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Near-normoglycaemia prevents the development of neuropathy. A 24-year prospective study from the diagnosis of type 1 diabetes

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Abstract

Objective: Complete prevention of diabetic neuropathies has not been previously demonstrated. We sought to determine whether long-term near-normoglycemia maintained from the diagnosis of type 1 diabetes prevents polyneuropathy and cardiac autonomic dysfunction.

Design: Prospective observational study over 24 years.

Setting: Ambulatory care.

Participants: Thirty-two newly diagnosed type 1 diabetic patients aged 20.3±1.0 years, duration of diabetes 2.7±0.3 weeks.

Intervention: Insulin therapy according to standards of care.

Primary and secondary outcome measures: Motor and sensory nerve conduction velocity (MNCV, SNCV), heart rate variability (HRV), and confirmed clinical polyneuropathy measured at 15 time points over 24 years and quantitative sensory testing (QST) determined over 20-22 years.

Results: Eleven patients were well controlled over 24 years with mean HbA1c<7.0% (6.5±0.1%; Group 1), whereas 21 patients were poorly controlled (mean HbA1c≥7.0%: 8.3±0.2%; Group 2). After 24 years, MNCV was faster in Group 1 vs Group 2 in the median (55.5±1.6 vs 48.9±1.6 m/s), ulnar (56.5±1.5 vs 49.3±1.7 m/s), and peroneal nerve (44.7±1.6 vs 36.8±2.5 m/s), while SNCV was faster in the median (53.6±1.6 vs 45.5±2.8 m/s), ulnar (54.7±1.8 vs 43.0±3.9 m/s), and sural nerve (44.5±1.8 vs 35.5±2.6 m/s) (all P<0.05). The annual decline in peroneal MNCV and sural SNCV in Group 1 was 6-fold and 3-fold faster in Group 2 than in Group 1, respectively. Likewise, impairment in QST and HRV developed at faster rates in Group 2. After 24 years, 64% of patients in Group 2 but none in Group 1 developed confirmed clinical polyneuropathy.

Conclusions: Near-normoglycemia maintained from the diagnosis of type 1 diabetes over 24 years effectively prevented the decline in hyperglycemia-related peripheral and autonomic nerve function and development of confirmed clinical polyneuropathy.

Strengths and limitations of this study

- This prospective observational study conducted over 24 years evaluated whether long-term near-normoglycemia maintained from the diagnosis of type 1 diabetes prevents polyneuropathy and cardiac autonomic dysfunction.
- Prospective assessment of neuropathy included detailed quantitative assessment of nerve function rather than nerve structure, since the latter became quantifiable only recently.
- The results of this study may provide a rationale for the design of future long-term clinical trials aimed at prevention of diabetic neuropathy.
- Allocation of patients to two groups markedly differing as to the degree of glycaemic control could only be done post-hoc, and the small study sample may be a source of bias.

INTRODUCTION

Approximately one third of patients with diabetes are affected by diabetic sensorimotor polyneuropathy (DSPN), which leads to considerable morbidity due to neuropathic pain and foot ulcers, while cardiovascular autonomic neuropathy (CAN) is associated with a 3-fold increased risk of mortality (1,2). Chronic cumulative glycaemic exposure is a major permissive, yet modifiable factor in the development and progression of diabetic microvascular complications (3), but explains only about one third of the variability of the severity of complications in the diabetic general population (4). The Diabetes Control and Complications Trial/ Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) confirmed that glycaemic control is a robust predictor of both DSPN and CAN in patients with type 1 diabetes (5). The persistent long-term favorable effects shown during EDIC of the prior intensive insulin therapy (IT) compared to conventional insulin therapy (CT) during DCCT on diabetic microvascular complications have been termed "metabolic memory" (6,7). In the DCCT, HbA1c explained 92% of the difference in risk of neuropathy between IT and CT (8).

On the other hand, DCCT/EDIC also revealed that for most patients with type 1 diabetes, current strategies for optimizing glucose control are insufficient to fully prevent or delay the development of DSPN and CAN (5,7). In fact, 25% of the former IT group and 35% of those in the former CT group had confirmed DSPN by 13-14 years of EDIC follow-up (9), while the corresponding rates for CAN were 29 and 35%, respectively (10). The mean HbA1c levels at EDIC years 13-14 were 7.8% in each of the former insulin treatment groups (9). Although IT prevented diabetic neuropathy only to a modest degree, there is general agreement that IT targeting HbA1c levels <7% should be implemented as early as possible in the course of type 1 diabetes (5,7,11).

Based on the DCCT/EDIC observations, it is conceivable that maintenance of the recommended HbA1c level <7% in the long-term from the diabetes onset could result in more effective prevention of neuropathy. However, detailed long-term prospective studies assessing the evolution of neuropathy from the diagnosis of type 1 diabetes onward are not available. The aim of the present study was to determine the long-term effects of near-normoglycaemia as compared with poor glycaemic control from the diagnosis of type 1 diabetes over the next 24 years on the development of DSPN and CAN using a comprehensive array of measures to detect early large and small nerve fiber dysfunction in conjunction with clinical assessment.

METHODS

Patients

Thirty-two inpatients with type 1 diabetes admitted to the clinical department of the German Diabetes Center at Heinrich Heine University, Düsseldorf, in 1985 (12) participated in this prospective observational study and were followed up for 24 years with outpatient visits at 3 months and 1,2,4,5,8,10,12,14,16,18,20,22, and 24 years following the baseline assessment. The study has been carried out in accordance with the principles of the Declaration of Helsinki as revised in 1983. Informed consent was obtained from all eligible subjects after the procedures involved were fully explained. Inclusion criteria were age below 40 years, type 1 diabetes according to the National Diabetes Data Group classification (13), known diabetes duration up to 2 months, and mean blood glucose 80-160 mg/dl at the baseline assessment. Exclusion criteria were causes of neuropathy other than diabetes, clinically relevant neurological diseases and use of medication with potential influence on nerve function. All patients underwent a standardized one-week diabetes teaching program.

To establish whether the development of neuropathy is related to the long-term degree of

glycaemic control, patients were grouped according to their mean HbA1c levels during the 24 years of follow-up (excluding baseline HbA1c) in line with the current recommendations by the American Diabetes Association (11). During the 24-year period, 11 patients had mean HbA1c levels within the recommended target <7.0% [<53.0 mmol/mol] (6.5±0.1% [47.1±1.0 mmol/mol]; Group 1), whereas 21 patients had mean HbAc levels ≥7.0% % [≥53.0 mmol/mol] (8.3±0.2% [67.2±2.2 mmol/mol]; Group 2). Ten healthy subjects (5 male, age: 37.3±2.8 years, height: 172±3.2 cm, weight: 72.9±3.5 kg, BMI: 24.5±0.9 kg/m²) served as controls for the nerve conduction studies. The clinical characteristics of the diabetic groups at baseline are shown in Table 1. There were no significant differences between the groups for any of the parameters listed. Compared to the controls both diabetic groups were younger and Group 2 had a lower BMI (P<0.05).

Electrophysiological tests

Motor nerve conduction velocity (MNCV) was measured in the median, ulnar, and peroneal nerves, while sensory nerve conduction velocity (SNCV) was determined in the median, ulnar, and sural nerves at baseline and each subsequent visit at a skin temperature of 32-34°C using surface electrodes (EMG 2000 electromyograph, Schwarzer-Picker, Munich, Germany; Sapphire, Medelec, Woking, U.K; VikingQuest EMG, Cardinal Health, Madison, WI, USA) as previously described (12).

Quantitative sensory testing (QST)

Vibration perception threshold (VPT) was measured from the second year at the second metacarpal bone and medial malleolus using the method of limits (Vibrameter, Somedic, Stockholm). Thermal perception thresholds (TPT) to warm and cold stimuli were determined from the 4th year at the thenar eminence and dorsum of the foot using the method of limits (Marstock stimulator, Somedic, Stockholm, Sweden; Path-Tester; Tönnies, Germany; TSA II

NeuroSensory Analyzer, Medoc, Ramat Yishai, Israel) as previously reported (12,14).

Clinical examination and confirmed DSPN

Neurological examination at baseline and each visit included assessment of neuropathic symptoms, vibration sensation using the Rydel-Seiffer tuning fork, thermal perception (TipTherm, GND, Düsseldorf, Germany), ankle reflexes, and pin-prick perception. Neuropathic deficits and symptoms were scored using the Neuropathy Disability Score (NDS) and the Neuropathy Symptom Score (NSS) (15). Criteria for the definition of confirmed clinical DSPN included NDS≥2 and reduced peroneal MNCV and/or sural SNCV below the 5th percentile and elevated malleolar VPT and/or warm TPT and/or cold TPT on the dorsum of the foot above the 95th percentile of healthy subjects (12,14).

Heart rate variability (HRV)

Coefficient of variation (CV) of HRV during spontaneous breathing over 5 min was assessed from baseline and CV during deep breathing over 1 min was measured from the first year (Neurocard-Analyzer, Argustron, Mettmann, Germany; ProSciCard, CPS Medical, Wetzlar, Germany; NeuroDiag II, Dr. Vetter, Baden-Baden, Germany; VariaCardio TF5 System, AMD Group, Buckinghamshire, UK) as previously described (12,16).

Retinal assessment

Color retinal photographs were produced by a CR3-45NM non-mydriatic retinal camera (Canon, Tokyo, Japan) and were judged by an experienced examiner.

Laboratory methods

HbA1c was measured at baseline and each visit using the HPLC technique (Diamat, Bio-Rad, Munich, Germany). Capillary blood glucose was measured by a hexokinase-based method (ACP 5040 autoanalyzer, Eppendorf, Hamburg, Germany; Cobas C 311 analyzer, Roche Diagnostics, Mannheim, Germany). Albuminuria was measured in 12 h urine samples using the

immuno-nephelometric technique (Array Protein System, Beckman, Fullerton, CA, USA) or turbidimetric method (Cobas C 311 analyzer, Roche Diagnostics, Mannheim, Germany) with a normal range: <20 µg/min. Islet cell antibodies were measured by indirect immunofluorescence using snap-frozen, unfixed human pancreatic sections of blood 0 donors (17).

Statistical analysis

Continuous data were expressed as mean±SEM. Categorical data were given as absolute or relative frequencies. Post-hoc comparisons of study endpoints between the two groups with mean HbA1c<7.0% and mean HbA1c≥7.0% from 3 months to 24 years of the study were performed using the t-test for two independent samples without missing data imputation. The level of significance was set at α=0.05.

RESULTS

Glycaemic control and microvascular complications

Insulin therapy was initiated in all patients with either subcutaneous or intravenous application of human or porcine regular insulins. After correction of the initial metabolic derangement, patients were treated with multiple daily insulin injections. During the study, two patients in each group switched to continuous subcutaneous insulin infusion. The mean HbA1c levels in the two groups studied are shown in Fig. 1A. HbA1c decreased in Group 1 from 8.8±0.6% at baseline to a mean of 6.5±0.1% throughout the 14 subsequent outpatient visits, while in Group 2 HbA1c decreased from 9.1±0.3% to 8.3±0.2%. The daily insulin dose in Group 1 and Group 2 was 28.3±5.1 and 37.2±4.3 IU/day at baseline, 49.6±5.1 and 68.3±4.7 IU/day ($p<0.05$) at 10 years, 49.7±5.7 and 64.7±6.8 IU/day at 20 years, and 52.1±6.4 and 69.3±8.3 IU/day at 24 years, respectively. The number of cigarettes smoked per day in Group 1 and Group 2 was 6.1±2.6 and 7.7±3.3 at 4 years, 9.5±3.0 and 9.2±3.7 at 10 years, 6.1±2.7 and 6.5±3.5 at 20 years, and 3.5±2.6 and 6.9±4.3 at 24

years, respectively.

At baseline, none of the patients had nephropathy or retinopathy. The percentages of patients with micro/macroalbuminuria in Group 1 and Group 2 were 0 and 20% at 12 years and 0 and 43% at 24 years, respectively. The percentages of patients with retinopathy in Group 1 and Group 2 were 0 and 17% at 12 years and 10% and 55% at 24 years, respectively.

The most frequently used medication at 24 years in Group 1 and Group 2 included ACE inhibitors/AT1 blockers: 33 and 55%, ß blockers: 22 and 36%, diuretics: 0 and 36%, calcium channel blockers: 0 and 27%, statins: 33 and 45%, and ASS: 0 and 27% of the patients, respectively.

Peripheral and cardiac autonomic nerve function

The course of median and peroneal MNCV and median and sural SNCV in the two diabetic groups and controls is illustrated in Fig. 1B-E. No significant differences in NCV between the diabetic groups were noted at baseline. Median MNCV was faster in Group 1 than Group 2 at 2,16,18, and 24 years (Fig. 1B), while for peroneal MNCV this was the case at 5,10-20, and 24 years (Fig. 1C) (all $P<0.05$). Median SNCV was higher in Group 1 than Group 2 at 1,4,8, and 12-24 years (Fig. 1D), while accordingly sural SNCV was higher at 8,10, and 14-24 years (Fig. 1E) (all $P<0.05$). NCV in the four nerves studied after 10 and 20 years did not differ between Group 1 and the healthy control group.

The course of QST, HRV, and confirmed DSPN is shown in Fig. 3. No significant differences between the groups in these measures were observed at the time of initial assessment. Thereafter, warm TPT was elevated in Group 2 vs Group 1 at 12,20, and 24 years (Fig. 2A), while cold TPT was increased Group 2 vs Group 1 at 12 and 24 years (Fig. 2B) (all $P<0.05$). CV of R-R intervals at rest was better in Group 1 vs Group 2 at 5,12,14,20, and 24 years (Fig. 2C), while CV during deep breathing was better in Group 1 vs Group 2 at 4,14,22, and 24 years (Fig. 2D) (all $P<0.05$).

Malleolar VPT was elevated in Group 2 vs Group 1 at 10,16-20, and 24 years (Fig. 2E) (all $P<0.05$). Confirmed DSPN was not present in either group up to 4 years. After 10,16, and 24 years, cumulative prevalence of confirmed clinical DSPN in Group 2 was 39%, 46%, and 64%, respectively, whereas in Group 1 only a transient occurrence of 1 case each meeting the criteria of confirmed clinical DSPN was observed at 10-14,18, and 20 years of follow-up (Fig. 2F).

The changes in the diabetic groups in NCV over 24 years, VPT over 22 years, TPT over 20 years, and CV of HRV at rest and during deep breathing over 24 and 23 years, respectively, as well as the changes in NCV in the control group over 20 years are listed in Table 2. The most pronounced decline in Group 2 was noted for peroneal MNCV and sural SNCV which was approximately 6-fold and 3-fold faster than in Group 1, respectively. SNCV declined slightly more rapidly (0.85-1.08 m/s/year) than MNCV (0.60-1.04 m/s/year) in Group 2. The magnitude of decline in MNCV and SNCV over 20 years in Group 1 was comparable to that seen in the control group. For the majority of the remaining measures, progression in Group 2 was 2.5-fold to 13-fold faster than in Group 1. The highest rate of deterioration in Group 2 was noted for cold TPT on the foot and malleolar VPT.

The numbers of patients lost to follow-up in Group 1 and Group 1 were 2 and 3 at 5 years, 1 and 4 at 12 years, 1 and 6 at 20 years, and 2 and 10 at 24 years, respectively. Among these, 2 patients from Group 2 died during follow-up, one female from diabetic ketoacidosis after 12 years and one male supposedly from severe hypoglycaemia after 18 years of follow-up. Other reasons for drop-out were inconvenience of regular follow-up and moving away from the area.

DISCUSSION

The results of this prospective study demonstrate that near-normoglycaemia maintained from the diagnosis of type 1 diabetes over the next 24 years effectively prevented a 3-fold and 6-fold faster

annual hyperglycaemia-related decline in sural SNCV and peroneal MNCV and preserved NCV at the expected age-induced level of change. In poorly controlled type 1 diabetes, MNCV, SNCV, and HRV started to deteriorate earlier (after approximately 4 years) than VPT and warm/cold TPT (after approximately 10-12 years), while the cumulative prevalence of confirmed clinical DSPN rose considerably from zero at diabetes diagnosis to one third after 12 years and almost two thirds after 24 years. In contrast, none of the patients who maintained near-normoglycaemia throughout 24 years presented with confirmed clinical DSPN after this period. Thus, the present findings are inasmuch novel as they indicate that near-normoglycaemia instituted at the time of diagnosis of type 1 diabetes and subsequently maintained over more than two decades may fully prevent the development of DSPN and diminished HRV.

