Endoneurial Capillary Abnormalities Presage Deterioration of Glucose Tolerance and Accompany Peripheral Neuropathy in Man

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To explore whether microangiopathy is associated with disturbed glucose tolerance and peripheral neuropathy, we assessed endoneurial capillary morphology in sural nerve biopsies from men with diabetes, impaired glucose tolerance (IGT), and normal glucose tolerance (NGT). Baseline morphology was related to glucose tolerance and neuropathy at baseline and at follow-up 6 years later. Capillary density (in number per millimeters squared) at baseline was higher in subjects with diabetes (n = 10) compared with those with NGT (n = 5) at follow-up (median [interquartile range]) (86.0 [24.3] vs. 54.9 [17.1]; P = 0.0200) and in those progressing from IGT to diabetes (n = 4) compared with those with persistent IGT (n = 4) (86.7 [25.2] vs. 54.1 [14.6]; P = 0.0433). The capillary luminal area (in micrometers squared) was lower in subjects with NGT progressing to IGT (n = 2) or subjects with IGT progressing to diabetes (n = 3) compared with subjects with constant NGT (n = 6) or constant IGT (n = 4) (11.9 [2.4] vs. 20.8 [7.8]; P = 0.0201). The capillary basement membrane area (in micrometers squared) was increased in patients with peripheral neuropathy (n = 10) compared with those without (n = 7) (114.6 [68.8] vs. 75.3 [28.7]; P = 0.0084). In conclusion, increased capillary density was associated with current or future diabetes, decreased capillary luminal area with future deterioration in glucose tolerance, and increased basement membrane area with peripheral neuropathy. *Diabetes* 52:2615–2622, 2003

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IGT, impaired glucose tolerance; MNFD, myelinated nerve fiber density; NDS, Neuropathy Disability Score; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; SNAP, sural nerve action potential; SNCV, sural nerve conduction velocity.

Peripheral neuropathy is common in diabetes, affecting ~50% of diabetic patients in the U.S. (1). Among 8,757 Italian diabetic patients attending outpatient clinics, the prevalence of clinical peripheral neuropathy was 32.3%, and this increased dramatically to 83.5% when quantitative neurological examination and nerve conduction studies were employed (2). Peripheral neuropathy is a leading and independent risk factor for mortality (3) and morbidity as a result of foot ulceration and amputation (4). Apart from improved glycemic control (5), there is no treatment for this condition.

Several recent studies (6–9) have demonstrated that subjects with impaired glucose tolerance (IGT) develop peripheral neuropathy. Indeed, in our recent study, subjects with IGT or type 2 diabetes and with peripheral neuropathy showed a comparable decrease in myelinated nerve fiber density (MNFD) (10). Both metabolic (11–14) and vascular (15–17) factors are important in the pathogenesis of peripheral neuropathy in diabetes. The exact relationship between the severity of hyperglycemia and these factors remains to be established. Diabetic patients without evidence of neuropathy demonstrate endoneurial microangiopathy. Microangiopathy may therefore precede the development of peripheral neuropathy (16). To clarify this issue, it is necessary to explore the relationship between glucose tolerance, peripheral neuropathy, and microvascular morphology.

Between 1975 and 1979, we initiated a prospective study in a population-based cohort of Swedish men aged 48 years who were defined as having diabetes, IGT, or normal glucose tolerance (NGT) after an oral glucose tolerance test (OGTT) (18). In this group, we previously reported an increase in skeletal muscle capillary density in subjects who progressed from IGT to diabetes (19). After 10–14 years from the start of this prospective study, we tested peripheral nerve function in representative subgroups (20) followed by sural nerve biopsy and assessment of the morphometry of myelinated nerve fibers in 30 subjects (10). Five to 6 years later, we reevaluated the subjects who previously underwent sural nerve biopsy with a new OGTT, measurements of plasma insulin concentrations, a neurological examination, neurophysiological tests, and a detailed evaluation of endoneurial capillary morphometry in their previously obtained baseline nerve biopsy. This study represents a unique opportunity to define the rela-

TABLE 1
Morphology and sural nerve function in subjects with diabetes, IGT, or NGT at baseline

