236] Vincent AM, Callaghan BC, Smith AL, Feldman EL. Diabetic neuropathy: cellular mechanisms as therapeutic targets. Nat Rev Neurol. 2011;7:573–83. 10.1038/nrneurol.2011.137

[237] Russell JW, Sullivan KA, Windebank AJ, Herrmann DN, Feldman EL. Neurons undergo apoptosis in animal and cell culture models of diabetes. Neurobiol Dis. 1999;6:347–63. 10.1006/nbdi.1999.0254

[238] Vincent AM, Hayes JM, McLean LL, Vivekanandan-Giri A, Pennathur S, Feldman EL. Dyslipidemia-induced neuropathy in mice: the role of oxLDL/LOX-1. Diabetes. 2009;58:2376–85. 10.2337/db09-0047

[239] Ziegler D, Bönhof GJ, Strom A, et al. Progression and regression of nerve fibre pathology and dysfunction early in diabetes over 5 years. Brain. 2021;144:3251–63. 10.1093/brain/awab330

[240] Oates PJ. Aldose reductase, still a compelling target for diabetic neuropathy. Curr Drug Targets. 2008;9:14–36. 10.2174/138945008783431781

[241] Donaghue KC, Margan SH, Chan AK, et al. The association of aldose reductase gene (AKR1B1) polymorphisms with diabetic neuropathy in adolescents. Diabet Med. 2005;22:1315–20. 10.1111/j.1464-5491.2005.01631.x

[242] Wen L, Yang C, Song T, Guo N, Tang L, Tian H. Association Between VEGF Gene Polymorphism and Diabetic Neuropathy Susceptibility. J Coll Physicians Surg Pak. 2025;35:1028–35. 10.29271/jcpsp.2025.08.1028

[243] Witzel, II, Jelinek HF, Khalaf K, Lee S, Khandoker AH, Alsafar H. Identifying Common Genetic Risk Factors of Diabetic Neuropathies. Front Endocrinol (Lausanne). 2015;6:88. 10.3389/fendo.2015.00088

[244] Cameron NE, Eaton SE, Cotter MA, Tesfaye S. Vascular factors and metabolic interactions in the pathogenesis of diabetic neuropathy. Diabetologia. 2001;44:1973–88. 10.1007/s001250100001

[245] Malik RA. Pathology of human diabetic neuropathy. Handb Clin Neurol. 2014;126:249–59. 10.1016/b978-0-444-53480-4.00016-3

[246] Chen L, Veves A. Micro- and Macrovascular Disease in Diabetic Neuropathy. In: Tesfaye S, Gibbons CH, Malik RA, Veves A, editors. Diabetic Neuropathy: Advances in Pathophysiology and Clinical Management. Cham: Springer International Publishing; 2023. p. 351–61.

[247] Ziegler D, Tesfaye S, Spallone V, et al. Screening, diagnosis and management of diabetic sensorimotor polyneuropathy in clinical practice: International expert consensus recommendations. Diabetes Res Clin Pract. 2022;186:109063. 10.1016/j.diabres.2021.109063

[248] Hansen CS, Määttä LL, Andersen ST, Charles MH. The Epidemiology of Diabetic Neuropathy. In: Tesfaye S, Gibbons CH, Malik RA, Veves A, editors. Diabetic Neuropathy: Advances in Pathophysiology and Clinical Management. Cham: Springer International Publishing; 2023. p. 5–36.

[249] Diabetes* ADAPPCf. 12. Retinopathy, Neuropathy, and Foot Care: Standards of Care in Diabetes—2026. Diabetes Care. 2025;49:S261–S76. 10.2337/dc26-S012

[250] Selvarajah D, Kar D, Khunti K, et al. Diabetic peripheral neuropathy: advances in diagnosis and strategies for screening and early intervention. Lancet Diabetes Endocrinol. 2019;7:938–48. 10.1016/s2213-8587(19)30081-6

[251] Shabeeb D, Najafi M, Hasanzadeh G, Hadian MR, Musa AE, Shirazi A. Electrophysiological measurements of diabetic peripheral neuropathy: A systematic review. Diabetes Metab Syndr. 2018;12:591–600. 10.1016/j.dsx.2018.03.026