There are no studies with which the present data can be directly compared, since previous studies did not focus on newly diagnosed type 1 diabetes but included patients with longer disease duration (3,5-10,12,18-26). The EURODIAB Prospective Complications Study (PCS) and Pittsburgh Epidemiology of Diabetes Complications Study including type 1 diabetes subjects with baseline duration of diabetes of 12.4 and 16.9 years, respectively, identified HbA1c as an independent predictor of incident DSPN over 7.3 and 6 years (18,19) and CAN over 7.3 and 4.7 years (20,21), respectively. In newly diagnosed diabetic children followed over 10 years, a correlation between HbA1c and measures of nerve function was found for peroneal MNCV but, in contrast to the present study, neither for sural SNCV nor HRV during normal and deep breathing (27). In a study of adolescents with a mean age of 15.5 years and diabetes duration of 6.8 years followed over approximately 13 years on average, HbA1c during the first year of follow-up was the strongest predictor for the development of clinical DSPN, but the rates of decline in nerve function in relation to HbA1c were not reported (22).

The Oslo Study recently reported that HbA1c was an important risk factor in the development of

DSPN over 27 years, but the mean diabetes duration at study start was 12.8 years, and mean HbA1c measured yearly was 8.0%. Dividing patients by low and high cumulative glycaemic exposure (3) after 27 years revealed a mean decrease in peroneal MNCV of 6.7. vs 13.0 m/s and sural SNCV of 8.1 vs 15.3 m/s, but the difference for these changes between the groups did not reach statistical significance (23). CAN was assessed at the 18-year follow-up revealing abnormalities in ≥2 out of 3 parameters in 29.4% of patients with mean HbA1c <8.4% compared to 66.7% in those with mean HbA1c ≥8.4%, but no prospective data was available (24). Likewise, in the Stockholm Diabetes Intervention Study (SDIS) including type 1 diabetic subjects with an initial mean diabetes duration of 17 years, peroneal MNCV and sural SNCV deteriorated after 10 years of both IT and CT (mean HbA1c: 7.2 vs 8.3%), albeit to a lesser degree in the former treatment group (25). Similar to the Oslo Study, CAN was assessed only once after 11.4 years, with only 3 out of 6 parameters were significantly better after IT than CT (26). Thus, in line with DCCT/EDIC (5,9,10), the development of DSPN and CAN in the Oslo Study and SDIS could not be entirely prevented but only slightly delayed.

In contrast to type 1 diabetes, the role of glycaemic control in the development of DSPN in type 2 diabetes is less clear. In newly diagnosed type 2 diabetes patients, the rates of definite or probable DSPN increased from 8.3% at baseline to 41.9% after 10 years, but mean HbA1c was not higher in patients who had DSPN at 10 years compared to those who did not (9.6 vs 8.9%) (28). In patients who developed parasympathetic CAN after 10 years (65%), HbA1c at 5 years was higher than in those who did not (9.2 vs 8.0%), but this difference was not found for sympathetic CAN (29).

The American Diabetes Association considers HbA1c levels <7% a reasonable treatment goal for many non-pregnant adults. More stringent HbA1c goals (<6.5%) are suggested for selected individual patients, if this can be achieved without significant hypoglycaemia or other adverse

effects of treatment (11). These recommendations entirely suit the well-controlled group in the present study, which maintained mean HbA1c at 6.5%.

Study limitations

The present study has some limitations. First, since this was an observational study, allocation of patients to the two groups of glycaemic control could only be done post-hoc. Second, the small study sample may be a source of bias. Third, prospective assessment of neuropathy included nerve function rather than nerve structure such as skin biopsy or corneal confocal microscopy (30), since the latter became available only recently. Nonetheless, we believe that the unequivocally preserved large and small fiber function in favor of near-normoglycaemia supports the notion that the degree of glycaemia governs the development of neuropathy over 24 years.

CONCLUSIONS

In conclusion, near-normoglycaemia maintained from the diagnosis of type 1 diabetes over the next 24 years was associated with a complete prevention of hyperglycaemia-induced slowing in nerve conduction, confirmed clinical DSPN, and reduced HRV. In contrast, poor glycaemic control was associated with a continuous and substantial increase in the cumulative prevalence of confirmed clinical DSPN over 24 years. Hence, poor glycaemic control constitutes the paramount permissive factor contributing to the evolution of neuropathy in type 1 diabetes. The annual rates of progression of NCV, QST, and HRV in well controlled compared with poorly controlled patients may provide a rationale for the design of future long-term clinical trials aimed at prevention of diabetic neuropathy.

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D.Z. designed the study. D.Z., M.B., and M.S.T. researched data. D.Z. wrote the manuscript. D.Z. and M.R. reviewed and edited the manuscript. D.Z. is the guarantor of this work and, as such, had full access to all of 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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Table 1: Demographic and clinical characteristic of the patients at baseline.

	Group 1 HbA1c <7.0%	**Group 2 HbA1c ≥7.0%**
n	11	21
Sex (m/f)	7/4	14/7
Age (years)	19.4±0.9	20.8±1.4
Height (cm)	175±2.2	176±2.0
Weight (kg)	68.6±6.4	64.0±3.1
BMI (kg/m²)	21.9±1.7	20.4±0.8
Systolic blood pressure (mmHg)	127±7.0	117±3.9
Diastolic blood pressure (mmHg)	81.3±6.6	69.4±3.9
Duration of symptoms (weeks)*	5.5±1.7	6.7±2.3
Duration of insulin treatment (weeks)	2.6±0.5	2.7±0.5
Insulin dose (IU/day)	28.3±5.1	37.2±4.3
HbA1c (%) HbA1c (mmol/mol)	8.8±0.6 72.6±5.2	9.1±0.3 76.5±2.9
Mean blood glucose (mg/dl)$^{+}$	111±8.0	121±4.2
Islet cell antibody positive (%)	75	84

*Symptoms due to hyperglycemia prior to diabetes diagnosis
$^{+}$Based on 5 values during the day of neurological assessment.

Table 2: Changes in motor and sensory nerve conduction velocity (MNCV, SNCV), vibration perception threshold (VPT), thermal perception threshold (TPT), and coefficient of variation (CV) of heart rate variability (HRV) during the study.

	Group (HbA1c)	Absolute change over 24 years	Absolute change per year	Percent change over 24 years	Percent change per year
Median MNCV	Control	-2.2*	-0.11	-3.8*	-0.19
	Group 1 (<7%)	-2.7	-0.11	-4.6	-0.19
	Group 2 (≥7%)	-8.2	-0.34	-14.4	-0.60
Ulnar MNCV	Control	-5.4*	-0.27	-8.7*	-0.44
	Group 1 (<7%)	-2.6	-0.11	-4.4	-0.18
	Group 2 (≥7%)	-11.5	-0.48	-18.9	-0.79
Peroneal MNCV	Control	-2.6*	-0.13	-5.3*	-0.27
	Group 1 (<7%)	-1.8	-0.08	-3.8	-0.16
	Group 2 (≥7%)	-11.8	-0.49	-24.9	-1.04
Median SNCV	Control	-1.5*	-0.08	-2.5*	-0.13
	Group 1 (<7%)	-5.5	-0.23	-9.2	-0.38
	Group 2 (≥7%)	-11.7	-0.49	-20.5	-0.85
Ulnar SNCV	Control	0.4*	0.02	0.7*	0.04
	Group 1 (<7%)	-0.4	-0.02	-0.7	-0.03
	Group 2 (≥7%)	-11.1	-0.46	-20.3	-0.85
Sural SNCV	Control	-4.8*	-0.24	-9.1*	-0.46
	Group 1 (<7%)	-4.2	-0.18	-8.5	-0.35
	Group 2 (≥7%)	-12.4	-0.52	-25.9	-1.08
Metacarpal VPT	Group 1 (<7%)	0.04^{+}	0.002	16.7^{+}	0.76
	Group 2 (≥7%)	0.24^{+}	0.01	114^{+}	5.18
Malleolar VPT	Group 1 (<7%)	0.47^{+}	0.02	162^{+}	7.36
	Group 2 (≥7%)	2.43^{+}	0.11	496^{+}	22.50
TPT Warm Foot	Group 1 (<7%)	0.40*	0.02	8.33*	0.42
	Group 2 (≥7%)	5.30*	0.27	110*	5.50
TPT Cold Foot	Group 1 (<7%)	0.70*	0.04	50.0*	2.50
	Group 2 (≥7%)	7.30*	0.37	608*	30.40
CV of HRV at rest	Group 1 (<7%)	-1.22	-0.05	-17.9	-0.75
	Group 2 (≥7%)	-2.69	-0.11	-46.1	-1.92
CV of HRV at deep breathing	Group 1 (<7%)	0.38$^{\#}$	0.02	3.9$^{\#}$	0.17
	Group 2 (≥7%)	-4.51$^{\#}$	-0.20	-49.1$^{\#}$	-2.13

*Change over 20 years, $^{+}$change over 22 years, $^{\#}$change over 23 years

Figure 1: HbA1c levels (A), median motor nerve conduction velocity (MNCV) (B), peroneal MNCV (C), median sensory nerve conduction velocity (SNCV) (D), and sural SNCV (E) over 24 years in Group 1 (mean HbAc<7.0%; n=11) and Group 2 (mean HbA1c≥7.0%; n=21), and healthy control subjects (n=11) over 20 years. * p<0.05 for Group 1 vs Group 2

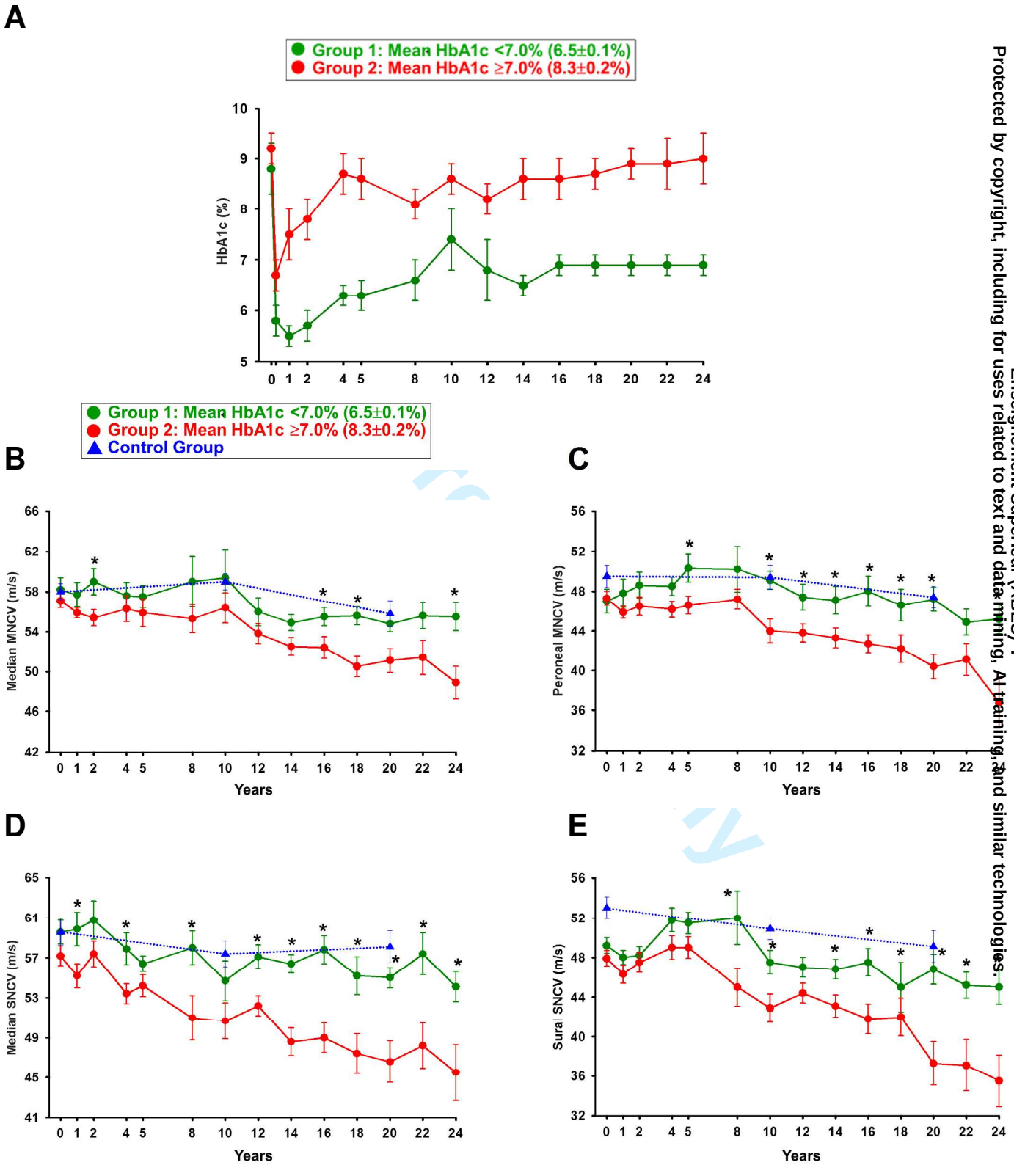
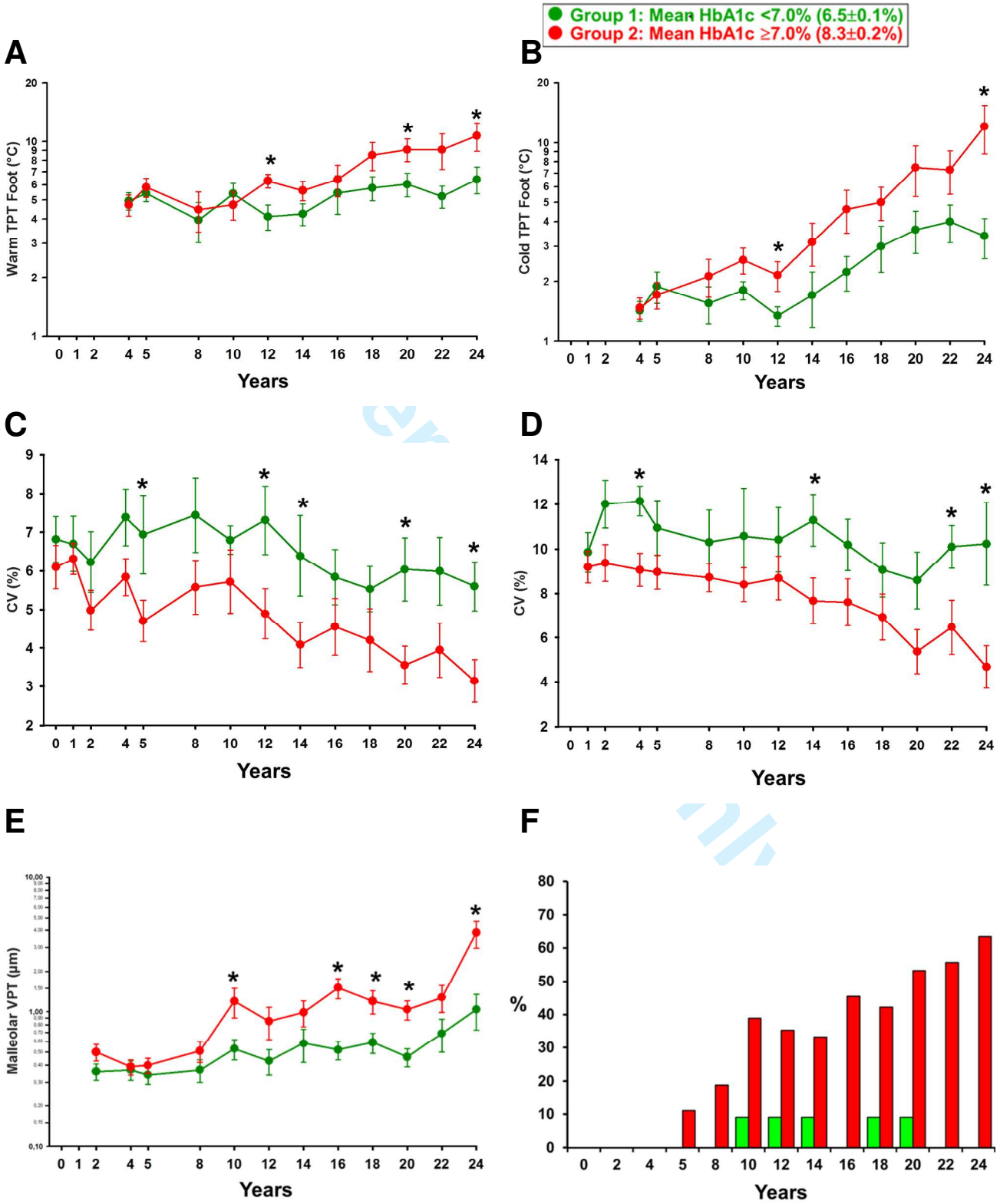


Figure 2: Warm thermal perception threshold (TPT) (A) and cold TPT (B) on the dorsum of the foot from the 4th year, coefficient of variation (CV) of R-R intervals at rest over 24 years (C) and CV during deep breathing from the first year (D), vibration perception threshold (VPT) from the second year (E), and cumulative prevalence of confirmed clinical diabetic sensorimotor polyneuropathy (DSPN) over 24 years (F). * p<0.05 for Group 1 vs Group 2



STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page number
1Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5-6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	6
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-7
Bias	9	Describe any efforts to address potential sources of bias	13
Study size	10	Explain how the study size was arrived at	n/a
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	8
		(*c*) Explain how missing data were addressed	8
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	8,10
		(*e*) Describe any sensitivity analyses	n/a

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10
		(b) Give reasons for non-participation at each stage	10
		(c) Consider use of a flow diagram	n/a
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	19
		(b) Indicate number of participants with missing data for each variable of interest	10
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	5
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	8-10
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8-10
		(*b*) Report category boundaries when continuous variables were categorized	n/a
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	n/a
Discussion			
Key results	18	Summarise key results with reference to study objectives	10-11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Near-normoglycaemia prevents the development of neuropathy. A 24-year prospective study from the diagnosis of type 1 diabetes

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Near-normoglycaemia prevents the development of neuropathy. A 24-year prospective study from the diagnosis of type 1 diabetes

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Abstract

Objective: Complete prevention of diabetic neuropathies has not been previously demonstrated. We sought to determine whether long-term near-normoglycemia maintained from the diagnosis of type 1 diabetes prevents polyneuropathy and cardiac autonomic dysfunction.