	Diabetes	IGT	NGT	*P*
n	10	10	10	—
Clinical features				
Age at biopsy (years)	62 (2)*	64 (2)	64 (1)	0.0195
Height (cm)	175.5 (14.0)	176.0 (6.0)	175.0 (7.0)	0.9881
BMI (kg/m^2)	27.3 (2.5)	26.6 (2.9)	26.1 (2.6)	0.6424
Prevalence of clinical neuropathy (%)	60 (6/10)	44 (4/9)	0	—
Capillary morphology				
Endoneurial capillary density (number/mm^2)	75.3 (22.5)	64.6 (30.5)	55.4 (16.6)	0.2864
Luminal area (μm^2)	15.1 (4.1)	14.7 (9.3)	18.6 (8.9)	0.4957
Basement membrane area (μm^2)	108.9 (97.8)	98.1 (36.0)	106.7 (76.8)	0.6375
Endothelial/pericyte nuclear ratio	2.4 (0.7)†	2.8 (1.9)‡	2.0 (0.6)§	0.0259
Nerve fiber morphology				
Light microscopy: MNFD (number/mm^2)	4,460 (1,415)	5,090 (1,045)	4,750 (1,651)	0.7015
Electron microscopy: MNFD (number/mm^2)	4,446 (793)	4,999 (797)	4,708 (1,574)	0.3222
Neurophysiology (sural nerve)				
SNAP (μv)	3.7 (3.5)‖	11.3 (10.6)	10.0 (11.6)	0.0306
SNCV (m/s)	41.0 (6.0)#	47.0 (3.0)	44.0 (2.7)	0.0454

Data are median (interquartile range). *P* values are by Kruskal-Wallis variance analysis. In Mann-Whitney *U* test: *$P = 0.0121$ vs. IGT and $P = 0.0297$ vs. NGT; †$P = 0.0141$ vs. NGT; ‡$P = 0.0299$ vs. NGT; §$P = 0.0069$ vs. diabetes or IGT; ‖$P = 0.0409$ vs. IGT and $P = 0.0142$ vs. NGT; #$P = 0.0140$ vs. IGT.

tion between glucose intolerance and the presence of endoneurial microangiopathy and the relation between endoneurial microangiopathy and the presence and subsequent development of neuropathy.

RESEARCH DESIGN AND METHODS

Between 1975 and 1979, 69 men with type 2 diabetes, 51 men with IGT, and 62 men with NGT were classified in the Malmö area and matched for age, height, and BMI. Between 1989 and 1991, they each underwent neurophysiological evaluation of peripheral nerve function (20). For the baseline examination in 1992–1993, after a neurological and neurophysiological examination, 10 subjects with diabetes, 10 with IGT, and 10 with NGT underwent a whole–sural nerve biopsy (10). At a follow-up examination between 1998 and 1999, 5–6 years after the biopsy, all subjects were invited to receive an OGTT and a detailed neurological and neurophysiological examination, but no second biopsy was performed. Among the 30 individuals, 22 accepted the complete follow-up investigation, 1 with IGT did not participate in the clinical examination, 3 died (2 with diabetes and 1 with NGT), 2 refused to participate in the clinical and neurophysiological examination (1 with diabetes and 1 with NGT), and 1 with diabetes had moved from the Malmö area. Accordingly, 7 of 10 subjects originally classified as having diabetes, 9 of 10 originally classified as having IGT, and 9 of 10 originally classified as having NGT at baseline were included at the follow-up examination.

Clinical examination. At baseline, the subjects were examined by a neurologist, and patients with absent ankle reflex and/or altered/reduced sensory perception and/or neuropathic symptoms in toes or feet were considered to have clinical peripheral neuropathy (10). At the follow-up examination, a modified version of Dyck's original Neuropathy Symptom Score and Neuropathy Disability Score (NDS) was used to define the incidence and severity of peripheral neuropathy (21). In our protocol, the subject was asked if the following symptoms were present in the feet: numbness, abnormal sensation of heat or cold, sensation of pins and needles, contact dysaesthesia to bed clothes, burning pain, lancinating pain, and dull pain. If positive, the subject was asked whether the symptoms occurred sometimes, often, or regularly at night. Each of the seven symptoms was scored; lack of symptoms was scored as 0, sometimes as 1, often as 2, and during most nights as 3. The scores were added into the Neuropathy Symptom Score, giving a range of 0–21. Sensory perception was assessed on the great toe, anterior foot, lateral malleolus, pretibia, and knee bilaterally with regard to the modalities of light touch (cotton wool), pin prick (needle), vibration (vibration fork), and cold (cold metal item) together with an evaluation of the patellar and ankle reflexes. Lack of sensation for the individual modality was given a score of 1. The sensory modalities were added into an NDS subscore A (NDS A) (the sum was divided by 2), giving a range of 0–20. Reflex findings were added into an NDS subscore B (NDS B) in which normal reflex was given a score of 0, reduced reflex a score of 1, and absent reflex a score of 2, giving a range of 0–8. NDS A and NDS B were also added together to create a composite NDS ranging from 0 to 28.

