Risk Factors for Incident Diabetic Polyneuropathy in a Cohort With Screen-Detected Type 2 Diabetes Followed for 13 Years: ADDITION-Denmark

Signe T. Andersen,[1] Daniel R. Witte,[1,2] Else-Marie Dalsgaard,[1] Henning Andersen,[3] Peter Nawroth,[4,5,6,7] Thomas Fleming,[4,5] Troels M. Jensen,[8] Nanna B. Finnerup,[9] Troels S. Jensen,[9] Torsten Lauritzen,[1] Eva L. Feldman,[10] Brian C. Callaghan,[10] and Morten Charles[1]

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OBJECTIVE

To study incident diabetic polyneuropathy (DPN) prospectively during the first 13 years after a screening-based diagnosis of type 2 diabetes and determine the associated risk factors for the development of DPN.

RESEARCH DESIGN AND METHODS

We assessed DPN longitudinally in the Danish arm of the Anglo-Danish-Dutch study of Intensive Treatment of Diabetes in Primary Care (ADDITION) using the Michigan Neuropathy Screening Instrument questionnaire (MNSIQ), defining DPN with scores ≥4. Risk factors present at the diabetes diagnosis associated with the risk of incident DPN were estimated using Cox proportional hazard models adjusted for trial randomization group, sex, and age.

RESULTS

Of the total cohort of 1,533 people, 1,445 completed the MNSIQ at baseline and 189 (13.1%) had DPN at baseline. The remaining 1,256 without DPN entered this study (median age 60.8 years [interquartile range 55.6; 65.6], 59% of whom were men). The cumulative incidence of DPN was 10% during 13 years of diabetes. Age (hazard ratio [HR] 1.03 [95% CI 1.00; 1.07]) (unit = 1 year), weight (HR 1.09 [95% CI 1.03; 1.16]) (unit = 5 kg), waist circumference (HR 1.14 [95% CI 1.05; 1.24]) (unit = 5 cm), BMI (HR 1.14 [95% CI 1.06; 1.23]) (unit = 2 kg/m^2), log$_2$ methylglyoxal (HR 1.45 [95% CI 1.12; 1.89]) (unit = doubling), HDL cholesterol (HR 0.82 [95% CI 0.69; 0.99]) (unit = 0.25 mmol/L), and LDL cholesterol (HR 0.92 [95% CI 0.86; 0.98]) (unit = 0.25 mmol/L) at baseline were significantly associated with the risk of incident DPN.

CONCLUSIONS

This study provides further epidemiological evidence for obesity as a risk factor for DPN. Moreover, low HDL cholesterol levels and higher levels of methylglyoxal, a marker of dicarbonyl stress, are identified as risk factors for the development of DPN.

The global burden of type 2 diabetes is increasing, and consequently the prevalence of the complications resulting from type 2 diabetes is likely to rise (1). Of these, diabetic polyneuropathy (DPN) is the most common complication (2). DPN increases the risk of chronic pain, falls, foot ulcers, and amputations; it also negatively affects the quality of life (3). Previous epidemiological studies have found that DPN occurs in 30–50% of

[1]Research Unit for General Practice and Section for General Medical Practice, Department of Public Health, Aarhus University, Aarhus, Denmark
[2]Danish Diabetes Academy, Odense, Denmark
[3]Department of Neurology, Aarhus University Hospital, Aarhus, Denmark
[4]Department of Medicine I and Clinical Chemistry, Heidelberg University Hospital, Heidelberg, Germany
[5]German Center for Diabetes Research, Neuherberg, Germany
[6]Institute for Diabetes and Cancer, Helmholtz Center Munich, Munich, Germany
[7]Joint Heidelberg–Institute for Diabetes and Cancer Translational Diabetes Program, Neuherberg, Germany
[8]Steno Diabetes Center Copenhagen, Gentofte, Denmark
[9]Danish Pain Research Center, Department of Clinical Medicine, Aarhus University, Aarhus, Denmark
[10]Department of Neurology, University of Michigan, Ann Arbor, MI

Corresponding author: Signe T. Andersen, sta@ph.au.dk.

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patients with diabetes over the course of their lives (1,4,5). However, there has been less research examining the incidence of DPN. In patients with newly and clinically diagnosed type 2 diabetes, what little data exist suggest an annual incidence of ~2% per year (6). Given the global burden of diabetes and the resultant burden of DPN, there is a critical need to identify risk factors for DPN.