Design: Prospective observational study over 24 years.

Setting: Ambulatory care.

Participants: Thirty-two newly diagnosed type 1 diabetic patients aged 20.3±1.0 years, duration of diabetes 2.7±0.3 weeks.

Intervention: Insulin therapy according to standards of care.

Primary and secondary outcome measures: Motor and sensory nerve conduction velocity (MNCV, SNCV), heart rate variability (HRV), and confirmed clinical polyneuropathy measured at 15 time points over 24 years and quantitative sensory testing (QST) determined over 20-22 years.

Results: Eleven patients were well controlled over 24 years with mean HbA1c<7.0% (6.5±0.1%; Group 1), whereas 21 patients were poorly controlled (mean HbA1c≥7.0%: 8.3±0.2%; Group 2). After 24 years, MNCV was faster in Group 1 vs Group 2 in the median (55.5±1.6 vs 48.9±1.6 m/s), ulnar (56.5±1.5 vs 49.3±1.7 m/s), and peroneal nerve (44.7±1.6 vs 36.8±2.5 m/s), while SNCV was faster in the median (53.6±1.6 vs 45.5±2.8 m/s), ulnar (54.7±1.8 vs 43.0±3.9 m/s), and sural nerve (44.5±1.8 vs 35.5±2.6 m/s) (all P<0.05). The annual decline in peroneal MNCV and sural SNCV in Group 1 was 6-fold and 3-fold faster in Group 2 than in Group 1, respectively. Likewise, impairment in QST and HRV developed at faster rates in Group 2. After 24 years, 64% of patients in Group 2 but none in Group 1 developed confirmed clinical polyneuropathy.

Conclusions: Near-normoglycemia maintained from the diagnosis of type 1 diabetes over 24 years effectively prevented the decline in hyperglycemia-related peripheral and autonomic nerve function and development of confirmed clinical polyneuropathy.

Strengths and limitations of this study

- This prospective observational study conducted over 24 years evaluated whether long-term near-normoglycemia maintained from the diagnosis of type 1 diabetes prevents polyneuropathy and cardiac autonomic dysfunction.
- Prospective assessment of neuropathy included detailed quantitative assessment of nerve function rather than nerve structure, since the latter became quantifiable only recently.
- The results of this study may provide a rationale for the design of future long-term clinical trials aimed at prevention of diabetic neuropathy.
- Allocation of patients to two groups markedly differing as to the degree of glycaemic control could only be done post-hoc, and the small study sample may be a source of bias.

INTRODUCTION

Approximately one third of patients with diabetes are affected by diabetic sensorimotor polyneuropathy (DSPN), which leads to considerable morbidity due to neuropathic pain and foot ulcers, while cardiovascular autonomic neuropathy (CAN) is associated with a 3-fold increased risk of mortality (1,2). Chronic cumulative glycaemic exposure is a major causative, yet modifiable factor in the development and progression of diabetic microvascular complications (3), but explains only about one third of the variability of the severity of complications in the diabetic general population (4). The Diabetes Control and Complications Trial/ Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) confirmed that glycaemic control is a robust predictor of both DSPN and CAN in patients with type 1 diabetes (5). The persistent long-term favorable effects shown during EDIC of the prior intensive insulin therapy (IT) compared to conventional insulin therapy (CT) during DCCT on diabetic microvascular complications have been termed "metabolic memory" (6,7). In the DCCT, HbA1c explained 92% of the difference in risk of neuropathy between IT and CT (8).

On the other hand, DCCT/EDIC also revealed that for most patients with type 1 diabetes, current strategies for optimizing glucose control are insufficient to fully prevent or delay the development of DSPN and CAN (5,7). In fact, 25% of the former IT group and 35% of those in the former CT group had confirmed DSPN by 13-14 years of EDIC follow-up (9), while the corresponding rates for CAN were 29 and 35%, respectively (10). The mean HbA1c levels at EDIC years 13-14 were 7.8% in each of the former insulin treatment groups (9). Although IT prevented diabetic neuropathy only to a modest degree, there is general agreement that IT targeting HbA1c levels <7% should be implemented as early as possible in the course of type 1 diabetes (5,7,11).

Based on the DCCT/EDIC observations, it is conceivable that maintenance of the recommended HbA1c level <7% in the long-term from the diabetes onset could result in more effective prevention of neuropathy. However, detailed long-term prospective studies assessing the evolution of neuropathy from the diagnosis of type 1 diabetes onward are not available. The aim of the present study was to determine the long-term effects of near-normoglycaemia as compared with poor glycaemic control from the diagnosis of type 1 diabetes over the next 24 years on the development of DSPN and CAN using a comprehensive array of measures to detect early large and small nerve fiber dysfunction in conjunction with clinical assessment.

METHODS

Patients

Thirty-two inpatients with type 1 diabetes admitted to the clinical department of the German Diabetes Center at Heinrich Heine University, Düsseldorf, in 1985 (12) participated in this prospective observational study and were followed up for 24 years with outpatient visits at 3 months and 1,2,4,5,8,10,12,14,16,18,20,22, and 24 years following the baseline assessment. The study has been carried out in accordance with the principles of the Declaration of Helsinki as revised in 1983. Informed consent was obtained from all eligible subjects after the procedures involved were fully explained. This observational natural history study included neurological assessments largely considered as standard of care in a specialised diabetes center. In 1985, no permission by the Institutional Review Board was required for this type of study. Inclusion criteria were age below 40 years, type 1 diabetes according to the National Diabetes Data Group classification (13), known diabetes duration up to 2 months, and mean blood glucose 80-160 mg/dl at the baseline assessment. Exclusion criteria were causes of neuropathy other than diabetes, clinically relevant neurological diseases and use of medication with potential influence

on nerve function. All patients underwent a standardized one-week diabetes teaching program.

To establish whether the development of neuropathy is related to the long-term degree of glycaemic control, patients were grouped according to their mean HbA1c levels during the 24 years of follow-up (excluding baseline HbA1c) in line with the current recommendations by the American Diabetes Association (11). During the 24-year period, 11 patients had mean HbA1c levels within the recommended target <7.0% [<53.0 mmol/mol] (6.5±0.1% [47.1±1.0 mmol/mol]; Group 1), whereas 21 patients had mean HbAc levels ≥7.0% % [≥53.0 mmol/mol] (8.3±0.2% [67.2±2.2 mmol/mol]; Group 2). Ten healthy subjects (5 male, age: 37.3±2.8 years, height: 172±3.2 cm, weight: 72.9±3.5 kg, BMI: 24.5±0.9 kg/m²) served as controls for the nerve conduction studies. The clinical characteristics of the diabetic groups at baseline are shown in Table 1. There were no significant differences between the groups for any of the parameters listed. Compared to the controls both diabetic groups were younger and Group 2 had a lower BMI ($P<0.05$).

Electrophysiological tests

Motor nerve conduction velocity (MNCV) was measured in the median, ulnar, and peroneal nerves, while sensory nerve conduction velocity (SNCV) was determined in the median, ulnar, and sural nerves at baseline and each subsequent visit at a skin temperature of 32-34°C using surface electrodes (EMG 2000 electromyograph, Schwarzer-Picker, Munich, Germany; Sapphire, Medelec, Woking, U.K; VikingQuest EMG, Cardinal Health, Madison, WI, USA) as previously described (12).

Quantitative sensory testing (QST)

Vibration perception threshold (VPT) was measured from the second year at the second metacarpal bone and medial malleolus using the method of limits (Vibrameter, Somedic, Stockholm). Thermal perception thresholds (TPT) to warm and cold stimuli were determined

from the 4th year at the thenar eminence and dorsum of the foot using the method of limits (Marstock stimulator, Somedic, Stockholm, Sweden; Path-Tester; Tönnies, Germany; TSA II NeuroSensory Analyzer, Medoc, Ramat Yishai, Israel) as previously reported (12,14).

Clinical examination and confirmed DSPN

Neurological examination at baseline and each visit included assessment of neuropathic symptoms, vibration sensation using the Rydel-Seiffer tuning fork, thermal perception (TipTherm, GND, Düsseldorf, Germany), ankle reflexes, and pin-prick perception. Neuropathic deficits and symptoms were scored using the Neuropathy Disability Score (NDS) and the Neuropathy Symptom Score (NSS) (15). Criteria for the definition of confirmed clinical DSPN included NDS≥2 and reduced peroneal MNCV and/or sural SNCV below the 5th percentile and elevated malleolar VPT and/or warm TPT and/or cold TPT on the dorsum of the foot above the 95th percentile of healthy subjects (12,14).

Heart rate variability (HRV)

Coefficient of variation (CV) of HRV during spontaneous breathing over 5 min was assessed from baseline and CV during deep breathing over 1 min was measured from the first year (Neurocard-Analyzer, Argustron, Mettmann, Germany; ProSciCard, CPS Medical, Wetzlar, Germany; NeuroDiag II, Dr. Vetter, Baden-Baden, Germany; VariaCardio TF5 System, AMD Group, Buckinghamshire, UK) as previously described (12,16).

The NCV, TPT, and HRV parameters were measured in the same way using equipment from different manufacturers and were comparable throughout the study.

Retinal assessment

Color retinal photographs were produced by a CR3-45NM non-mydriatic retinal camera (Canon, Tokyo, Japan) and were judged by an experienced examiner.

Laboratory methods

HbA1c was measured at baseline and each visit using the HPLC technique (Diamat, Bio-Rad, Munich, Germany). The examiners were not blinded to the HbA1c results at the individual time points. Capillary blood glucose was measured by a hexokinase-based method (ACP 5040 autoanalyzer, Eppendorf, Hamburg, Germany; Cobas C 311 analyzer, Roche Diagnostics, Mannheim, Germany). Albuminuria was measured in 12 h urine samples using the immuno-nephelometric technique (Array Protein System, Beckman, Fullerton, CA, USA) or turbidimetric method (Cobas C 311 analyzer, Roche Diagnostics, Mannheim, Germany) with a normal range: <20 µg/min. Islet cell antibodies were measured by indirect immunofluorescence using snap-frozen, unfixed human pancreatic sections of blood 0 donors (17).

Statistical analysis

Continuous data were expressed as mean±SEM. Categorical data were given as absolute or relative frequencies. Post-hoc comparisons of study endpoints between the two groups with mean HbA1c<7.0% and mean HbA1c≥7.0% from 3 months to 24 years of the study were performed using the t-test for two independent samples without missing data imputation. The level of significance was set at α=0.05.

RESULTS

Glycaemic control and microvascular complications

Insulin therapy was initiated in all patients with either subcutaneous or intravenous application of human or porcine regular insulins. After correction of the initial metabolic derangement, patients were treated with multiple daily insulin injections. During the study, two patients in each group switched to continuous subcutaneous insulin infusion. The mean HbA1c levels in the two groups studied are shown in Fig. 1A. HbA1c decreased in Group 1 from 8.8±0.6% at baseline to a mean of 6.5±0.1% throughout the 14 subsequent outpatient visits, while in Group 2 HbA1c decreased

from 9.1±0.3% to 8.3±0.2%. The daily insulin dose in Group 1 and Group 2 was 28.3±5.1 and 37.2±4.3 IU/day at baseline, 49.6±5.1 and 68.3±4.7 IU/day ($p<0.05$) at 10 years, 49.7±5.7 and 64.7±6.8 IU/day at 20 years, and 52.1±6.4 and 69.3±8.3 IU/day at 24 years, respectively. The number of cigarettes smoked per day in Group 1 and Group 2 was 6.1±2.6 and 7.7±3.3 at 4 years, 9.5±3.0 and 9.2±3.7 at 10 years, 6.1±2.7 and 6.5±3.5 at 20 years, and 3.5±2.6 and 6.9±4.3 at 24 years, respectively.

At baseline, none of the patients had nephropathy or retinopathy. The percentages of patients with micro/macroalbuminuria in Group 1 and Group 2 were 0 and 20% at 12 years and 0 and 43% at 24 years, respectively. The percentages of patients with retinopathy in Group 1 and Group 2 were 0 and 17% at 12 years and 10% and 55% at 24 years, respectively.

The most frequently used medication at 24 years in Group 1 and Group 2 included ACE inhibitors/AT1 blockers: 33 and 55%, ß blockers: 22 and 36%, diuretics: 0 and 36%, calcium channel blockers: 0 and 27%, statins: 33 and 45%, and ASS: 0 and 27% of the patients, respectively.

Peripheral and cardiac autonomic nerve function

The course of median and peroneal MNCV and median and sural SNCV in the two diabetic groups and controls is illustrated in Fig. 1B-E. No significant differences in NCV between the diabetic groups were noted at baseline. Median MNCV was faster in Group 1 than Group 2 at 2,16,18, and 24 years (Fig. 1B), while for peroneal MNCV this was the case at 5,10-20, and 24 years (Fig. 1C) (all $P<0.05$). Median SNCV was higher in Group 1 than Group 2 at 1,4,8, and 12-24 years (Fig. 1D), while accordingly sural SNCV was higher at 8,10, and 14-24 years (Fig. 1E) (all $P<0.05$). NCV in the four nerves studied after 10 and 20 years did not differ between Group 1 and the healthy control group.

The course of QST, HRV, and confirmed DSPN is shown in Fig. 3. No significant differences

between the groups in these measures were observed at the time of initial assessment. Thereafter, warm TPT was elevated in Group 2 vs Group 1 at 12,20, and 24 years (Fig. 2A), while cold TPT was increased Group 2 vs Group 1 at 12 and 24 years (Fig. 2B) (all $P<0.05$). CV of R-R intervals at rest was better in Group 1 vs Group 2 at 5,12,14,20, and 24 years (Fig. 2C), while CV during deep breathing was better in Group 1 vs Group 2 at 4,14,22, and 24 years (Fig. 2D) (all $P<0.05$). Malleolar VPT was elevated in Group 2 vs Group 1 at 10,16-20, and 24 years (Fig. 2E) (all $P<0.05$). Confirmed DSPN was not present in either group up to 4 years. After 10,16, and 24 years, cumulative prevalence of confirmed clinical DSPN in Group 2 was 39%, 46%, and 64%, respectively, whereas in Group 1 only a transient occurrence of 1 case each meeting the criteria of confirmed clinical DSPN was observed at 10-14,18, and 20 years of follow-up (Fig. 2F).