[252] England JD, Gronseth GS, Franklin G, et al. Distal symmetrical polyneuropathy: a definition for clinical research. A report of the American Academy of Neurology, the American Association of Electrodiagnostic Medicine, and the American Academy of Physical Medicine and Rehabilitation. Arch Phys Med Rehabil. 2005;86:167–74. 10.1016/j.apmr.2004.09.011

[253] Aso Y. Updates in diabetic neuropathy: A call for new diagnostic and treatment approaches. J Diabetes Investig. 2022;13:432–4. 10.1111/jdi.13711

[254] Lauria G, Lombardi R. Skin biopsy: a new tool for diagnosing peripheral neuropathy. Bmj. 2007;334:1159–62. 10.1136/bmj.39192.488125.BE

[255] Jensen TS, Karlsson P, Gylfadottir SS, et al. Painful and non-painful diabetic neuropathy, diagnostic challenges and implications for future management. Brain. 2021;144:1632–45. 10.1093/brain/awab079

[256] Callaghan BC, Kerber KA, Lisabeth LL, et al. Role of neurologists and diagnostic tests on the management of distal symmetric polyneuropathy. JAMA Neurol. 2014;71:1143–9. 10.1001/jamaneurol.2014.1279

[257] Rosenberg NR, Portegies P, de Visser M, Vermeulen M. Diagnostic investigation of patients with chronic polyneuropathy: evaluation of a clinical guideline. J Neurol Neurosurg Psychiatry. 2001;71:205–9. 10.1136/jnnp.71.2.205

[258] Castelli G, Desai KM, Cantone RE. Peripheral Neuropathy: Evaluation and Differential Diagnosis. Am Fam Physician. 2020;102:732–9.

[259] Samakidou G, Eleftheriadou I, Tentolouris A, Papanas N, Tentolouris N. Rare diabetic neuropathies: It is not only distal symmetrical polyneuropathy. Diabetes Res Clin Pract. 2021;177:108932. 10.1016/j.diabres.2021.108932

[260] Hsieh RY, Huang IC, Chen C, Sung JY. Effects of Oral Alpha-Lipoic Acid Treatment on Diabetic Polyneuropathy: A Meta-Analysis and Systematic Review. Nutrients. 2023;1510.3390/nu15163634

[261] Atmaca A, Ketenci A, Sahin I, et al. Expert opinion on screening, diagnosis and management of diabetic peripheral neuropathy: a multidisciplinary approach. Frontiers in Endocrinology. 2024;Volume 15 - 202410.3389/fendo.2024.1380929

[262] Filipowicz W. Traversing the RNA world. J Biol Chem. 2017;292:8122–35. 10.1074/jbc.X117.789065

[263] Rumora AE, Kim B, Feldman EL. A Role for Fatty Acids in Peripheral Neuropathy Associated with Type 2 Diabetes and Prediabetes. Antioxid Redox Signal. 2022;37:560–77. 10.1089/ars.2021.0155

[264] Periquet MI, Novak V, Collins MP, et al. Painful sensory neuropathy: prospective evaluation using skin biopsy. Neurology. 1999;53:1641–7. 10.1212/wnl.53.8.1641

[265] Hughes RA, Umapathi T, Gray IA, et al. A controlled investigation of the cause of chronic idiopathic axonal polyneuropathy. Brain. 2004;127:1723–30. 10.1093/brain/awh192

[266] Wiggin TD, Sullivan KA, Pop-Busui R, Amato A, Sima AAF, Feldman EL. Elevated Triglycerides Correlate With Progression of Diabetic Neuropathy. Diabetes. 2009;58:1634–40. 10.2337/db08-1771

[267] Tesfaye S, Stevens LK, Stephenson JM, et al. Prevalence of diabetic peripheral neuropathy and its relation to glycaemic control and potential risk factors: the EURODIAB IDDM Complications Study. Diabetologia. 1996;39:1377–84. 10.1007/s001250050586

[268] Bader S, Hasan S, Magliano DJ, et al. The association between smoking and diabetic neuropathy: a systematic review and meta-analysis. Syst Rev. 2025;14:249. 10.1186/s13643-025-02995-w