While hyperglycemia is the most well-documented risk factor for DPN in patients with type 1 diabetes, there is accumulating evidence supporting other risk factors in type 2 diabetes (6–9). Indeed, in type 2 diabetes, hyperglycemia is likely not the only DPN risk factor given the observed heterogeneity in DPN status at a given level of glycemic control. Moreover, interventions enhancing glucose control in patients with type 2 diabetes have had limited efficacy regarding the development of DPN (6). Other metabolic syndrome components such as obesity, hypertension, low HDL cholesterol, and hypertriglyceridemia are among the potential additional risk factors for the development of DPN (6,8,10). Cross-sectional studies in the U.S., China, and the Netherlands have supported the idea that obesity and other components of metabolic syndrome are potential risk factors for DPN (11–14).

In addition, other potential risk factors are linked to DPN as well: age, cigarette smoking, and alcohol consumption are all associated with DPN (7,9). Methylglyoxal, a highly reactive glucose-derived metabolite, is elevated in diabetes and is associated with insulin dysfunction, vascular dysfunction, and diabetes complications including DPN and neuropathic pain in clinical studies (15–17).

Previous studies examining potential risk factors have mainly been cross-sectional; longitudinal studies are a key tool to link these factors to the development of DPN. In the current study, we aimed to determine risk factors for the development of incident DPN present at the diagnosis of screen-detected type 2 diabetes, with a particular focus placed on components of metabolic syndrome and methylglyoxal. Using the Michigan Neuropathy Screening Instrument questionnaire (MNSIQ), we followed participants from the diagnosis of type 2 diabetes, found by screening longitudinally at multiple follow-up visits over the course of 13 years, to identify incident DPN and associated potential risk factors for the development of DPN.

RESEARCH DESIGN AND METHODS

Study Subjects

The current study is an observational, prospective cohort analysis of data from the Danish arm of the Anglo-Danish-Dutch study of Intensive Treatment in People with Screen-Detected Diabetes in Primary Care (ADDITION) (18) with study inclusion from 2001 to 2006. ADDITION and its outcome have previously been described in detail (18–20). ADDITION-Denmark enrolled patients with previously undiagnosed diabetes (aged 40–69 years) via a stepwise screening program in primary care starting with a self-administered risk score questionnaire. Exclusion criteria were as follows: previously diagnosed diabetes, pregnancy, lactation, being housebound, life expectancy of less than a year, or inability to give informed consent. In the Danish arm of the study, 1,533 participants at 190 participating general practices were enrolled.

General practices were randomized to deliver either the routine care for diabetes or an intensive multifactorial target-driven care until the trial was concluded in 2009 and subsequently follow the current guidelines for diabetes care.

Since the closure of ADDITION, participants have been followed observationally via questionnaires, registers, and a clinical follow-up examination carried out between 2015 and 2016—13 years after the trial baseline.

ADDITION Baseline Evaluations

A physical examination of each participant was performed by trained study examiners according to standardized study protocols to assess anthropometrics, blood pressure, and metabolic metrics from blood samples as previously described (18). Serum levels of methylglyoxal were measured via derivatization with 1,2-diamino-4,5-dimethoxybenzene and high-performance liquid chromatography of the quinoxaline adduct by fluorescence detection at the University of Heidelberg (21). From self-completed questionnaires, records of smoking status (current smoking, former smoking, or nonsmoker) and alcohol consumption (units of alcohol per week) were also obtained. Alcohol consumption was dichotomized into two categories reflecting alcohol consumption above or below the recommended weekly intake according to the Danish national health care authorities (<7 units alcohol per week for women and <14 units alcohol per week for men) (22). Records of prescribed medications (statins, antihypertensives, and aspirin) were provided by participants' general practitioners. Records of nonfatal cardiovascular disease (CVD) requiring hospitalization during the period of 10 years prior to inclusion in ADDITION (nonfatal myocardial infarction [53 participants] and nonfatal stroke [40 participants]) were obtained from national registers (ICD-8 codes 410–410.9, 430–434.9, and 437–437.9 and ICD-10 codes DI21–DI23 and DI60–DI64). CVD outcomes were dichotomized into a covariate reflecting events of either myocardial infarction or stroke.

DPN Assessments by the MNSIQ

The MNSIQ was developed as a screening tool to assess DPN in diabetes (23). It consists of a 15-item self-administered questionnaire, and it has been validated for the diagnosis of DPN (score ≥4) (24). In the current study, the MNSIQ was completed by participants at baseline and at the 6- and 13-year clinical examination; it was also mailed to participants at 12 years (Fig. 1).