The changes in the diabetic groups in NCV over 24 years, VPT over 22 years, TPT over 20 years, and CV of HRV at rest and during deep breathing over 24 and 23 years, respectively, as well as the changes in NCV in the control group over 20 years are listed in Table 2. The most pronounced decline in Group 2 was noted for peroneal MNCV and sural SNCV which was approximately 6-fold and 3-fold faster than in Group 1, respectively. SNCV declined slightly more rapidly (0.85-1.08 m/s/year) than MNCV (0.60-1.04 m/s/year) in Group 2. The magnitude of decline in MNCV and SNCV over 20 years in Group 1 was comparable to that seen in the control group. For the majority of the remaining measures, progression in Group 2 was 2.5-fold to 13-fold faster than in Group 1. The highest rate of deterioration in Group 2 was noted for cold TPT on the foot and malleolar VPT.

The numbers of patients lost to follow-up at the individual time points in Group 1 and Group 2 varied and were 2 and 3 at 5 years, 1 and 4 at 12 years, 1 and 6 at 20 years, and 2 and 10 at 24 years, respectively. Among these, 2 patients from Group 2 died during follow-up, one female from diabetic ketoacidosis after 12 years and one male supposedly from severe hypoglycaemia

after 18 years of follow-up. Other reasons for drop-out were inconvenience of regular follow-up and moving away from the area.

DISCUSSION

The results of this prospective study demonstrate that near-normoglycaemia maintained from the diagnosis of type 1 diabetes over the next 24 years effectively prevented a 3-fold and 6-fold faster annual hyperglycaemia-related decline in sural SNCV and peroneal MNCV and preserved NCV at the expected age-induced level of change. In poorly controlled type 1 diabetes, MNCV, SNCV, and HRV started to deteriorate earlier (after approximately 4 years) than VPT and warm/cold TPT (after approximately 10-12 years), while the cumulative prevalence of confirmed clinical DSPN rose considerably from zero at diabetes diagnosis to one third after 12 years and almost two thirds after 24 years. In contrast, none of the patients who maintained near-normoglycaemia throughout 24 years presented with confirmed clinical DSPN after this period. Thus, the present findings are inasmuch novel as they indicate that near-normoglycaemia instituted at the time of diagnosis of type 1 diabetes and subsequently maintained over more than two decades may fully prevent the development of DSPN and diminished HRV.

There are no studies with which the present data can be directly compared, since previous studies did not focus on newly diagnosed type 1 diabetes but included patients with longer disease duration (3,5-10,12,18-26). The EURODIAB Prospective Complications Study (PCS) and Pittsburgh Epidemiology of Diabetes Complications Study including type 1 diabetes subjects with baseline duration of diabetes of 12.4 and 16.9 years, respectively, identified HbA1c as an independent predictor of incident DSPN over 7.3 and 6 years (18,19) and CAN over 7.3 and 4.7 years (20,21), respectively. In newly diagnosed diabetic children followed over 10 years, a correlation between HbA1c and measures of nerve function was found for peroneal MNCV but,

in contrast to the present study, neither for sural SNCV nor HRV during normal and deep breathing (27). In a study of adolescents with a mean age of 15.5 years and diabetes duration of 6.8 years followed over approximately 13 years on average, HbA1c during the first year of follow-up was the strongest predictor for the development of clinical DSPN, but the rates of decline in nerve function in relation to HbA1c were not reported (22).

The Oslo Study recently reported that HbA1c was an important risk factor in the development of DSPN over 27 years, but the mean diabetes duration at study start was 12.8 years, and mean HbA1c measured yearly was 8.0%. Dividing patients by low and high cumulative glycaemic exposure (3) after 27 years revealed a mean decrease in peroneal MNCV of 6.7. vs 13.0 m/s and sural SNCV of 8.1 vs 15.3 m/s, but the difference for these changes between the groups did not reach statistical significance (23). CAN was assessed at the 18-year follow-up revealing abnormalities in ≥2 out of 3 parameters in 29.4% of patients with mean HbA1c <8.4% compared to 66.7% in those with mean HbA1c ≥8.4%, but no prospective data was available (24). Likewise, in the Stockholm Diabetes Intervention Study (SDIS) including type 1 diabetic subjects with an initial mean diabetes duration of 17 years, peroneal MNCV and sural SNCV deteriorated after 10 years of both IT and CT (mean HbA1c: 7.2 vs 8.3%), albeit to a lesser degree in the former treatment group (25). Similar to the Oslo Study, CAN was assessed only once after 11.4 years, with only 3 out of 6 parameters were significantly better after IT than CT (26). Thus, in line with DCCT/EDIC (5,9,10), the development of DSPN and CAN in the Oslo Study and SDIS could not be entirely prevented but only slightly delayed.

In contrast to type 1 diabetes, the role of glycaemic control in the development of DSPN in type 2 diabetes is less clear. In newly diagnosed type 2 diabetes patients, the rates of definite or probable DSPN increased from 8.3% at baseline to 41.9% after 10 years, but mean HbA1c was not higher in patients who had DSPN at 10 years compared to those who did not (9.6 vs 8.9%) (28). In

patients who developed parasympathetic CAN after 10 years (65%), HbA1c at 5 years was higher than in those who did not (9.2 vs 8.0%), but this difference was not found for sympathetic CAN (29).

The American Diabetes Association considers HbA1c levels <7% a reasonable treatment goal for many non-pregnant adults. More stringent HbA1c goals (<6.5%) are suggested for selected individual patients, if this can be achieved without significant hypoglycaemia or other adverse effects of treatment (11). These recommendations entirely suit the well-controlled group in the present study, which maintained mean HbA1c at 6.5%.

Study limitations

The present study has some limitations. First, since this was an observational study, allocation of patients to the two groups of glycaemic control could only be done post-hoc. Second, the small study sample may be a source of bias. Third, prospective assessment of neuropathy included nerve function rather than nerve structure such as skin biopsy or corneal confocal microscopy (30), since the latter became available only recently. Nonetheless, we believe that the unequivocally preserved large and small fiber function in favor of near-normoglycaemia supports the notion that the degree of glycaemia governs the development of neuropathy over 24 years.

CONCLUSIONS

In conclusion, near-normoglycaemia maintained from the diagnosis of type 1 diabetes over the next 24 years was associated with a complete prevention of hyperglycaemia-induced slowing in nerve conduction, confirmed clinical DSPN, and reduced HRV. In contrast, poor glycaemic control was associated with a continuous and substantial increase in the cumulative prevalence of confirmed clinical DSPN over 24 years. Hence, poor glycaemic control constitutes the paramount causative factor contributing to the evolution of neuropathy in type 1 diabetes. The annual rates of progression of NCV, QST, and HRV in well controlled compared with poorly controlled

patients may provide a rationale for the design of future long-term clinical trials aimed at prevention of diabetic neuropathy.

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D.Z. designed the study. D.Z., M.B., and M.S.T. researched data. D.Z. wrote the manuscript. D.Z. and M.R. reviewed and edited the manuscript. D.Z. is the guarantor of this work and, as such, had full access to all of 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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Table 1: Demographic and clinical characteristic of the patients at baseline.

	Group 1 HbA1c <7.0%	**Group 2 HbA1c ≥7.0%**
n	11	21
Sex (m/f)	7/4	14/7
Age (years)	19.4±0.9	20.8±1.4
Height (cm)	175±2.2	176±2.0
Weight (kg)	68.6±6.4	64.0±3.1
BMI (kg/m²)	21.9±1.7	20.4±0.8
Systolic blood pressure (mmHg)	127±7.0	117±3.9
Diastolic blood pressure (mmHg)	81.3±6.6	69.4±3.9
Duration of symptoms (weeks)*	5.5±1.7	6.7±2.3
Duration of insulin treatment (weeks)	2.6±0.5	2.7±0.5
Insulin dose (IU/day)	28.3±5.1	37.2±4.3
HbA1c (%) HbA1c (mmol/mol)	8.8±0.6 72.6±5.2	9.1±0.3 76.5±2.9
Mean blood glucose (mg/dl)$^{+}$	111±8.0	121±4.2
Islet cell antibody positive (%)	75	84

*Symptoms due to hyperglycemia prior to diabetes diagnosis
$^{+}$Based on 5 values during the day of neurological assessment.

Table 2: Changes in motor and sensory nerve conduction velocity (MNCV, SNCV), vibration perception threshold (VPT), thermal perception threshold (TPT), and coefficient of variation (CV) of heart rate variability (HRV) during the study.

	Group (HbA1c)	Absolute change over 24 years	Absolute change per year	Percent change over 24 years	Percent change per year
Median MNCV	Control	-2.2*	-0.11	-3.8*	-0.19
	Group 1 (<7%)	-2.7	-0.11	-4.6	-0.19
	Group 2 (≥7%)	-8.2	-0.34	-14.4	-0.60
Ulnar MNCV	Control	-5.4*	-0.27	-8.7*	-0.44
	Group 1 (<7%)	-2.6	-0.11	-4.4	-0.18
	Group 2 (≥7%)	-11.5	-0.48	-18.9	-0.79
Peroneal MNCV	Control	-2.6*	-0.13	-5.3*	-0.27
	Group 1 (<7%)	-1.8	-0.08	-3.8	-0.16
	Group 2 (≥7%)	-11.8	-0.49	-24.9	-1.04
Median SNCV	Control	-1.5*	-0.08	-2.5*	-0.13
	Group 1 (<7%)	-5.5	-0.23	-9.2	-0.38
	Group 2 (≥7%)	-11.7	-0.49	-20.5	-0.85
Ulnar SNCV	Control	0.4*	0.02	0.7*	0.04
	Group 1 (<7%)	-0.4	-0.02	-0.7	-0.03
	Group 2 (≥7%)	-11.1	-0.46	-20.3	-0.85
Sural SNCV	Control	-4.8*	-0.24	-9.1*	-0.46
	Group 1 (<7%)	-4.2	-0.18	-8.5	-0.35
	Group 2 (≥7%)	-12.4	-0.52	-25.9	-1.08
Metacarpal VPT	Group 1 (<7%)	0.04^{+}	0.002	16.7^{+}	0.76
	Group 2 (≥7%)	0.24^{+}	0.01	114^{+}	5.18
Malleolar VPT	Group 1 (<7%)	0.47^{+}	0.02	162^{+}	7.36
	Group 2 (≥7%)	2.43^{+}	0.11	496^{+}	22.50
TPT Warm Foot	Group 1 (<7%)	0.40*	0.02	8.33*	0.42
	Group 2 (≥7%)	5.30*	0.27	110*	5.50
TPT Cold Foot	Group 1 (<7%)	0.70*	0.04	50.0*	2.50
	Group 2 (≥7%)	7.30*	0.37	608*	30.40
CV of HRV at rest	Group 1 (<7%)	-1.22	-0.05	-17.9	-0.75
	Group 2 (≥7%)	-2.69	-0.11	-46.1	-1.92
CV of HRV at deep breathing	Group 1 (<7%)	0.38$^{\#}$	0.02	3.9$^{\#}$	0.17
	Group 2 (≥7%)	-4.51$^{\#}$	-0.20	-49.1$^{\#}$	-2.13

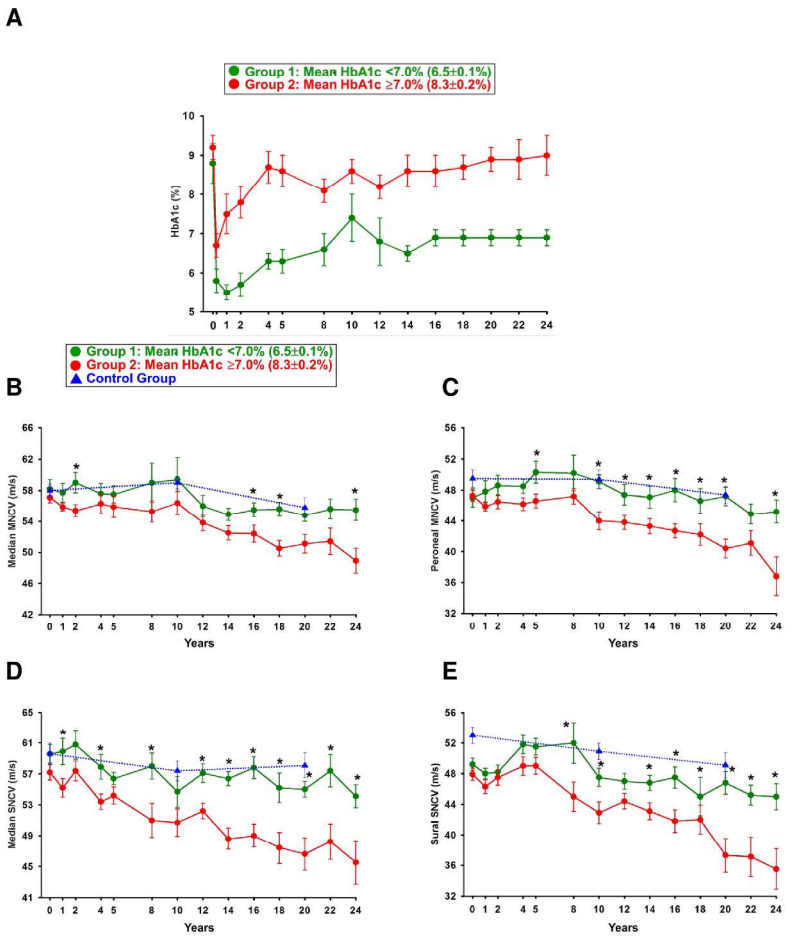
*Change over 20 years, $^{+}$change over 22 years, $^{\#}$change over 23 years

Legends to figures

Figure 1: HbA1c levels (A), median motor nerve conduction velocity (MNCV) (B), peroneal MNCV (C), median sensory nerve conduction velocity (SNCV) (D), and sural SNCV (E) over 24 years in Group 1 (mean HbAc<7.0%; n=11) and Group 2 (mean HbA1c≥7.0%; n=21), and healthy control subjects (n=11) over 20 years. * $p<0.05$ for Group 1 vs Group 2

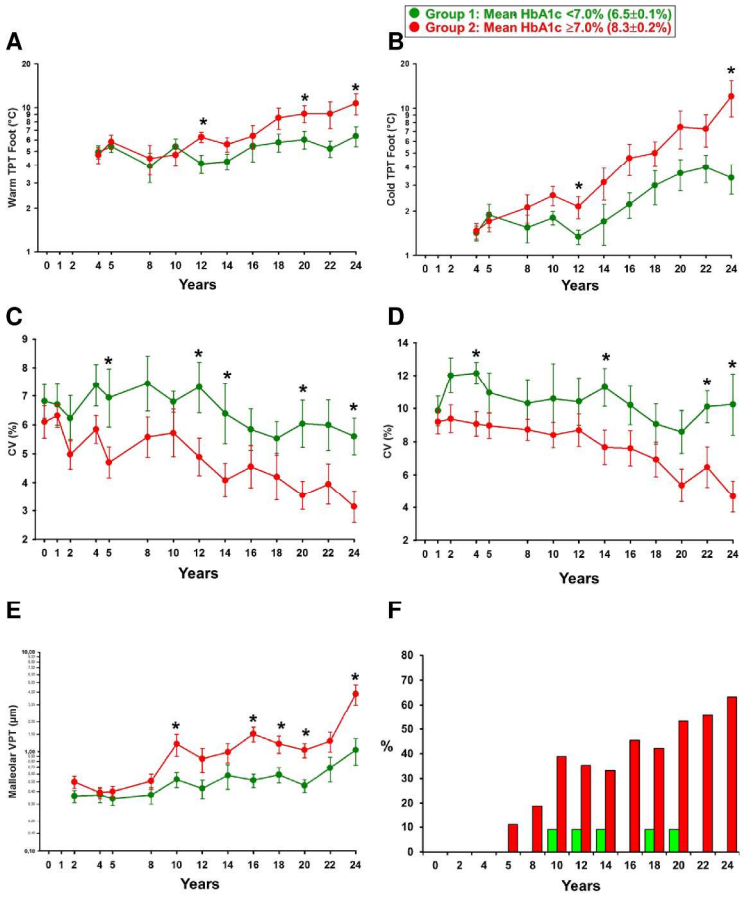
Figure 2: Warm thermal perception threshold (TPT) (A) and cold TPT (B) on the dorsum of the foot from the 4th year, coefficient of variation (CV) of R-R intervals at rest over 24 years (C) and CV during deep breathing from the first year (D), vibration perception threshold (VPT) from the second year (E), and cumulative prevalence of confirmed clinical diabetic sensorimotor polyneuropathy (DSPN) over 24 years (F). * $p<0.05$ for Group 1 vs Group 2

Fig. 1



899x1164mm (72 x 72 DPI)

Fig. 2



899x1164mm (72 x 72 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page number
1Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5-6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	6
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-7
Bias	9	Describe any efforts to address potential sources of bias	13
Study size	10	Explain how the study size was arrived at	n/a
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	8
		(*c*) Explain how missing data were addressed	8
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	8,10
		(*e*) Describe any sensitivity analyses	n/a

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10
		(b) Give reasons for non-participation at each stage	10
		(c) Consider use of a flow diagram	n/a
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	19
		(b) Indicate number of participants with missing data for each variable of interest	10
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	5
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	8-10
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8-10
		(*b*) Report category boundaries when continuous variables were categorized	n/a
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	n/a
Discussion			
Key results	18	Summarise key results with reference to study objectives	10-11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Near-normoglycaemia prevents the development of neuropathy. A 24-year prospective study from the diagnosis of type 1 diabetes

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Near-normoglycaemia prevents the development of neuropathy. A 24-year prospective study from the diagnosis of type 1 diabetes

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Abstract

Objective: Complete prevention of diabetic neuropathies has not been previously demonstrated. We sought to determine whether long-term near-normoglycemia maintained from the diagnosis of type 1 diabetes prevents polyneuropathy and cardiac autonomic dysfunction.