[269] Smith AG, Russell J, Feldman EL, et al. Lifestyle intervention for pre-diabetic neuropathy. Diabetes Care. 2006;29:1294–9. 10.2337/dc06-0224

[270] Singleton JR, Foster-Palmer S, Marcus RL. Exercise as Treatment for Neuropathy in the Setting of Diabetes and Prediabetic Metabolic Syndrome: A Review of Animal

Models and Human Trials. Curr Diabetes Rev. 2022;18:e230921196752. 10.2174/1573399817666210923125832

[271] Singleton JR, Marcus RL, Jackson JE, M KL, Graham TE, Smith AG. Exercise increases cutaneous nerve density in diabetic patients without neuropathy. Ann Clin Transl Neurol. 2014;1:844–9. 10.1002/acn3.125

[272] Long-term effects of lifestyle intervention or metformin on diabetes development and microvascular complications over 15-year follow-up: the Diabetes Prevention Program Outcomes Study. Lancet Diabetes Endocrinol. 2015;3:866–75. 10.1016/s2213-8587(15)00291-0

[273] Gong Q, Gregg EW, Wang J, et al. Long-term effects of a randomised trial of a 6-year lifestyle intervention in impaired glucose tolerance on diabetes-related microvascular complications: the China Da Qing Diabetes Prevention Outcome Study. Diabetologia. 2011;54:300–7. 10.1007/s00125-010-1948-9

[274] Chen X, Zhang J, Zhou Z. The effects of metabolic surgery on microvascular complications in obese patients with type 2 diabetes: a meta-analysis. Surg Obes Relat Dis. 2021;17:434–43. 10.1016/j.soard.2020.09.013

[275] Azmi S, Ferdousi M, Liu Y, et al. Bariatric surgery leads to an improvement in small nerve fibre damage in subjects with obesity. Int J Obes (Lond). 2021;45:631–8. 10.1038/s41366-020-00727-9

[276] Iqbal Z, Kalteniece A, Ferdousi M, et al. Corneal Keratocyte Density and Corneal Nerves Are Reduced in Patients With Severe Obesity and Improve After Bariatric Surgery. Invest Ophthalmol Vis Sci. 2021;62:20. 10.1167/iovs.62.1.20

[277] Herder C, Roden M, Ziegler D. Novel Insights into Sensorimotor and Cardiovascular Autonomic Neuropathy from Recent-Onset Diabetes and Population-Based Cohorts. Trends Endocrinol Metab. 2019;30:286–98. 10.1016/j.tem.2019.02.007

[278] González-Duarte A, Lem M, Díaz-Díaz E, Castillo C, Cárdenas-Soto K. The Efficacy of Pregabalin in the Treatment of Prediabetic Neuropathic Pain. Clin J Pain. 2016;32:927–32. 10.1097/ajp.0000000000000339

[279] Peng X, Haldar S, Deshpande S, Irani K, Kass DA. Wall stiffness suppresses Akt/eNOS and cytoprotection in pulse-perfused endothelium. Hypertension. 2003;41:378–81. 10.1161/01.hyp.0000049624.99844.3d

[280] Duprez DA. Arterial stiffness and endothelial function: key players in vascular health. Hypertension. 2010;55:612–3. 10.1161/hypertensionaha.109.144725

[281] Zieman SJ, Melenovsky V, Kass DA. Mechanisms, pathophysiology, and therapy of arterial stiffness. Arterioscler Thromb Vasc Biol. 2005;25:932–43. 10.1161/01.Atv.0000160548.78317.29

[282] Cavalcante JL, Lima JA, Redheuil A, Al-Mallah MH. Aortic stiffness: current understanding and future directions. J Am Coll Cardiol. 2011;57:1511–22. 10.1016/j.jacc.2010.12.017

[283] Endemann DH, Schiffrin EL. Endothelial dysfunction. J Am Soc Nephrol. 2004;15:1983–92. 10.1097/01.ASN.0000132474.50966.DA

[284] Paulus WJ, Tschope C. A novel paradigm for heart failure with preserved ejection fraction: comorbidities drive myocardial dysfunction and remodeling through coronary microvascular endothelial inflammation. J Am Coll Cardiol. 2013;62:263–71. 10.1016/j.jacc.2013.02.092