Upon their first abnormal MNSIQ score (≥4), participants were considered positive for DPN; DPN was treated as a binary outcome (participants were either positive or negative for DPN). Participants with abnormal MNSIQ but subsequently normal MNSIQ were still considered to have incident DPN, as DPN is rarely reversible and symptoms assessed by the MNSIQ may fluctuate. Participants who did not complete the MNSIQ at baseline and participants who had DPN at baseline based on the MNSIQ were excluded from the study. The date of DPN onset was defined as the midpoint between the first positive MNSIQ assessment and the previous negative MNSIQ assessment. Participants were censored in the Cox regression model at the date of DPN onset as defined above. Participants lost to follow-up (emigration, withdrawal from study, or death) were censored in the Cox regression model the day after their previously completed MNSIQ. All remaining participants were censored from the Cox regression on the day after their last MNSIQ. We truncated the cumulative incidence estimate at 13 years of follow-up owing to a very

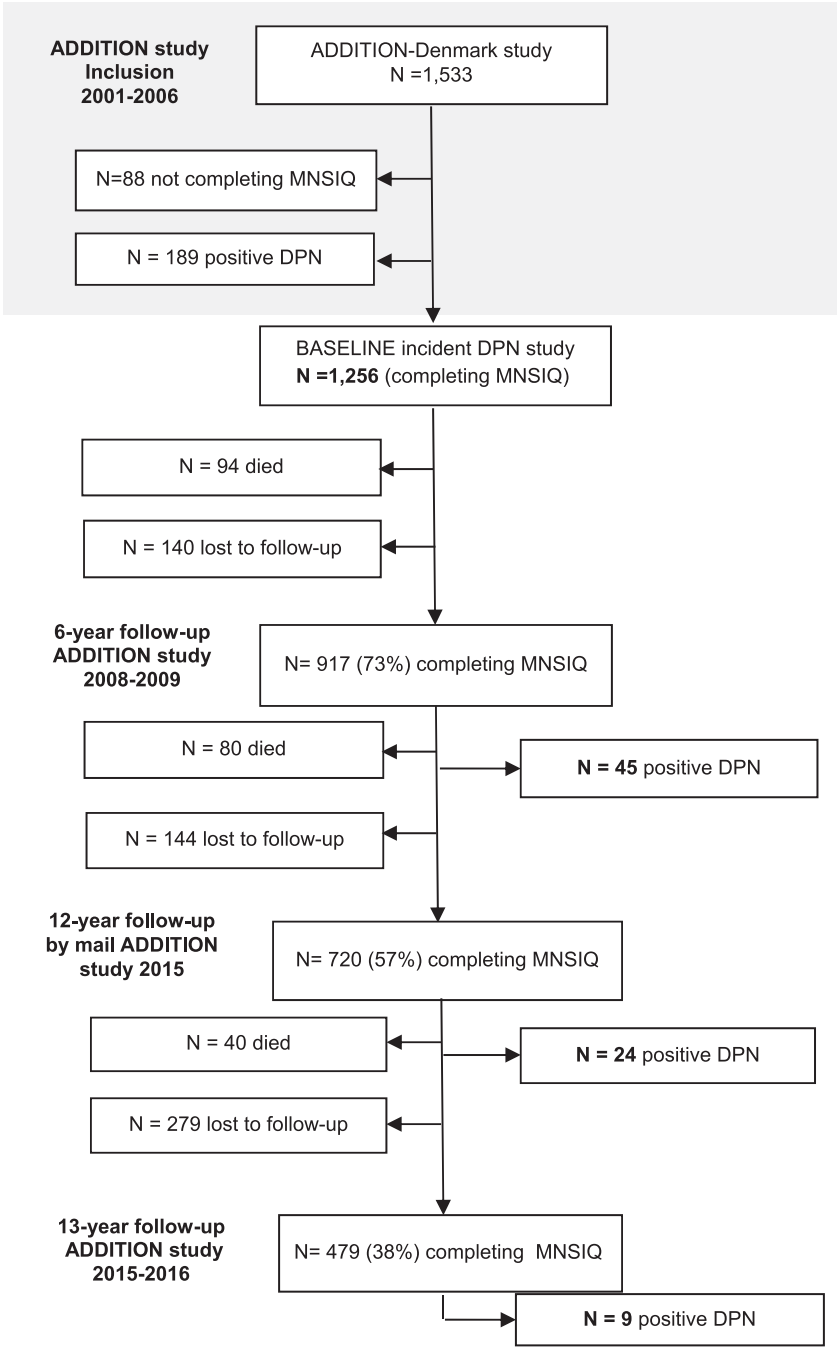

Figure 1—The flow of participants completing the MNSIQ: ADDITION-Denmark. The baseline cohort in the study of incident DPN comprises the 1,256 participants negative for DPN and completing the MNSIQ at inclusion in the ADDITION study. The numbers and proportions of participants from the baseline cohort completing subsequent MNSIQs are outlined. Positive DPN indicates a score ≥4 on the MNSIQ.

limited number of participants followed for a longer period.

Ethics

The study was approved by the Committee on Health Research Ethics in the Central Denmark Region (approval nos. 20000183 and 1-10-72-63-15) and by the Danish Data Protection Agency (approval no. 2005–57–0002, ID185). The study was conducted in accordance with the principles of the 1996 Declaration of Helsinki, and all study participants gave written informed consent.