Design: Prospective observational study over 24 years.

Setting: Ambulatory care.

Participants: Thirty-two newly diagnosed type 1 diabetic patients aged 20.3±1.0 years, duration of diabetes 2.7±0.3 weeks.

Intervention: Insulin therapy according to standards of care.

Primary and secondary outcome measures: Motor and sensory nerve conduction velocity (MNCV, SNCV), heart rate variability (HRV), and confirmed clinical polyneuropathy measured at 15 time points over 24 years and quantitative sensory testing (QST) determined over 20-22 years.

Results: Eleven patients were well controlled over 24 years with mean HbA1c<7.0% (6.5±0.1%; Group 1), whereas 21 patients were poorly controlled (mean HbA1c≥7.0%: 8.3±0.2%; Group 2). After 24 years, MNCV was faster in Group 1 vs Group 2 in the median (55.5±1.6 vs 48.9±1.6 m/s), ulnar (56.5±1.5 vs 49.3±1.7 m/s), and peroneal nerve (44.7±1.6 vs 36.8±2.5 m/s), while SNCV was faster in the median (53.6±1.6 vs 45.5±2.8 m/s), ulnar (54.7±1.8 vs 43.0±3.9 m/s), and sural nerve (44.5±1.8 vs 35.5±2.6 m/s) (all P<0.05). The annual decline in peroneal MNCV and sural SNCV in Group 1 was 6-fold and 3-fold faster in Group 2 than in Group 1, respectively. Likewise, impairment in QST and HRV developed at faster rates in Group 2. After 24 years, 64% of patients in Group 2 but none in Group 1 developed confirmed clinical polyneuropathy.

Conclusions: Near-normoglycemia maintained from the diagnosis of type 1 diabetes over 24 years effectively prevented the decline in hyperglycemia-related peripheral and autonomic nerve function and development of confirmed clinical polyneuropathy.

Strengths and limitations of this study

- This prospective observational study conducted over 24 years evaluated whether long-term near-normoglycemia maintained from the diagnosis of type 1 diabetes prevents polyneuropathy and cardiac autonomic dysfunction.
- Prospective assessment of neuropathy included detailed quantitative assessment of nerve function rather than nerve structure, since the latter became quantifiable only recently.
- The results of this study may provide a rationale for the design of future long-term clinical trials aimed at prevention of diabetic neuropathy.
- Allocation of patients to two groups markedly differing as to the degree of glycaemic control could only be done post-hoc, and the small study sample may be a source of bias.

INTRODUCTION

Approximately one third of patients with diabetes are affected by diabetic sensorimotor polyneuropathy (DSPN), which leads to considerable morbidity due to neuropathic pain and foot ulcers, while cardiovascular autonomic neuropathy (CAN) is associated with a 3-fold increased risk of mortality (1,2). Chronic cumulative glycaemic exposure is a major causative, yet modifiable factor in the development and progression of diabetic microvascular complications (3), but explains only about one third of the variability of the severity of complications in the diabetic general population (4). The Diabetes Control and Complications Trial/ Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) confirmed that glycaemic control is a robust predictor of both DSPN and CAN in patients with type 1 diabetes (5). The persistent long-term favorable effects shown during EDIC of the prior intensive insulin therapy (IT) compared to conventional insulin therapy (CT) during DCCT on diabetic microvascular complications have been termed "metabolic memory" (6,7). In the DCCT, HbA1c explained 92% of the difference in risk of neuropathy between IT and CT (8).

On the other hand, DCCT/EDIC also revealed that for most patients with type 1 diabetes, current strategies for optimizing glucose control are insufficient to fully prevent or delay the development of DSPN and CAN (5,7). In fact, 25% of the former IT group and 35% of those in the former CT group had confirmed DSPN by 13-14 years of EDIC follow-up (9), while the corresponding rates for CAN were 29 and 35%, respectively (10). The mean HbA1c levels at EDIC years 13-14 were 7.8% in each of the former insulin treatment groups (9). Although IT prevented diabetic neuropathy only to a modest degree, there is general agreement that IT targeting HbA1c levels <7% should be implemented as early as possible in the course of type 1 diabetes (5,7,11).

Based on the DCCT/EDIC observations, it is conceivable that maintenance of the recommended HbA1c level <7% in the long-term from the diabetes onset could result in more effective prevention of neuropathy. However, detailed long-term prospective studies assessing the evolution of neuropathy from the diagnosis of type 1 diabetes onward are not available. The aim of the present study was to determine the long-term effects of near-normoglycaemia as compared with poor glycaemic control from the diagnosis of type 1 diabetes over the next 24 years on the development of DSPN and CAN using a comprehensive array of measures to detect early large and small nerve fiber dysfunction in conjunction with clinical assessment.

METHODS

Patients

Thirty-two inpatients with type 1 diabetes admitted to the clinical department of the German Diabetes Center at Heinrich Heine University, Düsseldorf, in 1985 (12) participated in this prospective observational study and were followed up for 24 years with outpatient visits at 3 months and 1,2,4,5,8,10,12,14,16,18,20,22, and 24 years following the baseline assessment. The study has been carried out in accordance with the principles of the Declaration of Helsinki as revised in 1983. Informed consent was obtained from all eligible subjects after the procedures involved were fully explained. Inclusion criteria were age below 40 years, type 1 diabetes according to the National Diabetes Data Group classification (13), known diabetes duration up to 2 months, and mean blood glucose 80-160 mg/dl at the baseline assessment. Exclusion criteria were causes of neuropathy other than diabetes, clinically relevant neurological diseases and use of medication with potential influence on nerve function. All patients underwent a standardized one-week diabetes teaching program.

To establish whether the development of neuropathy is related to the long-term degree of

glycaemic control, patients were grouped according to their mean HbA1c levels during the 24 years of follow-up (excluding baseline HbA1c) in line with the current recommendations by the American Diabetes Association (11). During the 24-year period, 11 patients had mean HbA1c levels within the recommended target <7.0% [<53.0 mmol/mol] (6.5±0.1% [47.1±1.0 mmol/mol]; Group 1), whereas 21 patients had mean HbAc levels ≥7.0% % [≥53.0 mmol/mol] (8.3±0.2% [67.2±2.2 mmol/mol]; Group 2). Ten healthy subjects (5 male, age: 37.3±2.8 years, height: 172±3.2 cm, weight: 72.9±3.5 kg, BMI: 24.5±0.9 kg/m²) served as controls for the nerve conduction studies. The clinical characteristics of the diabetic groups at baseline are shown in Table 1. There were no significant differences between the groups for any of the parameters listed. Compared to the controls both diabetic groups were younger and Group 2 had a lower BMI (P<0.05).

Electrophysiological tests

Motor nerve conduction velocity (MNCV) was measured in the median, ulnar, and peroneal nerves, while sensory nerve conduction velocity (SNCV) was determined in the median, ulnar, and sural nerves at baseline and each subsequent visit at a skin temperature of 32-34°C using surface electrodes (EMG 2000 electromyograph, Schwarzer-Picker, Munich, Germany; Sapphire, Medelec, Woking, U.K; VikingQuest EMG, Cardinal Health, Madison, WI, USA) as previously described (12).

Quantitative sensory testing (QST)

Vibration perception threshold (VPT) was measured from the second year at the second metacarpal bone and medial malleolus using the method of limits (Vibrameter, Somedic, Stockholm). Thermal perception thresholds (TPT) to warm and cold stimuli were determined from the 4th year at the thenar eminence and dorsum of the foot using the method of limits (Marstock stimulator, Somedic, Stockholm, Sweden; Path-Tester; Tönnies, Germany; TSA II

NeuroSensory Analyzer, Medoc, Ramat Yishai, Israel) as previously reported (12,14).

Clinical examination and confirmed DSPN

Neurological examination at baseline and each visit included assessment of neuropathic symptoms, vibration sensation using the Rydel-Seiffer tuning fork, thermal perception (TipTherm, GND, Düsseldorf, Germany), ankle reflexes, and pin-prick perception. Neuropathic deficits and symptoms were scored using the Neuropathy Disability Score (NDS) and the Neuropathy Symptom Score (NSS) (15). Criteria for the definition of confirmed clinical DSPN included NDS≥2 and reduced peroneal MNCV and/or sural SNCV below the 5th percentile and elevated malleolar VPT and/or warm TPT and/or cold TPT on the dorsum of the foot above the 95th percentile of healthy subjects (12,14).

Heart rate variability (HRV)

Coefficient of variation (CV) of HRV during spontaneous breathing over 5 min was assessed from baseline and CV during deep breathing over 1 min was measured from the first year (Neurocard-Analyzer, Argustron, Mettmann, Germany; ProSciCard, CPS Medical, Wetzlar, Germany; NeuroDiag II, Dr. Vetter, Baden-Baden, Germany; VariaCardio TF5 System, AMD Group, Buckinghamshire, UK) as previously described (12,16).

The NCV, TPT, and HRV parameters were measured in the same way using equipment from different manufacturers and were comparable throughout the study.

Retinal assessment

Color retinal photographs were produced by a CR3-45NM non-mydriatic retinal camera (Canon, Tokyo, Japan) and were judged by an experienced examiner.

Laboratory methods

HbA1c was measured at baseline and each visit using the HPLC technique (Diamat, Bio-Rad, Munich, Germany). The examiners were not blinded to the HbA1c results at the individual time

points. Capillary blood glucose was measured by a hexokinase-based method (ACP 5040 autoanalyzer, Eppendorf, Hamburg, Germany; Cobas C 311 analyzer, Roche Diagnostics, Mannheim, Germany). Albuminuria was measured in 12 h urine samples using the immuno-nephelometric technique (Array Protein System, Beckman, Fullerton, CA, USA) or turbidimetric method (Cobas C 311 analyzer, Roche Diagnostics, Mannheim, Germany) with a normal range: <20 µg/min. Islet cell antibodies were measured by indirect immunofluorescence using snap-frozen, unfixed human pancreatic sections of blood 0 donors (17).

Statistical analysis

Continuous data were expressed as mean±SEM. Categorical data were given as absolute or relative frequencies. Post-hoc comparisons of study endpoints between the two groups with mean HbA1c<7.0% and mean HbA1c≥7.0% from 3 months to 24 years of the study were performed using the t-test for two independent samples without missing data imputation. The level of significance was set at α=0.05.

RESULTS

Glycaemic control and microvascular complications

Insulin therapy was initiated in all patients with either subcutaneous or intravenous application of human or porcine regular insulins. After correction of the initial metabolic derangement, patients were treated with multiple daily insulin injections according to standards of care throughout the study. During the study, two patients in each group switched to continuous subcutaneous insulin infusion. The mean HbA1c levels in the two groups studied are shown in Fig. 1A. HbA1c decreased in Group 1 from 8.8±0.6% at baseline to a mean of 6.5±0.1% throughout the 14 subsequent outpatient visits, while in Group 2 HbA1c decreased from 9.1±0.3% to 8.3±0.2%. The daily insulin dose in Group 1 and Group 2 was 28.3±5.1 and 37.2±4.3 IU/day at baseline,

49.6±5.1 and 68.3±4.7 IU/day ($p<0.05$) at 10 years, 49.7±5.7 and 64.7±6.8 IU/day at 20 years, and 52.1±6.4 and 69.3±8.3 IU/day at 24 years, respectively. The number of cigarettes smoked per day in Group 1 and Group 2 was 6.1±2.6 and 7.7±3.3 at 4 years, 9.5±3.0 and 9.2±3.7 at 10 years, 6.1±2.7 and 6.5±3.5 at 20 years, and 3.5±2.6 and 6.9±4.3 at 24 years, respectively.

At baseline, none of the patients had nephropathy or retinopathy. The percentages of patients with micro/macroalbuminuria in Group 1 and Group 2 were 0 and 20% at 12 years and 0 and 43% at 24 years, respectively. The percentages of patients with retinopathy in Group 1 and Group 2 were 0 and 17% at 12 years and 10% and 55% at 24 years, respectively.

The most frequently used medication at 24 years in Group 1 and Group 2 included ACE inhibitors/AT1 blockers: 33 and 55%, ß blockers: 22 and 36%, diuretics: 0 and 36%, calcium channel blockers: 0 and 27%, statins: 33 and 45%, and ASS: 0 and 27% of the patients, respectively.

Peripheral and cardiac autonomic nerve function

The course of median and peroneal MNCV and median and sural SNCV in the two diabetic groups and controls is illustrated in Fig. 1B-E. No significant differences in NCV between the diabetic groups were noted at baseline. Median MNCV was faster in Group 1 than Group 2 at 2,16,18, and 24 years (Fig. 1B), while for peroneal MNCV this was the case at 5,10-20, and 24 years (Fig. 1C) (all $P<0.05$). Median SNCV was higher in Group 1 than Group 2 at 1,4,8, and 12-24 years (Fig. 1D), while accordingly sural SNCV was higher at 8,10, and 14-24 years (Fig. 1E) (all $P<0.05$). NCV in the four nerves studied after 10 and 20 years did not differ between Group 1 and the healthy control group.

The course of QST, HRV, and confirmed DSPN is shown in Fig. 2. No significant differences between the groups in these measures were observed at the time of initial assessment. Thereafter, warm TPT was elevated in Group 2 vs Group 1 at 12,20, and 24 years (Fig. 2A), while cold TPT

was increased Group 2 vs Group 1 at 12 and 24 years (Fig. 2B) (all P<0.05). CV of R-R intervals at rest was better in Group 1 vs Group 2 at 5,12,14,20, and 24 years (Fig. 2C), while CV during deep breathing was better in Group 1 vs Group 2 at 4,14,22, and 24 years (Fig. 2D) (all P<0.05). Malleolar VPT was elevated in Group 2 vs Group 1 at 10,16-20, and 24 years (Fig. 2E) (all P<0.05). Confirmed DSPN was not present in either group up to 4 years. After 10,16, and 24 years, cumulative prevalence of confirmed clinical DSPN in Group 2 was 39%, 46%, and 64%, respectively, whereas in Group 1 only a transient occurrence of 1 case each meeting the criteria of confirmed clinical DSPN was observed at 10-14,18, and 20 years of follow-up (Fig. 2F).

The changes in the diabetic groups in NCV over 24 years, VPT over 22 years, TPT over 20 years, and CV of HRV at rest and during deep breathing over 24 and 23 years, respectively, as well as the changes in NCV in the control group over 20 years are listed in Table 2. The most pronounced decline in Group 2 was noted for peroneal MNCV and sural SNCV which was approximately 6-fold and 3-fold faster than in Group 1, respectively. SNCV declined slightly more rapidly (0.85-1.08 m/s/year) than MNCV (0.60-1.04 m/s/year) in Group 2. The magnitude of decline in MNCV and SNCV over 20 years in Group 1 was comparable to that seen in the control group. For the majority of the remaining measures, progression in Group 2 was 2.5-fold to 13-fold faster than in Group 1. The highest rate of deterioration in Group 2 was noted for cold TPT on the foot and malleolar VPT.