OGTT. Whole blood glucose was measured fasting (0 min) and 120 min after 75 g glucose was ingested. In accordance with World Health Organization criteria (22), a fasting blood glucose concentration >6.1 mmol/l and/or a 120-min blood glucose >11.0 mmol/l defined diabetes. A fasting value of <6.1 mmol/l combined with a 120-min value of 7.8–11.0 mmol/l defined IGT, and a fasting blood glucose <6.1 mmol/l combined with a 120-min value of <7.8 mmol/l defined NGT. OGTTs were conducted in all subjects except one with previously classified IGT. This subject was considered to have IGT in all analyses except those involving glucose tolerance status at follow-up, from which he was excluded.

Plasma insulin assay. Plasma insulin was measured by radioimmunoassay, according to the method of Heding (23), at fasting (0 min), 40 min, and 120 min after the 75-g dose of glucose was ingested.

Neurophysiology. Stimuli were applied to the sural nerve at the calf ~15 cm proximal to the lateral malleolus. Antidromic sensory neurography of the sural nerve was performed with subcutaneous needle electrodes placed at the lateral malleolus with a reference electrode placed 3 cm distally.

Sural nerve biopsy: processing and morphometry. The surgical procedure for the whole–sural nerve biopsy performed at baseline has been previously described in detail (24). Briefly, the sural nerve was exposed posterior to the lateral malleolus during local 1% lignocaine anesthesia and a whole, 5- to 6-cm length of sural nerve was obtained and divided into five 1-cm segments. The first, fourth, and fifth (proximal to distal) segments were immediately frozen in liquid nitrogen and stored at −70°C. The second and third segments were fixed in 0.1 mol/l cacodylate buffered (pH 7.3) 2.5% gluteraldehyde. The fixed 1-cm segments were rinsed and further divided into three equal segments, postfixed in 1% osmium [4% sucrose, 1.5% $K_3Fe(CN)_6$ in cacodylate buffer], dehydrated in ascending concentrations of ethanol (50–100%), and infiltrated with Epon 812 resin using propylene oxide as an intermediary before setting in resin blocks.

We have previously reported on the morphologic findings of the myelinated fibers conducted at the University of Michigan Nerve Biopsy Laboratory, Michigan (10). In the current study, we report on the morphometric evaluation of endoneurial capillaries conducted in Manchester, U.K., by one of us (R.M.). The biopsies were recorded with random identification numbers to conceal their identities from the analysis. The study was approved by the Ethics Committee of Lund University.

Endoneurial capillaries. The endoneurial capillary density was determined directly by light microscopy from semithin sections stained with Thionin and Acradine Orange. Sampling was not used because all endoneurial capillaries were counted directly in all fascicles of the whole nerve and related to the fascicular area to calculate a density. Electron micrographs (magnification ×6,000) were prepared on an average of 10 randomly selected endoneurial capillaries per biopsy (range 8–12; no difference between the three groups of different glucose tolerances), and the luminal, endothelial cellular, and basement membrane area were derived by tracing the image analysis cursor around each capillary profile. The endothelial cell profile nuclear number and pericyte cell nuclear number per capillary were counted directly from each

TABLE 2
Morphology and sural nerve function in subjects with diabetes, IGT, or NGT at follow-up

	Diabetes	IGT	NGT	*P*
n	12	7	6	—
Capillary morphology				
Endoneurial capillary density (number/mm^2)	86.0 (24.3)*	56.1 (22.8)	54.9 (17.1)	0.0380
Luminal area (μm^2)	14.0 (5.2)	13.3 (14.5)	20.8 (4.9)	0.0787
Basement membrane area (μm^2)	80.7 (42.8)	98.4 (26.7)	146.0 (58.4)	0.3065
Endothelial/pericyte nuclear ratio	2.4 (2.1)	2.5 (1.9)	2.0 (0.4)	0.1631
Nerve fiber morphology				
Light microscopy: MNFD (number/mm^2)	5,090 (914)	5,134 (1,400)	4,358 (1,276)	0.5425
Electron microscopy: MNFD (number/mm^2)	4,847 (763)	4,999 (1,530)	3,997 (1,428)	0.4329
Neurophysiology (sural nerve) at follow-up study				
SNAP (μv)	4.0 (7.5)	7.5 (4.0)	6.5 (2.0)	0.6276
SNCV (m/s)	44.5 (8.0)	48.5 (5.0)	48.0 (7.0)	0.0904