Statistical Analysis

Characteristics of participants are reported by incident DPN status during the total follow-up time of 13 years and by censoring status (lost to follow-up) in the Cox regression models before the second MNSIQ assessment (Tables 1 and 2). Data are represented as the median and interquartile range (IQR) for continuous variables and as frequencies and proportions for categorical variables. Covariates were compared using Kruskal-Wallis and χ^2 tests as appropriate.

Cox proportional hazards models were used to estimate the risk of time-to-incident DPN associated with each potential risk factor for DPN as measured at baseline; the time since diabetes diagnosis was used as the underlying timescale. Risk factors included sex, age, HbA_{1c}, systolic blood pressure (SBP), diastolic blood pressure (DBP), height, weight, waist circumference, BMI, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, methylglyoxal, smoking status, and alcohol consumption. All models were adjusted for trial randomization group, sex, and age. The assumption of proportionality was assessed via inspection of a log-log plot of survival time by analysis time; no violation of this assumption was detected.

Effect modification by sex was tested using a Wald test. We also tested for modification by the other covariates under study. The linearity of the associations with continuous risk factors was confirmed by testing the statistical significance of quadratic terms. The risk of incident DPN was expressed as clinically relevant differences in continuous risk factors as well as the doubling of the methylglyoxal levels. The risk of incident DPN for the dichotomous risk factors of alcohol and smoking was expressed for participants with alcohol consumption above the recommended weekly intake and for participants being current or former smokers compared with participants with alcohol consumption not exceeding the recommended weekly intake and with nonsmokers, respectively. Sensitivity analyses were performed using multivariate logistic regression models to estimate the associations of risk factors with incident DPN (as estimated by the Cox regression models). This was done for the various potential risk factors with a binary answer of incident DPN during the total follow-up of 13 years as the outcome and with adjustment for trial randomization group, sex, and age. These analyses allowed us to study risk factors for incident DPN without accounting for time-to-incident DPN for the 1,022 participants completing the MNSIQ at a minimum of two time points.

Table 1—Baseline characteristics of participants by incident DPN status during 13 years of follow-up: ADDITION-Denmark

Characteristics	Participants without incident DPN	Participants with incident DPN	*N*
Number (%) of participants	1,178 (81.5)	78 (5.4)	1,256
Intensive randomization group	686 (58.2)	44 (56.4)	1,256
Male sex	693 (58.8)	42 (53.8)	1,256
Age (years)	60.9 (55.6; 65.7)	60.6 (56.5; 63.9)	1,209
HbA_{1c} (%)	6.3 (6.0; 6.9)	6.4 (6.0; 6.9)	1,214
HbA_{1c} (mmol/mol)	45 (42; 52)	46 (42; 52)	1,214
SBP (mmHg)	148.0 (135.2; 162.3)*	150.5 (134.0; 167.0)*	1,206
DBP (mmHg)	87.3 (80.7; 94.7)*	86.5 (77.0; 97.3)*	1,206
Height (cm)	170.6 (163.5; 176.9)	168.6 (163.9; 176.8)	1,206
Weight (kg)	87.4 (77.2; 98.8)*	90.5 (83.3; 101.7)*	1,206
Waist circumference (cm)	103.5 (95.0; 112.5)*	106.3 (101.5; 112.0)*	1,203
BMI (kg/m^2)	30.0 (27.0; 33.0)*	32.0 (28.0; 34.0)*	1,206
Total cholesterol (mmol/L)	5.6 (4.9; 6.4)*	5.5 (4.8; 6.2)*	1,158
LDL cholesterol (mmol/L)	3.4 (2.8; 4.0)*	3.1 (2.5; 3.9)*	1,098
HDL cholesterol (mmol/L)	1.3 (1.2; 1.6)	1.3 (1.0; 1.6)	1,133
Triglycerides (mmol/L)	1.6 (1.1; 2.2)	1.7 (1.3; 2.6)	1,147
Methylglyoxal (nmol/L)	254 (175; 406)	272 (177; 404)	1,065
Smoking			
Nonsmoker	320 (27.5)	27 (35.1)	1,242
Former smoker	404 (34.7)	19 (24.7)	1,242
Current smoker	441 (37.9)	31 (40.3)	1,242
Alcohol†	330 (31.0)	19 (27.5)	1,134
Treatment with statins	145 (12.4)	8 (10.3)	1,252
Treatment with antihypertensives	494 (42.1)	37 (47.4)	1,252
Treatment with aspirin	141 (12.0)	11 (14.1)	1,252
History of CVD‡	67 (5.7)	8 (10.3)	1,256

Categorical data are *n* (%), and continuous data are median (IQR). *N*, number of observations. *$P < 0.05$. †Weekly alcohol consumption exceeding recommended intake (>7 units in women and >14 units in men). ‡History of CVD: nonfatal myocardial infarction or stroke up to 10 years prior to the diagnosis of type 2 diabetes.