The numbers of patients lost to follow-up at the individual time points in Group 1 and Group 2 varied and were 2 and 3 at 5 years, 1 and 4 at 12 years, 1 and 6 at 20 years, and 2 and 10 at 24 years, respectively. Among these, 2 patients from Group 2 died during follow-up, one female from diabetic ketoacidosis after 12 years and one male supposedly from severe hypoglycaemia after 18 years of follow-up. Other reasons for drop-out were inconvenience of regular follow-up and moving away from the area.

DISCUSSION

The results of this prospective study demonstrate that near-normoglycaemia maintained from the diagnosis of type 1 diabetes over the next 24 years effectively prevented a 3-fold and 6-fold faster annual hyperglycaemia-related decline in sural SNCV and peroneal MNCV and preserved NCV at the expected age-induced level of change. In poorly controlled type 1 diabetes, MNCV, SNCV, and HRV started to deteriorate earlier (after approximately 4 years) than VPT and warm/cold TPT (after approximately 10-12 years), while the cumulative prevalence of confirmed clinical DSPN rose considerably from zero at diabetes diagnosis to one third after 12 years and almost two thirds after 24 years. In contrast, none of the patients who maintained near-normoglycaemia throughout 24 years presented with confirmed clinical DSPN after this period. Thus, the present findings are inasmuch novel as they indicate that near-normoglycaemia instituted at the time of diagnosis of type 1 diabetes and subsequently maintained over more than two decades may fully prevent the development of DSPN and diminished HRV.

There are no studies with which the present data can be directly compared, since previous studies did not focus on newly diagnosed type 1 diabetes but included patients with longer disease duration (3,5-10,12,18-26). The EURODIAB Prospective Complications Study (PCS) and Pittsburgh Epidemiology of Diabetes Complications Study including type 1 diabetes subjects with baseline duration of diabetes of 12.4 and 16.9 years, respectively, identified HbA1c as an independent predictor of incident DSPN over 7.3 and 6 years (18,19) and CAN over 7.3 and 4.7 years (20,21), respectively. In newly diagnosed diabetic children followed over 10 years, a correlation between HbA1c and measures of nerve function was found for peroneal MNCV but, in contrast to the present study, neither for sural SNCV nor HRV during normal and deep breathing (27). In a study of adolescents with a mean age of 15.5 years and diabetes duration of 6.8 years followed over approximately 13 years on average, HbA1c during the first year of

follow-up was the strongest predictor for the development of clinical DSPN, but the rates of decline in nerve function in relation to HbA1c were not reported (22).

The Oslo Study recently reported that HbA1c was an important risk factor in the development of DSPN over 27 years, but the mean diabetes duration at study start was 12.8 years, and mean HbA1c measured yearly was 8.0%. Dividing patients by low and high cumulative glycaemic exposure (3) after 27 years revealed a mean decrease in peroneal MNCV of 6.7. vs 13.0 m/s and sural SNCV of 8.1 vs 15.3 m/s, but the difference for these changes between the groups did not reach statistical significance (23). CAN was assessed at the 18-year follow-up revealing abnormalities in ≥2 out of 3 parameters in 29.4% of patients with mean HbA1c <8.4% compared to 66.7% in those with mean HbA1c ≥8.4%, but no prospective data was available (24). Likewise, in the Stockholm Diabetes Intervention Study (SDIS) including type 1 diabetic subjects with an initial mean diabetes duration of 17 years, peroneal MNCV and sural SNCV deteriorated after 10 years of both IT and CT (mean HbA1c: 7.2 vs 8.3%), albeit to a lesser degree in the former treatment group (25). Similar to the Oslo Study, CAN was assessed only once after 11.4 years, with only 3 out of 6 parameters were significantly better after IT than CT (26). Thus, in line with DCCT/EDIC (5,9,10), the development of DSPN and CAN in the Oslo Study and SDIS could not be entirely prevented but only slightly delayed.

In contrast to type 1 diabetes, the role of glycaemic control in the development of DSPN in type 2 diabetes is less clear. In newly diagnosed type 2 diabetes patients, the rates of definite or probable DSPN increased from 8.3% at baseline to 41.9% after 10 years, but mean HbA1c was not higher in patients who had DSPN at 10 years compared to those who did not (9.6 vs 8.9%) (28). In patients who developed parasympathetic CAN after 10 years (65%), HbA1c at 5 years was higher than in those who did not (9.2 vs 8.0%), but this difference was not found for sympathetic CAN (29).

The American Diabetes Association considers HbA1c levels <7% a reasonable treatment goal for many non-pregnant adults. More stringent HbA1c goals (<6.5%) are suggested for selected individual patients, if this can be achieved without significant hypoglycaemia or other adverse effects of treatment (11). These recommendations entirely suit the well-controlled group in the present study, which maintained mean HbA1c at 6.5%.

Study limitations

The present study has some limitations. First, since this was an observational study, allocation of patients to the two groups of glycaemic control could only be done post-hoc. Second, the small study sample may be a source of bias, particularly in view of the long study duration which also inevitably resulted in some drop-outs. Third, prospective assessment of neuropathy included nerve function rather than nerve structure such as skin biopsy or corneal confocal microscopy (30), since the latter became available only recently. Nonetheless, we believe that the unequivocally preserved large and small fiber function in favor of near-normoglycaemia supports the notion that the degree of glycaemia governs the development of neuropathy over 24 years.

CONCLUSIONS

In conclusion, near-normoglycaemia maintained from the diagnosis of type 1 diabetes over the next 24 years was associated with a complete prevention of hyperglycaemia-induced slowing in nerve conduction, confirmed clinical DSPN, and reduced HRV. In contrast, poor glycaemic control was associated with a continuous and substantial increase in the cumulative prevalence of confirmed clinical DSPN over 24 years. Hence, poor glycaemic control constitutes the paramount causative factor contributing to the evolution of neuropathy in type 1 diabetes. The annual rates of progression of NCV, QST, and HRV in well controlled compared with poorly controlled patients may provide a rationale for the design of future long-term clinical trials aimed at prevention of diabetic neuropathy.

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D.Z. designed the study. D.Z., M.B., and M.S.T. researched data. D.Z. wrote the manuscript. D.Z. and M.R. reviewed and edited the manuscript. D.Z. is the guarantor of this work and, as such, had full access to all of 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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Table 1: Demographic and clinical characteristic of the patients at baseline.

	Group 1 HbA1c <7.0%	Group 2 HbA1c ≥7.0%
n	11	21
Sex (m/f)	7/4	14/7
Age (years)	19.4±0.9	20.8±1.4
Height (cm)	175±2.2	176±2.0
Weight (kg)	68.6±6.4	64.0±3.1
BMI (kg/m²)	21.9±1.7	20.4±0.8
Systolic blood pressure (mmHg)	127±7.0	117±3.9
Diastolic blood pressure (mmHg)	81.3±6.6	69.4±3.9
Duration of symptoms (weeks)*	5.5±1.7	6.7±2.3
Duration of insulin treatment (weeks)	2.6±0.5	2.7±0.5
Insulin dose (IU/day)	28.3±5.1	37.2±4.3
HbA1c (%) HbA1c (mmol/mol)	8.8±0.6 72.6±5.2	9.1±0.3 76.5±2.9
Mean blood glucose (mg/dl)[+]	111±8.0	121±4.2
Islet cell antibody positive (%)	75	84

*Symptoms due to hyperglycemia prior to diabetes diagnosis
[+]Based on 5 values during the day of neurological assessment.

Table 2: Changes in motor and sensory nerve conduction velocity (MNCV, SNCV), vibration perception threshold (VPT), thermal perception threshold (TPT), and coefficient of variation (CV) of heart rate variability (HRV) during the study.

	Group (HbA1c)	Absolute change over 24 years	Absolute change per year	Percent change over 24 years	Percent change per year
Median MNCV (m/s or %)	Control	-2.2*	-0.11	-3.8*	-0.19
	Group 1 (<7%)	-2.7	-0.11	-4.6	-0.19
	Group 2 (≥7%)	-8.2	-0.34	-14.4	-0.60
Ulnar MNCV (m/s or %)	Control	-5.4*	-0.27	-8.7*	-0.44
	Group 1 (<7%)	-2.6	-0.11	-4.4	-0.18
	Group 2 (≥7%)	-11.5	-0.48	-18.9	-0.79
Peroneal MNCV (m/s or %)	Control	-2.6*	-0.13	-5.3*	-0.27
	Group 1 (<7%)	-1.8	-0.08	-3.8	-0.16
	Group 2 (≥7%)	-11.8	-0.49	-24.9	-1.04
Median SNCV (m/s or %)	Control	-1.5*	-0.08	-2.5*	-0.13
	Group 1 (<7%)	-5.5	-0.23	-9.2	-0.38
	Group 2 (≥7%)	-11.7	-0.49	-20.5	-0.85
Ulnar SNCV (m/s or %)	Control	0.4*	0.02	0.7*	0.04
	Group 1 (<7%)	-0.4	-0.02	-0.7	-0.03
	Group 2 (≥7%)	-11.1	-0.46	-20.3	-0.85
Sural SNCV (m/s or %)	Control	-4.8*	-0.24	-9.1*	-0.46
	Group 1 (<7%)	-4.2	-0.18	-8.5	-0.35
	Group 2 (≥7%)	-12.4	-0.52	-25.9	-1.08
Metacarpal VPT (µm or %)	Group 1 (<7%)	0.04^{+}	0.002	16.7^{+}	0.76
	Group 2 (≥7%)	0.24^{+}	0.01	114^{+}	5.18
Malleolar VPT (µm or %)	Group 1 (<7%)	0.47^{+}	0.02	162^{+}	7.36
	Group 2 (≥7%)	2.43^{+}	0.11	496^{+}	22.50
TPT Warm Foot (°C or %)	Group 1 (<7%)	0.40*	0.02	8.33*	0.42
	Group 2 (≥7%)	5.30*	0.27	110*	5.50
TPT Cold Foot (°C or %)	Group 1 (<7%)	0.70*	0.04	50.0*	2.50
	Group 2 (≥7%)	7.30*	0.37	608*	30.40
CV of HRV at rest (% for CV or %)	Group 1 (<7%)	-1.22	-0.05	-17.9	-0.75
	Group 2 (≥7%)	-2.69	-0.11	-46.1	-1.92
CV of HRV at deep breathing (% for CV or %)	Group 1 (<7%)	0.38$^{\#}$	0.02	3.9$^{\#}$	0.17
	Group 2 (≥7%)	-4.51$^{\#}$	-0.20	-49.1$^{\#}$	-2.13

*Change over 20 years, $^{+}$change over 22 years, $^{\#}$change over 23 years

Legends to figures

Figure 1: HbA1c levels (A), median motor nerve conduction velocity (MNCV) (B), peroneal MNCV (C), median sensory nerve conduction velocity (SNCV) (D), and sural SNCV (E) over 24 years in Group 1 (mean HbAc<7.0%; n=11) and Group 2 (mean HbA1c≥7.0%; n=21), and healthy control subjects (n=11) over 20 years. * $p<0.05$ for Group 1 vs Group 2

Figure 2: Warm thermal perception threshold (TPT) (A) and cold TPT (B) on the dorsum of the foot from the 4th year, coefficient of variation (CV) of R-R intervals at rest over 24 years (C) and CV during deep breathing from the first year (D), vibration perception threshold (VPT) from the second year (E), and cumulative prevalence of confirmed clinical diabetic sensorimotor polyneuropathy (DSPN) over 24 years (F). * $p<0.05$ for Group 1 vs Group 2

Fig. 1

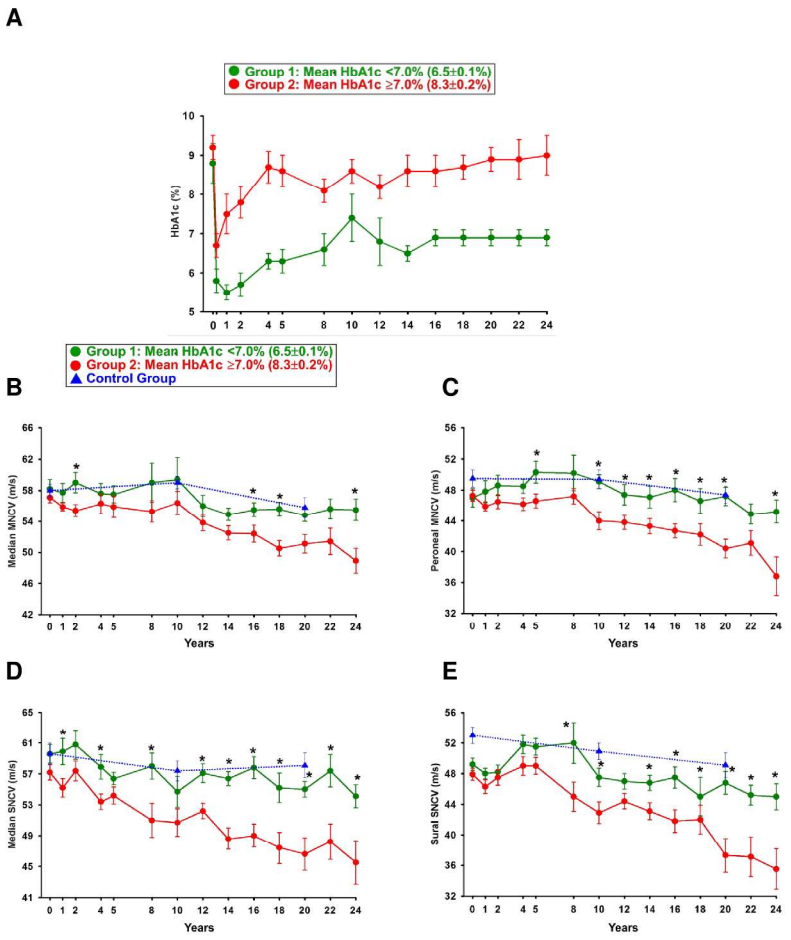

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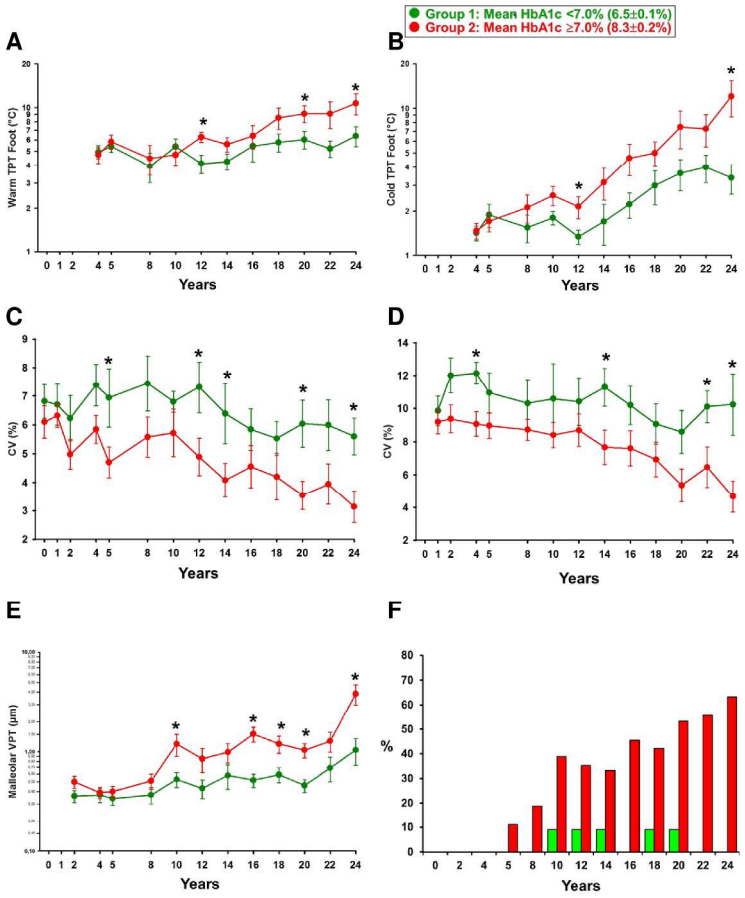
Fig. 2



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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page number
1Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5-6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	6
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-7
Bias	9	Describe any efforts to address potential sources of bias	13
Study size	10	Explain how the study size was arrived at	n/a
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	8
		(*c*) Explain how missing data were addressed	8
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	8,10
		(*e*) Describe any sensitivity analyses	n/a

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10
		(b) Give reasons for non-participation at each stage	10
		(c) Consider use of a flow diagram	n/a
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	19
		(b) Indicate number of participants with missing data for each variable of interest	10
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	5
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	8-10
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8-10
		(*b*) Report category boundaries when continuous variables were categorized	n/a
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	n/a
Discussion			
Key results	18	Summarise key results with reference to study objectives	10-11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Near-normoglycaemia and the development of neuropathy. A 24-year prospective study from the diagnosis of type 1 diabetes

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Near-normoglycaemia and development of neuropathy. A 24-year prospective study from the diagnosis of type 1 diabetes

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Abstract

Objective: Complete prevention of diabetic neuropathies has not been previously demonstrated. We sought to determine whether long-term near-normoglycemia maintained from the diagnosis of type 1 diabetes is associated with polyneuropathy and cardiac autonomic dysfunction.