[285] O'Rourke MF, Safar ME, Dzau V. The Cardiovascular Continuum extended: aging effects on the aorta and microvasculature. Vasc Med. 2010;15:461–8. 10.1177/1358863X10382946

[286] Cuijpers I, Simmonds SJ, van Bilsen M, et al. Microvascular and lymphatic dysfunction in HFpEF and its associated comorbidities. Basic Res Cardiol. 2020;115:39. 10.1007/s00395-020-0798-y

[287] Jeggle P, Callies C, Tarjus A, et al. Epithelial sodium channel stiffens the vascular endothelium in vitro and in Liddle mice. Hypertension. 2013;61:1053–9. 10.1161/HYPERTENSIONAHA.111.199455

[288] Muniyappa R, Chen H, Montagnani M, Sherman A, Quon MJ. Endothelial dysfunction due to selective insulin resistance in vascular endothelium: insights from mechanistic modeling. Am J Physiol Endocrinol Metab. 2020;319:E629–E46. 10.1152/ajpendo.00247.2020

[289] Lamprou S, Koletsos N, Mintziori G, et al. Microvascular and Endothelial Dysfunction in Prediabetes. Life (Basel). 2023;1310.3390/life13030644

[290] Liang X, Li D, Wang Z, et al. Aortic Stiffness Measured by Carotid Femoral-Pulse Wave Velocity at Different Stages of Normal Glucose, Prediabetes, and Diabetes Mellitus: A Systematic Review and Meta-Analysis. Rev Cardiovasc Med. 2024;25:339. 10.31083/j.rcm2509339

[291] Gagliardino JJ, Salazar MR, Espeche WG, et al. Arterial Stiffness: Its Relation with Prediabetes and Metabolic Syndrome and Possible Pathogenesis. J Clin Med. 2021;1010.3390/jcm10153251

[292] McNulty M, Mahmud A, Feely J. Advanced glycation end-products and arterial stiffness in hypertension. Am J Hypertens. 2007;20:242–7. 10.1016/j.amjhyper.2006.08.009

[293] Katakami N. Mechanism of Development of Atherosclerosis and Cardiovascular Disease in Diabetes Mellitus. J Atheroscler Thromb. 2018;25:27–39. 10.5551/jat.RV17014

[294] Mancia G, Kreutz R, Brunstrom M, et al. 2023 ESH Guidelines for the management of arterial hypertension The Task Force for the management of arterial hypertension of the European Society of Hypertension: Endorsed by the International Society of Hypertension (ISH) and the European Renal Association (ERA). J Hypertens. 2023;41:1874–2071. 10.1097/HJH.0000000000003480

[295] Chirinos JA, Segers P, Hughes T, Townsend R. Large-Artery Stiffness in Health and Disease: JACC State-of-the-Art Review. J Am Coll Cardiol. 2019;74:1237–63. 10.1016/j.jacc.2019.07.012

[296] Milan A, Zocaro G, Leone D, et al. Current assessment of pulse wave velocity: comprehensive review of validation studies. J Hypertens. 2019;37:1547–57. 10.1097/HJH.0000000000002081

[297] Ghasemzadeh N, Zafari AM. A brief journey into the history of the arterial pulse. Cardiol Res Pract. 2011;2011:164832. 10.4061/2011/164832

[298] Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. J Am Coll Cardiol. 2010;55:1318–27. 10.1016/j.jacc.2009.10.061

[299] McEvoy JW, McCarthy CP, Bruno RM, et al. 2024 ESC Guidelines for the management of elevated blood pressure and hypertension. Eur Heart J. 2024;45:3912–4018. 10.1093/eurheartj/ehae178

[300] Laurent S, Cockcroft J, Van Bortel L, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J. 2006;27:2588–605. 10.1093/eurheartj/ehl254

[301] Weber T, Wassertheurer S, Hametner B, Parragh S, Eber B. Noninvasive methods to assess pulse wave velocity: comparison with the invasive gold standard and

relationship with organ damage. J Hypertens. 2015;33:1023–31. 10.1097/HJH.0000000000000518

[302] Fiori G, Fuiano F, Scorza A, Conforto S, Sciuto SA. Non-Invasive Methods for PWV Measurement in Blood Vessel Stiffness Assessment. IEEE Rev Biomed Eng. 2022;15:169–83. 10.1109/RBME.2021.3092208