RESULTS

Of the initial cohort of 1,533 enrolled participants, 88 (5.7%) did not complete the MNSIQ at baseline and 189 (13.1%) had DPN at baseline (MNSIQ ≥4). We included the remaining 1,256 participants in the study. Participant median age was 60.8 years (IQR 55.6; 65.6), 1,180 (93.9%) participants were of white Caucasian ethnicity, and 735 (58.5%) were men. The cumulative incidence of DPN during 13 years of follow-up was 10% (*n* = 78) (Fig. 1), and the corresponding annual incidence was 0.7% (7 cases per 1,000 person-years). The subsequent MNSIQs were conducted after a median of 6.1 years (IQR 5.4; 6.9), 11.4 years (IQR 10.5; 12.2), and 12.8 years (IQR 11.8; 13.5), and 73%, 57%, and 38% of participants, respectively, completed these MNSIQs. The median follow-up time was 10.7 years (IQR 5.8; 12.7). The mean change in MNSIQ scores was 0.28 (SD 1.50) for the longest interval in MNSIQ scores for each participant.

The baseline characteristics of participants by incident DPN status during the total follow-up of 13 years are shown in Table 1. Measures of obesity (weight, waist circumference, and BMI), as well as SBP, were significantly higher at baseline in patients with incident DPN than in those without incident DPN. In contrast, measures of DBP, total cholesterol, and LDL cholesterol at baseline were significantly lower for participants with incident DPN than for participants without incident DPN.

Table 2 shows the baseline characteristics of participants by censoring status (lost to follow-up) in the Cox regression models before the second MNSIQ versus those who completed 6–13 years of follow-up. We found that participants who were censored prior to the second MNSIQ tended to be older and shorter, and the group had a higher percentage of females. In addition, they had a higher median BMI compared with participants followed for 6–13 years.

No effect modification by sex or other covariates was found.

Increased age was associated with an increased risk of incident DPN (hazard ratio [HR] 1.03 [95% CI 1.00; 1.07]) (per 1 year of age), whereas sex was not significantly associated with the risk of incident DPN (HR 0.68 [95% CI 0.47; 1.01]) for men compared with women. HRs for incident DPN per clinically relevant changes in the different continuous risk factors and per doubling of methylglyoxal levels are summarized in Table 3. Increased weight, waist circumference, and BMI were all significantly associated with a higher risk of incident DPN. Lower levels of both HDL cholesterol and LDL cholesterol were associated with a higher risk of incident DPN, and higher levels of methylglyoxal were associated with a higher risk of incident DPN. HRs for incident DPN for dichotomous risk factors are summarized in Supplementary Table 1. No statistically significant associations with the risk of incident DPN were found for the dichotomous risk factors of alcohol and smoking.

A sensitivity analysis based on multivariate logistic regression analysis confirmed the associations between incident

Table 2—Baseline characteristics of participants by censoring status (lost to follow-up) before the second MNSIQ: ADDITION-Denmark

Characteristics	Censored before the second MNSIQ	Followed for 6–13 years	*N*
Number (%) of participants	234 (18.6)	1,022 (81.4)	1,256
Intensive randomization group	127 (54.3)	603 (59.0)	1,256
Male sex	123 (52.6)*	612 (59.9)*	1,256
Age (years)	62.8 (57.7; 67.6)*	60.5 (55.4; 65.1)*	1,256
HbA_{1c} (%)	6.3 (5.9; 6.9)	6.4 (6.0; 7.0)	1,214
HbA_{1c} (mmol/mol)	45.4 (41.0; 51.9)	46.4 (42.1; 53.0)	1,214
SBP (mmHg)	147.0 (132.7; 164.0)	148.3 (135.7; 162.7)	1,206
DBP (mmHg)	85.7 (79.0; 94.0)	87.3 (80.7; 95.0)	1,206
Height (cm)	168.7 (161.0; 175.4)*	170.9 (164.2; 177.0)*	1,239
Weight (kg)	88.4 (77.3; 101.1)	87.3 (77.6; 98.7)	1,206
Waist circumference (cm)	104.5 (96.0; 116.0)	103.5 (95.5; 111.5)	1,203
BMI (kg/m^2)	31.0 (27.0; 35.0)*	30.0 (27.0; 33.0)*	1,206
Total cholesterol (mmol/L)	5.7 (4.9; 6.6)	5.6 (4.9; 6.4)	1,158
LDL cholesterol (mmol/L)	3.4 (2.7; 4.0)	3.3 (2.7; 4.0)	1,098
HDL cholesterol (mmol/L)	1.3 (1.2; 1.7)	1.3 (1.1; 1.6)	1,133
Triglycerides (mmol/L)	1.7 (1.1; 2.5)	1.6 (1.1; 2.2)	1,147
Methylglyoxal (nmol/L)	260 (188; 436)	254 (172; 399)	1,065
Smoking	*	*	1,242
Nonsmoker	47 (20.3)	300 (29.7)	
Former smoker	109 (47.2)	314 (31.1)	
Current smoker	75 (32.5)	397 (39.3)	
Alcohol†	72 (34.3)	277 (30.0)	1,134
Treatment with statins	20 (8.6)	133 (13.0)	1,252
Treatment with antihypertensives	99 (42.7)	432 (42.4)	1,252
Treatment with aspirin	30 (12.9)	122 (12.0)	1,252
History of CVD‡	15 (6.4)	60 (5.9)	1,256