Design: Prospective observational study over 24 years.

Setting: Ambulatory care.

Participants: Thirty-two newly diagnosed type 1 diabetic patients aged 20.3±1.0 years, duration of diabetes 2.7±0.3 weeks.

Intervention: Insulin therapy according to standards of care.

Primary and secondary outcome measures: Motor and sensory nerve conduction velocity (MNCV, SNCV), heart rate variability (HRV), and confirmed clinical polyneuropathy measured at 15 time points over 24 years and quantitative sensory testing (QST) determined over 20-22 years.

Results: Eleven patients were well controlled over 24 years with mean HbA1c<7.0% (6.5±0.1%; Group 1), whereas 21 patients were poorly controlled (mean HbA1c≥7.0%: 8.3±0.2%; Group 2). After 24 years, MNCV was faster in Group 1 vs Group 2 in the median (55.5±1.6 vs 48.9±1.6 m/s), ulnar (56.5±1.5 vs 49.3±1.7 m/s), and peroneal nerve (44.7±1.6 vs 36.8±2.5 m/s), while SNCV was faster in the median (53.6±1.6 vs 45.5±2.8 m/s), ulnar (54.7±1.8 vs 43.0±3.9 m/s), and sural nerve (44.5±1.8 vs 35.5±2.6 m/s) (all P<0.05). The annual decline in peroneal MNCV and sural SNCV in Group 1 was 6-fold and 3-fold faster in Group 2 than in Group 1, respectively. Likewise, impairment in QST and HRV developed at faster rates in Group 2. After 24 years, 64% of patients in Group 2 but none in Group 1 developed confirmed clinical polyneuropathy.

Conclusions: Near-normoglycemia maintained from the diagnosis of type 1 diabetes over 24 years was associated with a complete prevention of the decline in hyperglycemia-related peripheral and autonomic nerve function and development of confirmed clinical polyneuropathy.

Strengths and limitations of this study

- This prospective observational study conducted over 24 years evaluated whether long-term near-normoglycemia maintained from the diagnosis of type 1 diabetes prevents polyneuropathy and cardiac autonomic dysfunction.
- Prospective assessment of neuropathy included detailed quantitative assessment of nerve function rather than nerve structure, since the latter became quantifiable only recently.
- The results of this study may provide a rationale for the design of future long-term clinical trials aimed at prevention of diabetic neuropathy.
- Allocation of patients to two groups markedly differing as to the degree of glycaemic control could only be done post-hoc, and the small study sample may be a source of bias.

INTRODUCTION

Approximately one third of patients with diabetes are affected by diabetic sensorimotor polyneuropathy (DSPN), which leads to considerable morbidity due to neuropathic pain and foot ulcers, while cardiovascular autonomic neuropathy (CAN) is associated with a 3-fold increased risk of mortality (1,2). Chronic cumulative glycaemic exposure is a major causative, yet modifiable factor in the development and progression of diabetic microvascular complications (3), but explains only about one third of the variability of the severity of complications in the diabetic general population (4). The Diabetes Control and Complications Trial/ Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) confirmed that glycaemic control is a robust predictor of both DSPN and CAN in patients with type 1 diabetes (5). The persistent long-term favorable effects shown during EDIC of the prior intensive insulin therapy (IT) compared to conventional insulin therapy (CT) during DCCT on diabetic microvascular complications have been termed “metabolic memory” (6,7). In the DCCT, HbA1c explained 92% of the difference in risk of neuropathy between IT and CT (8). On the other hand, DCCT/EDIC also revealed that for most patients with type 1 diabetes, current strategies for optimizing glucose control are insufficient to fully prevent or delay the development of DSPN and CAN (5,7). In fact, 25% of the former IT group and 35% of those in the former CT group had confirmed DSPN by 13-14 years of EDIC follow-up (9), while the corresponding rates for CAN were 29 and 35%, respectively (10). The mean HbA1c levels at EDIC years 13-14 were 7.8% in each of the former insulin treatment groups (9). Although IT prevented diabetic neuropathy only to a modest degree, there is general agreement that IT targeting HbA1c levels <7% should be implemented as early as possible in the course of type 1 diabetes (5,7,11).

Based on the DCCT/EDIC observations, it is conceivable that maintenance of the recommended HbA1c level <7% in the long-term from the diabetes onset could result in more effective

prevention of neuropathy. However, detailed long-term prospective studies assessing the evolution of neuropathy from the diagnosis of type 1 diabetes onward are not available. The aim of the present study was to determine the long-term development of DSPN and CAN during near-normoglycaemia compared to poor glycaemic control from the diagnosis of type 1 diabetes over the next 24 years on the using a comprehensive array of measures to detect early large and small nerve fiber dysfunction in conjunction with clinical assessment.

METHODS

Patients

Thirty-two inpatients with type 1 diabetes admitted to the clinical department of the German Diabetes Center at Heinrich Heine University, Düsseldorf, in 1985 (12) participated in this prospective observational study and were followed up for 24 years with outpatient visits at 3 months and 1,2,4,5,8,10,12,14,16,18,20,22, and 24 years following the baseline assessment. The study has been carried out in accordance with the principles of the Declaration of Helsinki as revised in 1983. Informed consent was obtained from all eligible subjects after the procedures involved were fully explained. Inclusion criteria were age below 40 years, type 1 diabetes according to the National Diabetes Data Group classification (13), known diabetes duration up to 2 months, and mean blood glucose 80-160 mg/dl at the baseline assessment. Exclusion criteria were causes of neuropathy other than diabetes, clinically relevant neurological diseases and use of medication with potential influence on nerve function. All patients underwent a standardized one-week diabetes teaching program.

To establish whether the development of neuropathy is related to the long-term degree of glycaemic control, patients were grouped according to their mean HbA1c levels during the 24 years of follow-up (excluding baseline HbA1c) in line with the current recommendations by the

American Diabetes Association (11). During the 24-year period, 11 patients had mean HbA1c levels within the recommended target <7.0% [<53.0 mmol/mol] (6.5±0.1% [47.1±1.0 mmol/mol]; Group 1), whereas 21 patients had mean HbAc levels ≥7.0% % [≥53.0 mmol/mol] (8.3±0.2% [67.2±2.2 mmol/mol]; Group 2). Ten healthy subjects (5 male, age: 37.3±2.8 years, height: 172±3.2 cm, weight: 72.9±3.5 kg, BMI: 24.5±0.9 kg/m²) served as controls for the nerve conduction studies. The clinical characteristics of the diabetic groups at baseline are shown in Table 1. There were no significant differences between the groups for any of the parameters listed. Compared to the controls both diabetic groups were younger and Group 2 had a lower BMI ($P<0.05$).

Electrophysiological tests

Motor nerve conduction velocity (MNCV) was measured in the median, ulnar, and peroneal nerves, while sensory nerve conduction velocity (SNCV) was determined in the median, ulnar, and sural nerves at baseline and each subsequent visit at a skin temperature of 32-34°C using surface electrodes (EMG 2000 electromyograph, Schwarzer-Picker, Munich, Germany; Sapphire, Medelec, Woking, U.K; VikingQuest EMG, Cardinal Health, Madison, WI, USA) as previously described (12).

Quantitative sensory testing (QST)

Vibration perception threshold (VPT) was measured from the second year at the second metacarpal bone and medial malleolus using the method of limits (Vibrameter, Somedic, Stockholm). Thermal perception thresholds (TPT) to warm and cold stimuli were determined from the 4th year at the thenar eminence and dorsum of the foot using the method of limits (Marstock stimulator, Somedic, Stockholm, Sweden; Path-Tester; Tönnies, Germany; TSA II NeuroSensory Analyzer, Medoc, Ramat Yishai, Israel) as previously reported (12,14).

Clinical examination and confirmed DSPN

Neurological examination at baseline and each visit included assessment of neuropathic symptoms, vibration sensation using the Rydel-Seiffer tuning fork, thermal perception (TipTherm, GND, Düsseldorf, Germany), ankle reflexes, and pin-prick perception. Neuropathic deficits and symptoms were scored using the Neuropathy Disability Score (NDS) and the Neuropathy Symptom Score (NSS) (15). Criteria for the definition of confirmed clinical DSPN included NDS≥2 and reduced peroneal MNCV and/or sural SNCV below the 5th percentile and elevated malleolar VPT and/or warm TPT and/or cold TPT on the dorsum of the foot above the 95th percentile of healthy subjects (12,14).

Heart rate variability (HRV)

Coefficient of variation (CV) of HRV during spontaneous breathing over 5 min was assessed from baseline and CV during deep breathing over 1 min was measured from the first year (Neurocard-Analyzer, Argustron, Mettmann, Germany; ProSciCard, CPS Medical, Wetzlar, Germany; NeuroDiag II, Dr. Vetter, Baden-Baden, Germany; VariaCardio TF5 System, AMD Group, Buckinghamshire, UK) as previously described (12,16).

The NCV, TPT, and HRV parameters were measured in the same way using equipment from different manufacturers and were comparable throughout the study.

Retinal assessment

Color retinal photographs were produced by a CR3-45NM non-mydriatic retinal camera (Canon, Tokyo, Japan) and were judged by an experienced examiner.

Laboratory methods

HbA1c was measured at baseline and each visit using the HPLC technique (Diamat, Bio-Rad, Munich, Germany). The examiners were not blinded to the HbA1c results at the individual time points. Capillary blood glucose was measured by a hexokinase-based method (ACP 5040 autoanalyzer, Eppendorf, Hamburg, Germany; Cobas C 311 analyzer, Roche Diagnostics,

Mannheim, Germany). Albuminuria was measured in 12 h urine samples using the immuno-nephelometric technique (Array Protein System, Beckman, Fullerton, CA, USA) or turbidimetric method (Cobas C 311 analyzer, Roche Diagnostics, Mannheim, Germany) with a normal range: <20 µg/min. Islet cell antibodies were measured by indirect immunofluorescence using snap-frozen, unfixed human pancreatic sections of blood 0 donors (17).

Statistical analysis

Continuous data were expressed as mean±SEM. Categorical data were given as absolute or relative frequencies. Post-hoc comparisons of study endpoints between the two groups with mean HbA1c<7.0% and mean HbA1c≥7.0% from 3 months to 24 years of the study were performed using the t-test for two independent samples without missing data imputation. The level of significance was set at α=0.05.

RESULTS

Glycaemic control and microvascular complications

Insulin therapy was initiated in all patients with either subcutaneous or intravenous application of human or porcine regular insulins. After correction of the initial metabolic derangement, patients were treated with multiple daily insulin injections according to standards of care throughout the study. During the study, two patients in each group switched to continuous subcutaneous insulin infusion. The mean HbA1c levels in the two groups studied are shown in Fig. 1A. HbA1c decreased in Group 1 from 8.8±0.6% at baseline to a mean of 6.5±0.1% throughout the 14 subsequent outpatient visits, while in Group 2 HbA1c decreased from 9.1±0.3% to 8.3±0.2%. The daily insulin dose in Group 1 and Group 2 was 28.3±5.1 and 37.2±4.3 IU/day at baseline, 49.6±5.1 and 68.3±4.7 IU/day ($p<0.05$) at 10 years, 49.7±5.7 and 64.7±6.8 IU/day at 20 years, and 52.1±6.4 and 69.3±8.3 IU/day at 24 years, respectively. The number of cigarettes smoked per day in Group 1

and Group 2 was 6.1±2.6 and 7.7±3.3 at 4 years, 9.5±3.0 and 9.2±3.7 at 10 years, 6.1±2.7 and 6.5±3.5 at 20 years, and 3.5±2.6 and 6.9±4.3 at 24 years, respectively.

At baseline, none of the patients had nephropathy or retinopathy. The percentages of patients with micro/macroalbuminuria in Group 1 and Group 2 were 0 and 20% at 12 years and 0 and 43% at 24 years, respectively. The percentages of patients with retinopathy in Group 1 and Group 2 were 0 and 17% at 12 years and 10% and 55% at 24 years, respectively.

The most frequently used medication at 24 years in Group 1 and Group 2 included ACE inhibitors/AT1 blockers: 33 and 55%, ß blockers: 22 and 36%, diuretics: 0 and 36%, calcium channel blockers: 0 and 27%, statins: 33 and 45%, and ASS: 0 and 27% of the patients, respectively.

Peripheral and cardiac autonomic nerve function

The course of median and peroneal MNCV and median and sural SNCV in the two diabetic groups and controls is illustrated in Fig. 1B-E. No significant differences in NCV between the diabetic groups were noted at baseline. Median MNCV was faster in Group 1 than Group 2 at 2,16,18, and 24 years (Fig. 1B), while for peroneal MNCV this was the case at 5,10-20, and 24 years (Fig. 1C) (all $P<0.05$). Median SNCV was higher in Group 1 than Group 2 at 1,4,8, and 12-24 years (Fig. 1D), while accordingly sural SNCV was higher at 8,10, and 14-24 years (Fig. 1E) (all $P<0.05$). NCV in the four nerves studied after 10 and 20 years did not differ between Group 1 and the healthy control group.

The course of QST, HRV, and confirmed DSPN is shown in Fig. 2. No significant differences between the groups in these measures were observed at the time of initial assessment. Thereafter, warm TPT was elevated in Group 2 vs Group 1 at 12,20, and 24 years (Fig. 2A), while cold TPT was increased Group 2 vs Group 1 at 12 and 24 years (Fig. 2B) (all $P<0.05$). CV of R-R intervals at rest was better in Group 1 vs Group 2 at 5,12,14,20, and 24 years (Fig. 2C), while CV during deep

breathing was better in Group 1 vs Group 2 at 4,14,22, and 24 years (Fig. 2D) (all P<0.05). Malleolar VPT was elevated in Group 2 vs Group 1 at 10,16-20, and 24 years (Fig. 2E) (all P<0.05). Confirmed DSPN was not present in either group up to 4 years. After 10,16, and 24 years, cumulative prevalence of confirmed clinical DSPN in Group 2 was 39%, 46%, and 64%, respectively, whereas in Group 1 only a transient occurrence of 1 case each meeting the criteria of confirmed clinical DSPN was observed at 10-14,18, and 20 years of follow-up (Fig. 2F).

The changes in the diabetic groups in NCV over 24 years, VPT over 22 years, TPT over 20 years, and CV of HRV at rest and during deep breathing over 24 and 23 years, respectively, as well as the changes in NCV in the control group over 20 years are listed in Table 2. The most pronounced decline in Group 2 was noted for peroneal MNCV and sural SNCV which was approximately 6-fold and 3-fold faster than in Group 1, respectively. SNCV declined slightly more rapidly (0.85-1.08 m/s/year) than MNCV (0.60-1.04 m/s/year) in Group 2. The magnitude of decline in MNCV and SNCV over 20 years in Group 1 was comparable to that seen in the control group. For the majority of the remaining measures, progression in Group 2 was 2.5-fold to 13-fold faster than in Group 1. The highest rate of deterioration in Group 2 was noted for cold TPT on the foot and malleolar VPT.

The numbers of patients lost to follow-up at the individual time points in Group 1 and Group 2 varied and were 2 and 3 at 5 years, 1 and 4 at 12 years, 1 and 6 at 20 years, and 2 and 10 at 24 years, respectively. Among these, 2 patients from Group 2 died during follow-up, one female from diabetic ketoacidosis after 12 years and one male supposedly from severe hypoglycaemia after 18 years of follow-up. Other reasons for drop-out were inconvenience of regular follow-up and moving away from the area.