[303] Firmino SM, Goulart CDL, Gregorio JP, et al. Discriminative value of pulse wave velocity for arterial stiffness and cardiac injury in prediabetic patients. J Vasc Bras. 2023;22:e20230076. 10.1590/1677-5449.202300762

[304] Climie RE, van Sloten TT, Bruno RM, et al. Macrovasculature and Microvasculature at the Crossroads Between Type 2 Diabetes Mellitus and Hypertension. Hypertension. 2019;73:1138–49. 10.1161/HYPERTENSIONAHA.118.11769

[305] Boutouyrie P, Chowienczyk P, Humphrey JD, Mitchell GF. Arterial Stiffness and Cardiovascular Risk in Hypertension. Circ Res. 2021;128:864–86. 10.1161/CIRCRESAHA.121.318061

[306] Cardoso CR, Moran CB, Marinho FS, Ferreira MT, Salles GF. Increased aortic stiffness predicts future development and progression of peripheral neuropathy in patients with type 2 diabetes: the Rio de Janeiro Type 2 Diabetes Cohort Study. Diabetologia. 2015;58:2161–8. 10.1007/s00125-015-3658-9

[307] Chen Q, Chiheb S, Fysekidis M, et al. Arterial stiffness is elevated in normotensive type 2 diabetic patients with peripheral neuropathy. Nutr Metab Cardiovasc Dis. 2015;25:1041–9. 10.1016/j.numecd.2015.08.001

[308] Yokoyama H, Yokota Y, Tada J, Kanno S. Diabetic neuropathy is closely associated with arterial stiffening and thickness in Type 2 diabetes. Diabet Med. 2007;24:1329–35. 10.1111/j.1464-5491.2007.02278.x

[309] Aso K, Miyata M, Kubo T, et al. Brachial-ankle pulse wave velocity is useful for evaluation of complications in type 2 diabetic patients. Hypertens Res. 2003;26:807–13. 10.1291/hypres.26.807

[310] Ha BK, Kim BG, Kim DH, et al. Relationships between Brachial-Ankle Pulse Wave Velocity and Peripheral Neuropathy in Type 2 Diabetes. Diabetes Metab J. 2012;36:443–51. 10.4093/dmj.2012.36.6.443

[311] Szczyrba S, Kozera GM, Neubauer-Geryk J, Wolnik B, Nyka WM, Bieniaszewski L. Diabetic symmetric polyneuropathy is associated with increased aortal stiffening but not cerebral angiopathy in type 1 diabetes. J Diabetes Complications. 2015;29:73–6. 10.1016/j.jdiacomp.2014.10.002

[312] Schlesinger S, Neuenschwander M, Barbaresko J, et al. Prediabetes and risk of mortality, diabetes-related complications and comorbidities: umbrella review of meta-analyses of prospective studies. Diabetologia. 2022;65:275–85. 10.1007/s00125-021-05592-3

[313] Anastasiou G, Liberopoulos E, Tentolouris N, Papanas N. Diabetic Sensorimotor Polyneuropathy: An Overview on Epidemiology, Risk Factors, Classification, Diagnosis, and Treatment. Int J Low Extrem Wounds. 2024:15347346241240513. 10.1177/15347346241240513

[314] Anastasiou G, Papanas N, Barkas F, et al. Distal symmetrical polyneuropathy in prediabetes is associated with abdominal obesity and insulin resistance. Diabetes Res Clin Pract. 2026;233:113140. 10.1016/j.diabres.2026.113140

[315] Callaghan BC, Reynolds EL, Banerjee M, et al. The Prevalence and Determinants of Cognitive Deficits and Traditional Diabetic Complications in the Severely Obese. Diabetes Care. 2020;43:683–90. 10.2337/dc19-1642

[316] Association AD. Standards of Medical Care in Diabetes—2019 Abridged for Primary Care Providers. Clinical Diabetes. 2019;37:11–34. 10.2337/cd18-0105

[317] Grundy SM, Cleeman JI, Daniels SR, et al. Diagnosis and Management of the Metabolic Syndrome. Circulation. 2005;112:e285–e90. doi:10.1161/CIRCULATIONAHA.105.169405