Categorical data are *n* (%), and continuous data are median (IQR). *N*, number of observations. *$P < 0.05$. The distribution of smoking categories (nonsmoker, former smoker, and current smoker) compared between groups. †Weekly alcohol consumption exceeding recommended intake (>7 units in women and >14 units in men). ‡History of CVD: nonfatal myocardial infarction or stroke up to 10 years prior to the diagnosis of type 2 diabetes.

DPN and weight, waist circumference, BMI, and HDL cholesterol levels (Supplementary Table 2). Lastly, we compared the effect of intensive multifactorial treatment with that of routine care on the risk of incident DPN. We found no difference in the HR for incident DPN for participants in the intensive treatment group compared with those undergoing routine treatment (HR 0.94 [95% CI 0.59; 1.51]).

CONCLUSIONS

To our knowledge, this is the first observational, longitudinal cohort study examining risk factors for incident DPN present at the time of a type 2 diabetes diagnosis by screening. The prospective design of the study provides stronger epidemiological support for the identified risk factors compared with previous studies, which were primarily cross-sectional. Using MNSIQ for the diagnosis of DPN, we found that participants who were negative for DPN at baseline had a 10% cumulative incidence of DPN over the next 13 years (corresponding with an annual incidence of 0.7%). A higher risk of incident DPN was linked with increased age and weight, larger waist circumference, and higher BMI. In addition, higher levels of methylglyoxal were also associated with increased risk of DPN. Conversely, we found that lower levels of HDL and LDL cholesterol were associated with a higher risk of DPN. In sensitivity analysis, without time to incident DPN accounted for, increased weight, waist circumference, and BMI as well as lower levels of HDL cholesterol remained statistically significant risk factors for incident DPN.

Our observation that markers of obesity (weight, waist circumference, and BMI) are potential risk factors for DPN is consistent with previous reports from cross-sectional studies of patients with and patients without type 2 diabetes in populations from the U.S., China, and the Netherlands (9,11,13,25–27).

Other reported risk factors for DPN include high blood pressure, smoking, alcohol consumption, hyperlipidemia, and low HDL cholesterol (7–9,25). In the current study, we found no support for smoking status or alcohol consumption as risk factors for DPN. In addition, while significant differences in both SBP and DBP were observed between participants who developed DPN and those who did not, neither metric proved to be a significant risk factor for the development of disease. However, we found that lower HDL cholesterol levels are a risk factor for DPN. This is consistent with the Health ABC (Health, Aging, and Body Composition) study, which showed that lower levels of HDL cholesterol are associated with DPN (11). Other studies examining HDL cholesterol as a risk factor for DPN have been inconclusive, however (12,13), suggesting that further investigation is needed. We found a nonstatistically significant lower HR for incident DPN for men compared with women (HR 0.68 [95% CI 0.47;

Table 3—Risk of incident DPN by clinically relevant changes in continuous potential risk factors for incident DPN and by doubling of methylglyoxal present at the diagnosis of type 2 diabetes found by screening: ADDITION-Denmark

	HR of incident DPN (95% CI)†
HbA_{1c} (unit = 1%)	0.93 (0.75; 1.15)
HbA_{1c} (unit = 10 mmol/mol)	0.94 (0.77; 1.14)
SBP (unit = 10 mmHg)	1.02 (0.90; 1.16)
DBP (unit = 5 mmHg)	0.96 (0.83; 1.12)
Height (unit = 5 cm)	0.97 (0.83; 1.13)
Weight (unit = 5 kg)	1.09 (1.03; 1.16)*
Waist circumference (unit = 5 cm)	1.14 (1.05; 1.24)*
BMI (unit = 2 kg/m^2)	1.14 (1.06; 1.23)*
Total cholesterol (unit = 0.5 mmol/L)	0.90 (0.80; 1.01)
LDL cholesterol (unit = 0.25 mmol/L)	0.92 (0.86; 0.98)*
HDL cholesterol (unit = 0.25 mmol/L)	0.82 (0.69; 0.99)*
Triglycerides (unit = 0.5 mmol/L)	1.04 (0.98; 1.09)
Log_2 methylglyoxal (unit = doubling)	1.45 (1.12; 1.89)*

The risk of incident DPN is expressed by HR (95% CI) from Cox proportional hazard models adjusted for trial randomization group, sex, and age. *P value <0.05. †The HRs can be converted from HR per x units (e.g., per 10 mmHg in SBP) to HR per y units (e.g., per 1 mmHg in SBP) using the following equation: $HR^{(y/x)}$. For example, the HR for SBP per 1 mmHg is $(1.02)^{1/10} = 1.00$. The same equation applies to the CI. The χ^2 test and P values are unchanged by a change of scale.