DISCUSSION

The results of this prospective study demonstrate that near-normoglycaemia maintained from the

diagnosis of type 1 diabetes over the next 24 years was associated with an effective prevention of a 3-fold and 6-fold faster annual hyperglycaemia-related decline in sural SNCV and peroneal MNCV and preservation of NCV at the expected age-induced level of change. In poorly controlled type 1 diabetes, MNCV, SNCV, and HRV started to deteriorate earlier (after approximately 4 years) than VPT and warm/cold TPT (after approximately 10-12 years), while the cumulative prevalence of confirmed clinical DSPN rose considerably from zero at diabetes diagnosis to one third after 12 years and almost two thirds after 24 years. In contrast, none of the patients who maintained near-normoglycaemia throughout 24 years presented with confirmed clinical DSPN after this period. Thus, the present findings are inasmuch novel as they indicate that near-normoglycaemia instituted at the time of diagnosis of type 1 diabetes and subsequently maintained over more than two decades may fully prevent the development of DSPN and diminished HRV.

There are no studies with which the present data can be directly compared, since previous studies did not focus on newly diagnosed type 1 diabetes but included patients with longer disease duration (3,5-10,12,18-26). The EURODIAB Prospective Complications Study (PCS) and Pittsburgh Epidemiology of Diabetes Complications Study including type 1 diabetes subjects with baseline duration of diabetes of 12.4 and 16.9 years, respectively, identified HbA1c as an independent predictor of incident DSPN over 7.3 and 6 years (18,19) and CAN over 7.3 and 4.7 years (20,21), respectively. In newly diagnosed diabetic children followed over 10 years, a correlation between HbA1c and measures of nerve function was found for peroneal MNCV but, in contrast to the present study, neither for sural SNCV nor HRV during normal and deep breathing (27). In a study of adolescents with a mean age of 15.5 years and diabetes duration of 6.8 years followed over approximately 13 years on average, HbA1c during the first year of follow-up was the strongest predictor for the development of clinical DSPN, but the rates of decline in nerve function in

relation to HbA1c were not reported (22).

The Oslo Study recently reported that HbA1c was an important risk factor in the development of DSPN over 27 years, but the mean diabetes duration at study start was 12.8 years, and mean HbA1c measured yearly was 8.0%. Dividing patients by low and high cumulative glycaemic exposure (3) after 27 years revealed a mean decrease in peroneal MNCV of 6.7. vs 13.0 m/s and sural SNCV of 8.1 vs 15.3 m/s, but the difference for these changes between the groups did not reach statistical significance (23). CAN was assessed at the 18-year follow-up revealing abnormalities in ≥2 out of 3 parameters in 29.4% of patients with mean HbA1c <8.4% compared to 66.7% in those with mean HbA1c ≥8.4%, but no prospective data was available (24). Likewise, in the Stockholm Diabetes Intervention Study (SDIS) including type 1 diabetic subjects with an initial mean diabetes duration of 17 years, peroneal MNCV and sural SNCV deteriorated after 10 years of both IT and CT (mean HbA1c: 7.2 vs 8.3%), albeit to a lesser degree in the former treatment group (25). Similar to the Oslo Study, CAN was assessed only once after 11.4 years, with only 3 out of 6 parameters were significantly better after IT than CT (26). Thus, in line with DCCT/EDIC (5,9,10), the development of DSPN and CAN in the Oslo Study and SDIS could not be entirely prevented but only slightly delayed.

In contrast to type 1 diabetes, the role of glycaemic control in the development of DSPN in type 2 diabetes is less clear. In newly diagnosed type 2 diabetes patients, the rates of definite or probable DSPN increased from 8.3% at baseline to 41.9% after 10 years, but mean HbA1c was not higher in patients who had DSPN at 10 years compared to those who did not (9.6 vs 8.9%) (28). In patients who developed parasympathetic CAN after 10 years (65%), HbA1c at 5 years was higher than in those who did not (9.2 vs 8.0%), but this difference was not found for sympathetic CAN (29).

The American Diabetes Association considers HbA1c levels <7% a reasonable treatment goal for many non-pregnant adults. More stringent HbA1c goals (<6.5%) are suggested for selected

individual patients, if this can be achieved without significant hypoglycaemia or other adverse effects of treatment (11). These recommendations entirely suit the well-controlled group in the present study, which maintained mean HbA1c at 6.5%.

Study limitations

The present study has some limitations. First, since this was an observational study, allocation of patients to the two groups of glycaemic control could only be done post-hoc. Second, the small study sample may be a source of bias, particularly in view of the long study duration which also inevitably resulted in some drop-outs. Third, prospective assessment of neuropathy included nerve function rather than nerve structure such as skin biopsy or corneal confocal microscopy (30), since the latter became available only recently. Nonetheless, we believe that the unequivocally preserved large and small fiber function in favor of near-normoglycaemia supports the notion that the degree of glycaemia governs the development of neuropathy over 24 years.

CONCLUSIONS

In conclusion, near-normoglycaemia maintained from the diagnosis of type 1 diabetes over the next 24 years was associated with a complete prevention of hyperglycaemia-induced slowing in nerve conduction, confirmed clinical DSPN, and reduced HRV. In contrast, poor glycaemic control was associated with a continuous and substantial increase in the cumulative prevalence of confirmed clinical DSPN over 24 years. Hence, poor glycaemic control constitutes the paramount causative factor contributing to the evolution of neuropathy in type 1 diabetes. The annual rates of progression of NCV, QST, and HRV in well controlled compared with poorly controlled patients may provide a rationale for the design of future long-term clinical trials aimed at prevention of diabetic neuropathy.

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No potential conflicts of interest relevant to this article were reported.

D.Z. designed the study. D.Z., M.B., and M.S.T. researched data. D.Z. wrote the manuscript. D.Z. and M.R. reviewed and edited the manuscript. D.Z. is the guarantor of this work and, as such, had full access to all of 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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Table 1: Demographic and clinical characteristic of the patients at baseline.

	Group 1 HbA1c <7.0%	**Group 2 HbA1c ≥7.0%**
n	11	21
Sex (m/f)	7/4	14/7
Age (years)	19.4±0.9	20.8±1.4
Height (cm)	175±2.2	176±2.0
Weight (kg)	68.6±6.4	64.0±3.1
BMI (kg/m²)	21.9±1.7	20.4±0.8
Systolic blood pressure (mmHg)	127±7.0	117±3.9
Diastolic blood pressure (mmHg)	81.3±6.6	69.4±3.9
Duration of symptoms (weeks)*	5.5±1.7	6.7±2.3
Duration of insulin treatment (weeks)	2.6±0.5	2.7±0.5
Insulin dose (IU/day)	28.3±5.1	37.2±4.3
HbA1c (%) HbA1c (mmol/mol)	8.8±0.6 72.6±5.2	9.1±0.3 76.5±2.9
Mean blood glucose (mg/dl)[+]	111±8.0	121±4.2
Islet cell antibody positive (%)	75	84

*Symptoms due to hyperglycemia prior to diabetes diagnosis
[+]Based on 5 values during the day of neurological assessment.

Table 2: Changes in motor and sensory nerve conduction velocity (MNCV, SNCV), vibration perception threshold (VPT), thermal perception threshold (TPT), and coefficient of variation (CV) of heart rate variability (HRV) during the study.

	Group (HbA1c)	Absolute change over 24 years	Absolute change per year	Percent change over 24 years	Percent change per year
Median MNCV (m/s or %)	Control	-2.2*	-0.11	-3.8*	-0.19
	Group 1 (<7%)	-2.7	-0.11	-4.6	-0.19
	Group 2 (≥7%)	-8.2	-0.34	-14.4	-0.60
Ulnar MNCV (m/s or %)	Control	-5.4*	-0.27	-8.7*	-0.44
	Group 1 (<7%)	-2.6	-0.11	-4.4	-0.18
	Group 2 (≥7%)	-11.5	-0.48	-18.9	-0.79
Peroneal MNCV (m/s or %)	Control	-2.6*	-0.13	-5.3*	-0.27
	Group 1 (<7%)	-1.8	-0.08	-3.8	-0.16
	Group 2 (≥7%)	-11.8	-0.49	-24.9	-1.04
Median SNCV (m/s or %)	Control	-1.5*	-0.08	-2.5*	-0.13
	Group 1 (<7%)	-5.5	-0.23	-9.2	-0.38
	Group 2 (≥7%)	-11.7	-0.49	-20.5	-0.85
Ulnar SNCV (m/s or %)	Control	0.4*	0.02	0.7*	0.04
	Group 1 (<7%)	-0.4	-0.02	-0.7	-0.03
	Group 2 (≥7%)	-11.1	-0.46	-20.3	-0.85
Sural SNCV (m/s or %)	Control	-4.8*	-0.24	-9.1*	-0.46
	Group 1 (<7%)	-4.2	-0.18	-8.5	-0.35
	Group 2 (≥7%)	-12.4	-0.52	-25.9	-1.08
Metacarpal VPT (µm or %)	Group 1 (<7%)	0.04^{+}	0.002	16.7^{+}	0.76
	Group 2 (≥7%)	0.24^{+}	0.01	114^{+}	5.18
Malleolar VPT (µm or %)	Group 1 (<7%)	0.47^{+}	0.02	162^{+}	7.36
	Group 2 (≥7%)	2.43^{+}	0.11	496^{+}	22.50
TPT Warm Foot (°C or %)	Group 1 (<7%)	0.40*	0.02	8.33*	0.42
	Group 2 (≥7%)	5.30*	0.27	110*	5.50
TPT Cold Foot (°C or %)	Group 1 (<7%)	0.70*	0.04	50.0*	2.50
	Group 2 (≥7%)	7.30*	0.37	608*	30.40
CV of HRV at rest (% for CV or %)	Group 1 (<7%)	-1.22	-0.05	-17.9	-0.75
	Group 2 (≥7%)	-2.69	-0.11	-46.1	-1.92
CV of HRV at deep breathing (% for CV or %)	Group 1 (<7%)	0.38$^{\#}$	0.02	3.9$^{\#}$	0.17
	Group 2 (≥7%)	-4.51$^{\#}$	-0.20	-49.1$^{\#}$	-2.13

*Change over 20 years, $^{+}$change over 22 years, $^{\#}$change over 23 years

Legends to figures

Figure 1: HbA1c levels (A), median motor nerve conduction velocity (MNCV) (B), peroneal MNCV (C), median sensory nerve conduction velocity (SNCV) (D), and sural SNCV (E) over 24 years in Group 1 (mean HbAc<7.0%; n=11) and Group 2 (mean HbA1c≥7.0%; n=21), and healthy control subjects (n=11) over 20 years. * $p<0.05$ for Group 1 vs Group 2

Figure 2: Warm thermal perception threshold (TPT) (A) and cold TPT (B) on the dorsum of the foot from the 4th year, coefficient of variation (CV) of R-R intervals at rest over 24 years (C) and CV during deep breathing from the first year (D), vibration perception threshold (VPT) from the second year (E), and cumulative prevalence of confirmed clinical diabetic sensorimotor polyneuropathy (DSPN) over 24 years (F). * $p<0.05$ for Group 1 vs Group 2

Fig. 1

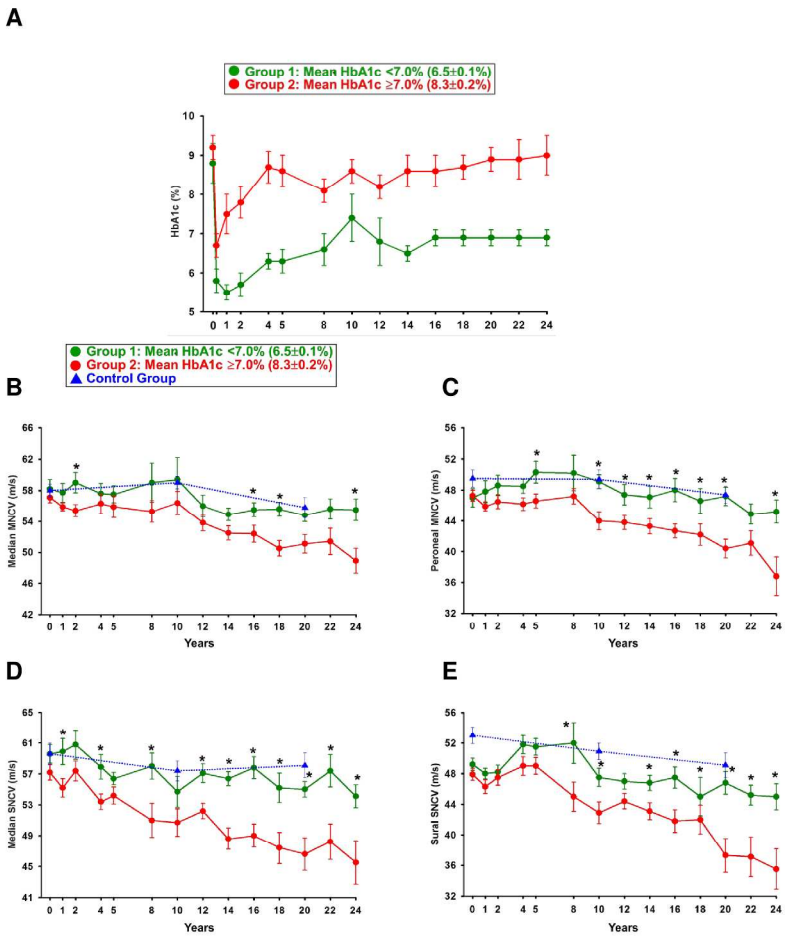

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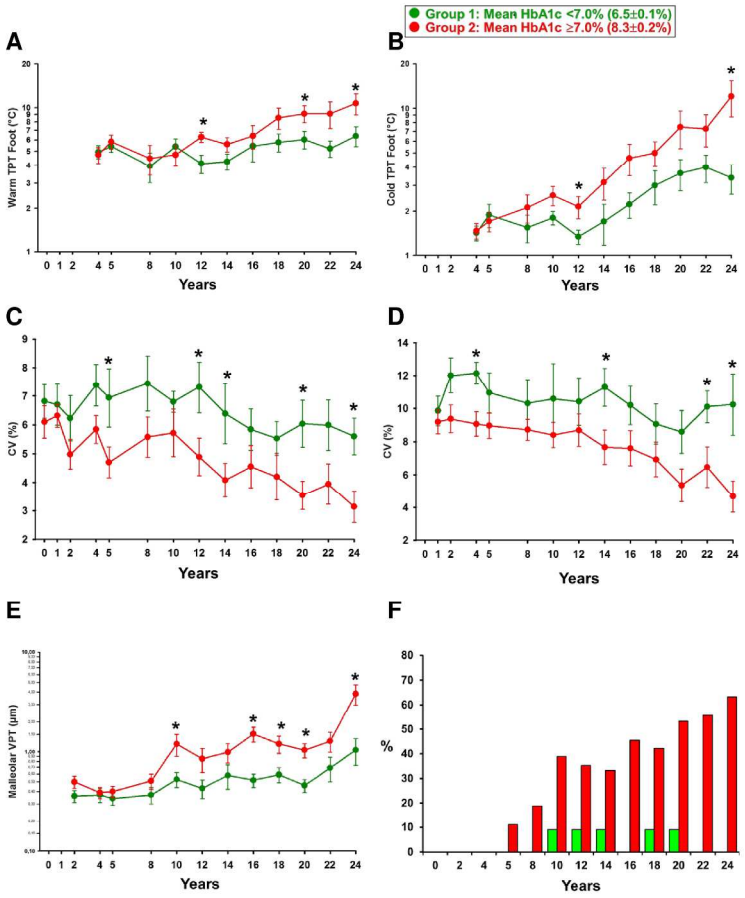
Fig. 2



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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page number
1Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5-6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	6
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-7
Bias	9	Describe any efforts to address potential sources of bias	13
Study size	10	Explain how the study size was arrived at	n/a
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	8
		(*c*) Explain how missing data were addressed	8
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	8,10
		(*e*) Describe any sensitivity analyses	n/a

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10
		(b) Give reasons for non-participation at each stage	10
		(c) Consider use of a flow diagram	n/a
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	19
		(b) Indicate number of participants with missing data for each variable of interest	10
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	5
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	8-10
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8-10
		(*b*) Report category boundaries when continuous variables were categorized	n/a
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	n/a
Discussion			
Key results	18	Summarise key results with reference to study objectives	10-11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.