[318] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28:412–9. 10.1007/bf00280883

[319] Laurent S, Cockcroft J, Van Bortel L, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J. 2006;27:2588–605. 10.1093/eurheartj/ehl254

[320] Panagiotakos DB, Pitsavos C, Stefanadis C. Dietary patterns: A Mediterranean diet score and its relation to clinical and biological markers of cardiovascular disease risk. Nutrition, Metabolism and Cardiovascular Diseases. 2006;16:559–68. https://doi.org/10.1016/j.numecd.2005.08.006

[321] Truong QC, Cervin M, Choo CC, et al. Examining the validity of the Mini-Mental State Examination (MMSE) and its domains using network analysis. Psychogeriatrics. 2024;24:259–71. 10.1111/psyg.13069

[322] Butlin M, Qasem A. Large Artery Stiffness Assessment Using SphygmoCor Technology. Pulse (Basel). 2017;4:180–92. 10.1159/000452448

[323] Dyck PJ. Detection, characterization, and staging of polyneuropathy: assessed in diabetics. Muscle Nerve. 1988;11:21–32. 10.1002/mus.880110106

[324] Meijer JW, Smit AJ, Sonderen EV, Groothoff JW, Eisma WH, Links TP. Symptom scoring systems to diagnose distal polyneuropathy in diabetes: the Diabetic Neuropathy Symptom score. Diabet Med. 2002;19:962–5. 10.1046/j.1464-5491.2002.00819.x

[325] Maffei L, Premrou V, Roldan P, et al. Vibration perception threshold in the screening of sensorimotor distal symmetric polyneuropathy: the need of more accurate age-specific reference values. J Diabetes Sci Technol. 2014;8:621–2. 10.1177/1932296814527818

[326] Wiles PG, Pearce SM, Rice PJ, Mitchell JM. Vibration perception threshold: influence of age, height, sex, and smoking, and calculation of accurate centile values. Diabet Med. 1991;8:157–61. 10.1111/j.1464-5491.1991.tb01563.x

[327] Bloom S, Till S, Sönksen P, Smith S. Use of a biothesiometer to measure individual vibration thresholds and their variation in 519 non-diabetic subjects. Br Med J (Clin Res Ed). 1984;288:1793–5. 10.1136/bmj.288.6433.1793

[328] Tan LS. The clinical use of the 10g monofilament and its limitations: a review. Diabetes Res Clin Pract. 2010;90:1–7. 10.1016/j.diabres.2010.06.021

[329] Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. Behav Res Methods. 2009;41:1149–60. 10.3758/BRM.41.4.1149

[330] Ziegler D, Rathmann W, Dickhaus T, Meisinger C, Mielck A. Neuropathic pain in diabetes, prediabetes and normal glucose tolerance: the MONICA/KORA Augsburg Surveys S2 and S3. Pain Med. 2009;10:393–400. 10.1111/j.1526-4637.2008.00555.x

[331] Ziegler D, Rathmann W, Meisinger C, Dickhaus T, Mielck A. Prevalence and risk factors of neuropathic pain in survivors of myocardial infarction with pre-diabetes and diabetes. The KORA Myocardial Infarction Registry. Eur J Pain. 2009;13:582–7. 10.1016/j.ejpain.2008.07.007

[332] Herder C, Kannenberg JM, Huth C, et al. Proinflammatory Cytokines Predict the Incidence and Progression of Distal Sensorimotor Polyneuropathy: KORA F4/FF4 Study. Diabetes Care. 2017;40:569–76. 10.2337/dc16-2259

[333] The Lancet Diabetes E. Prediabetes: much more than just a risk factor. Lancet Diabetes Endocrinol. 2025;13:165. 10.1016/S2213-8587(25)00034-8

[334] Savelieff MG, Elafros MA, Viswanathan V, Jensen TS, Bennett DL, Feldman EL. The global and regional burden of diabetic peripheral neuropathy. Nat Rev Neurol. 2025;21:17–31. 10.1038/s41582-024-01041-y

[335] Han L, Ji L, Chang J, et al. Peripheral neuropathy is associated with insulin resistance independent of metabolic syndrome. Diabetol Metab Syndr. 2015;7:14. 10.1186/s13098-015-0010-y