1.01]). Potentially, this finding might reflect the use of the MNSIQ for diagnosing DPN, since other studies have found women to report more symptoms than men in general (28). Another explanation for the finding of a lower risk of DPN in men might be a lower burden of risk factors in men compared with women in the ADDITION cohort, reflecting the screening method used in ADDITION; 1 out of 5 points in the risk score questionnaire initiating the screening for type 2 diabetes is derived from being male.

In contrast to the prevailing view of hyperlipidemia as a risk factor for DPN (3,7,8), we found that lower levels of LDL cholesterol are associated with a higher risk of DPN development. The reason for this unexpected finding is unknown. Adjustment for statin treatment in our Cox model did not eliminate the association between LDL cholesterol and DPN. Notably, a big change in statin treatment took place during this study, as only a small number of participants were treated with statins at the diagnosis of type 2 diabetes (12%), whereas a large proportion of participants (79%) were treated after the diabetes diagnosis. No data on the dosage of statins or the exact date for onset of statin treatment were available for this study. Statin treatment and a history of CVD prior to the diagnosis of diabetes were associated with lower levels of baseline LDL cholesterol, suggesting that LDL cholesterol levels at baseline may be acting as an indicator of a different risk profile among participants. Alternatively, statins might protect against the development of DPN and may be acting as a confounder (29,30). Our data do not allow us to determine whether our finding regarding LDL cholesterol is solely explained by the actual LDL cholesterol levels or, rather, by an effect of statin treatment or other therapeutic interventions. Longitudinal measures of DPN correlated with longitudinal measures of LDL cholesterol and statin treatment are required to further explore the correlation between LDL cholesterol and DPN.

Despite previous studies identifying hyperglycemia as a risk factor for the development of DPN (7,31,32), we found no association between HbA_{1c} levels and incident DPN. This is likely explained by little variation—and low levels of HbA_{1c} at baseline compared with levels usually found in studies of clinically diagnosed diabetes. Low levels of hyperglycemia were retained in the cohort at 6-year follow-up; $<$50% of this cohort had HbA_{1c} levels $>$6.5% (47.5 mmol/mol) at the diagnosis of diabetes, and at the 6-year follow-up mean levels of HbA_{1c} of $\sim$6.5% (47.5 mmol/mol) were seen. These data therefore reflect a cohort exposed to lower levels of hyperglycemia over time than cohorts with clinically diagnosed type 2 diabetes used in previous studies.

The current study also found that higher levels of methylglyoxal are a risk factor for the development of DPN. This supports previous literature linking methylglyoxal to DPN and other late diabetes complications (15–17). It has been shown that modification of critical arginine residue(s) in voltage-gated sodium channel $Na_v1.8$ is associated with increased electrical excitability and facilitates firing of nociceptive neurons (16). Similar findings have been reported for transient receptor potential cation channel, subfamily V, member 1 (TRPV1) (33) and subfamily A, member 1 (TRPA1) (34). For TRPV1, it has been shown that methylglyoxal modification of cysteine residues leads to increased calcium influx, slowing of conduction velocity in unmyelinated peripheral nerve fibers, and the release of proinflammatory neuropeptides. The differential modification of these channel proteins may therefore provide an explanation for the coexistence of positive and negative clinical symptoms (e.g., pain and numbness) observed in DPN. However, no association between methylglyoxal and prevalent DPN or prevalent cardiac autonomic neuropathy was found in the cross-sectional study at the 6-year follow-up in ADDITION-Denmark (35). Another study using this cohort explored changes in methylglyoxal levels from baseline to the 6-year follow-up; they found that methylglyoxal decreases with no difference between randomization groups (36). The clinical and pathogenic implications of our finding of methylglyoxal as a risk factor for DPN needs to be further elucidated.

As in the cross-sectional 6-year follow-up in ADDITION-Denmark, we found no differences between randomization groups in the trial analysis (37). This is likely due to the relatively small differences in treatment intensity achieved in this pragmatic trial as a consequence of tightening of the Danish guidelines of diabetes care (which made routine treatment very similar to the intensive treatment group) during the trial follow-up period. It is also possible that early treatment intensification has only a small effect on the development of DPN (6,19,20), as our finding is consistent with findings from other trials studying the effect of intensive treatment on DPN development (6).

The incidence of DPN found in our prospective study is low (0.7% per year) compared with previous studies of people with newly and clinically diagnosed type 2 diabetes (which observed an

incidence of ~2% per year) (5,6,9). Several factors may explain this. First, this study examines a cohort with a lower degree of hyperglycemia than cohorts of patients with clinically diagnosed diabetes. Second, the use of diabetes screening means that participants in all likelihood had diabetes identified several years before they otherwise would have presented (38). Third, participants lost to follow-up prior to the second MNSIQ may introduce selection bias resulting in an underestimation of the true incidence of DPN, as they are typically older and have higher BMIs. Fourth, the low incidence may be attributed to the use of MNSIQ rather than a neurological history and examination in conjunction with nerve conduction studies or validated small-fiber measures (39). The agreement between symptoms and a clinical examination, nerve conduction studies, or intra-epidermal nerve fiber density might be low, indicating that symptoms might reflect a different aspect of this disease. However, the MNSIQ is a validated measure of DPN based on the Epidemiology of Diabetes Interventions and Complications (EDIC) study that used >1,100 patients with long-standing type 1 diabetes and a high prevalence of DPN of 33% (24). In that study, an MNSIQ score ≥4 yielded a sensitivity of 40%, a specificity of 92%, a positive predictive value of 69%, and a negative predictive value of 78% compared with clinically defined DPN (24). Moreover, the diagnostic performance of the MNSIQ was not assessed in our study, and our cohort consists of older subjects with type 2 diabetes compared with the younger cohort of people with type 1 diabetes in the EDIC study. Finally, as a result of the relatively low sensitivity reported by the MNSIQ in the EDIC study, use of this instrument as a diagnostic tool for DPN should be expected to underestimate the true incidence of DPN.

We consider the Cox regression analyses superior for examining the association between risk factors and incident DPN compared with logistic regression, since the Cox model takes into account the individual follow-up time of participants and thus makes better use of the available data. In the current study, the data were analyzed as time-to-event data, though we only had data at baseline, 6, 12, and 13 years (with great variation in time points for participants owing to the time span of the follow-up examinations). However, DPN is a very slow-progressing disease, which mitigates the effects of infrequent assessments for DPN. Furthermore, any misclassification of the true incident date of the onset of DPN is most likely random, since the assessments of DPN in this study were determined by the study protocol and not influenced by any individual factors featured by study participants. The potential misclassification of the time of onset of DPN might cause imprecision in our estimates of associations for risk factors but would not cause spurious associations to appear. Since the study attempted to correlate time to DPN development with associated risk factors present at the diagnosis of screen-detected diabetes, we deemed this potential source of random error acceptable.

Moreover, potential selection bias due to participants lost to follow-up is likely causing some degree of underestimation of both the incidence of DPN and the effect size of the identified risk factors of obesity, as participants lost to follow-up are older and have higher levels of BMI. However, a relatively high level of participation was seen in this study.

In conclusion, our study provides stronger epidemiological evidence for obesity as a risk factor for DPN. This finding supports several previous cross-sectional studies (9,11,13,25–27). In addition, lower levels of HDL and LDL cholesterol at diabetes diagnosis were associated with higher risk of DPN development, but previous studies reveal conflicting results, and potential confounding by indication through statin treatment exists. Lastly, this study further implicates increased methylglyoxal levels at diabetes diagnosis as a risk factor for DPN (15–17).

The major strengths of our study are its large size and high level of participation, the unselected nature of the cohort, the prospective design from an early stage of type 2 diabetes found by screening, the long duration of follow-up, and the examination of participants receiving diabetes care in a primary care setting. Despite these strengths, our results might not apply to cohorts of younger people or cohorts where diabetes and other risk factors for DPN are poorly controlled. DPN progression might be different in younger people, although the age of onset of type 2 diabetes has previously been shown to have no clear impact on the development of microvascular complications (40). Future prospective studies applying even more stringent definitions of DPN, as well as those that study DPN development by longitudinal measures of DPN correlated with changes in risk factors over time, would enhance the evidence for the risk factors identified in this study.

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Author Contributions. S.T.A. carried out all statistical analysis and wrote the manuscript. D.R.W., H.A., T.L., B.C.C., and M.C. helped design the study, provided input on statistical analysis, contributed to the discussion, and reviewed and edited the manuscript. E.-M.D., N.B.F., T.S.J., and E.L.F. contributed to the discussion and reviewed and edited the manuscript. P.N. and T.F. led the analysis of methylglyoxal and together with T.M.J. contributed to the discussion and reviewed and edited the manuscript. S.T.A. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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