[336] Callaghan BC, Xia R, Banerjee M, et al. Metabolic Syndrome Components Are Associated With Symptomatic Polyneuropathy Independent of Glycemic Status. Diabetes Care. 2016;39:801–7. 10.2337/dc16-0081

[337] Zheng M, Wang P. Role of insulin receptor substance-1 modulating PI3K/Akt insulin signaling pathway in Alzheimer's disease. 3 Biotech. 2021;11:179. 10.1007/s13205-021-02738-3

[338] Feldman EL, Callaghan BC, Pop-Busui R, et al. Diabetic neuropathy. Nat Rev Dis Primers. 2019;5:41. 10.1038/s41572-019-0092-1

[339] Chirinos JA, Segers P, Hughes T, Townsend R. Large-Artery Stiffness in Health and Disease: JACC State-of-the-Art Review. Journal of the American College of Cardiology. 2019;74:1237–63. https://doi.org/10.1016/j.jacc.2019.07.012

[340] Henry RM, Kostense PJ, Spijkerman AM, et al. Arterial stiffness increases with deteriorating glucose tolerance status: the Hoorn Study. Circulation. 2003;107:2089–95. 10.1161/01.Cir.0000065222.34933.Fc

[341] Yeboah K, Agyekum JA, Owusu Mensah RNA, et al. Arterial Stiffness Is Associated with Peripheral Sensory Neuropathy in Diabetes Patients in Ghana. J Diabetes Res. 2018;2018:2320737. 10.1155/2018/2320737

[342] Wang L, Zhi F, Gao B, et al. Association between lipid profiles and arterial stiffness: A secondary analysis based on a cross-sectional study. J Int Med Res. 2020;48:300060520938188. 10.1177/0300060520938188

[343] Doonan RJ, Hausvater A, Scallan C, Mikhailidis DP, Pilote L, Daskalopoulou SS. The effect of smoking on arterial stiffness. Hypertens Res. 2010;33:398–410. 10.1038/hr.2010.25

[344] Tentolouris A, Eleftheriadou I, Grigoropoulou P, et al. The association between pulse wave velocity and peripheral neuropathy in patients with type 2 diabetes mellitus. J Diabetes Complications. 2017;31:1624–9. 10.1016/j.jdiacomp.2017.07.010

[345] Beros A, Sluyter J, Scragg RKR. Association of arterial stiffness and neuropathy in diabetes: a systematic review and meta-analysis. BMJ Open Diabetes Res Care. 2023;1110.1136/bmjdrc-2022-003140

[346] Lyle AN, Raaz U. Killing Me Unsoftly: Causes and Mechanisms of Arterial Stiffness. Arterioscler Thromb Vasc Biol. 2017;37:e1–e11. 10.1161/atvbaha.116.308563

[347] Lee J, Yun JS, Ko SH. Advanced Glycation End Products and Their Effect on Vascular Complications in Type 2 Diabetes Mellitus. Nutrients. 2022;1410.3390/nu14153086

[348] Tian X, Zuo Y, Chen S, et al. Hypertension, Arterial Stiffness, and Diabetes: a Prospective Cohort Study. Hypertension. 2022;79:1487–96. 10.1161/hypertensionaha.122.19256

[349] Papachristou S, Pafili K, Papanas N. Skin AGEs and diabetic neuropathy. BMC Endocr Disord. 2021;21:28. 10.1186/s12902-021-00697-7

[350] Callaghan BC, Xia R, Reynolds E, et al. Association Between Metabolic Syndrome Components and Polyneuropathy in an Obese Population. JAMA Neurol. 2016;73:1468–76. 10.1001/jamaneurol.2016.3745

[351] Callaghan BC, Reynolds E, Banerjee M, Chant E, Villegas-Umana E, Feldman EL. Central Obesity is Associated With Neuropathy in the Severely Obese. Mayo Clin Proc. 2020;95:1342–53. 10.1016/j.mayocp.2020.03.025

[352] Perkins BA, Orszag A, Ngo M, Ng E, New P, Bril V. Prediction of incident diabetic neuropathy using the monofilament examination: a 4-year prospective study. Diabetes Care. 2010;33:1549–54. 10.2337/dc09-1835