Data are median (interquartile range). *P* values are by Kruskal-Wallis variance analysis. In Mann-Whitney *U* test: *$P = 0.0200$ vs. NGT.

micrograph. The methodology for quantifying microangiopathy has been described in detail elsewhere (25–28). The following variables were analyzed: endoneurial capillary density (in number per millimeters squared), luminal area (in micrometers squared), basement membrane area (in micrometers squared), and endothelial/pericyte nuclear ratio. For technical reasons, capillary density could not be assessed in three subjects: one with diabetes, one with IGT, and one with NGT at baseline. Similarly, endothelial/pericyte ratio, luminal area, and basement membrane area could not be assessed in three subjects: two with IGT and one with NGT at baseline. In addition, MNFD could not be assessed in four subjects, one with diabetes, one with IGT, and two with NGT at baseline.

Statistical analyses. Data are presented as median (interquartile range). Differences were compared using the Kruskal-Wallis and Mann-Whitney *U* tests. Correlations were analyzed by Spearman rank-correlation test.

RESULTS

OGTT at follow-up. All seven subjects with diabetes still had diabetes, whereas five of the nine IGT subjects had developed diabetes, and three of the nine NGT subjects had developed IGT. Accordingly, among the 25 subjects at follow-up, 12 had diabetes, 7 had IGT, and 6 had NGT. At follow-up, plasma insulin concentrations were significantly higher in diabetic subjects compared with NGT subjects at fasting (22.0 [14.0] vs. 9.5 [10.0] μU/ml; $P = 0.0050$) and 120 min (68.0 [65.0] vs. 34.0 [29.0] μU/ml; $P = 0.0342$), and in IGT subjects compared with NGT subjects at 120 min (76.0 [23.0] vs. 34.0 [29.0] μU/ml; $P = 0.0282$).

Clinical characteristics in relation to glucose tolerance. Subjects with diabetes were slightly younger than other subjects (Table 1), but there were no significant differences in height or BMI. As previously reported in the baseline study (10), subjects with diabetes had significantly lower sural nerve action potential (SNAP) amplitudes compared with those of the subjects with IGT ($P = 0.0409$) and NGT ($P = 0.0142$). In addition, subjects with diabetes had significantly lower sural nerve conduction velocities (SNCVs) compared with those of subjects with IGT at baseline ($P = 0.0140$) (Table 1). However, MNFD did not differ significantly between the three groups with different glucose tolerances. At follow-up, there were no significant differences in sural nerve electrophysiology or MNFD between the three groups with different glucose tolerances (Table 2).

Clinical characteristics in relation to clinical peripheral neuropathy. The prevalence of clinical peripheral neuropathy was 60% (6 of 10) in the diabetic group, 44% (4 of 9) in the IGT group, and 0% in the NGT group at baseline (Table 1). There were no significant differences in age,

TABLE 3
Morphology and sural nerve function in subjects with diabetes or IGT and with or without clinical peripheral neuropathy at the baseline study

	Peripheral neuropathy		
	With	Without	*P*
n	10	9	—
Age at biopsy (years)	63 (2)	63 (2)	0.8671
Height (cm)	178.0 (9.0)	173.0 (9.5)	0.0652
BMI	26.8 (2.1)	26.8 (4.6)	0.8065
Capillary morphology			
Endoneurial capillary density (number/mm^2)	72.0 (16.9)	84.5 (33.9)	0.4350
Luminal area (μm^2)	14.9 (5.1)	12.7 (4.9)	0.5912
Basement membrane area (μm^2)	114.6 (68.8)	75.3 (28.7)	0.0084
Endothelial/pericyte nuclear ratio	2.4 (0.9)	2.7 (2.5)	0.2037
Nerve fiber morphology			
Light microscopy: MNFD (number/mm^2)	3,853 (944)	5,385 (614)	0.0005
Electron microscopy: MNFD (number/mm^2)	4,340 (748)	5,193 (686)	0.0008
Neurophysiology (sural nerve) at baseline study			
SNAP (μv)	2.4 (3.9)	12.6 (8.1)	0.0029
SNCV (m/s)	43.0 (4.0)	45.0 (4.2)	0.1238

Data are median (interquartile range). *P* values are by Mann-Whitney *U* test.

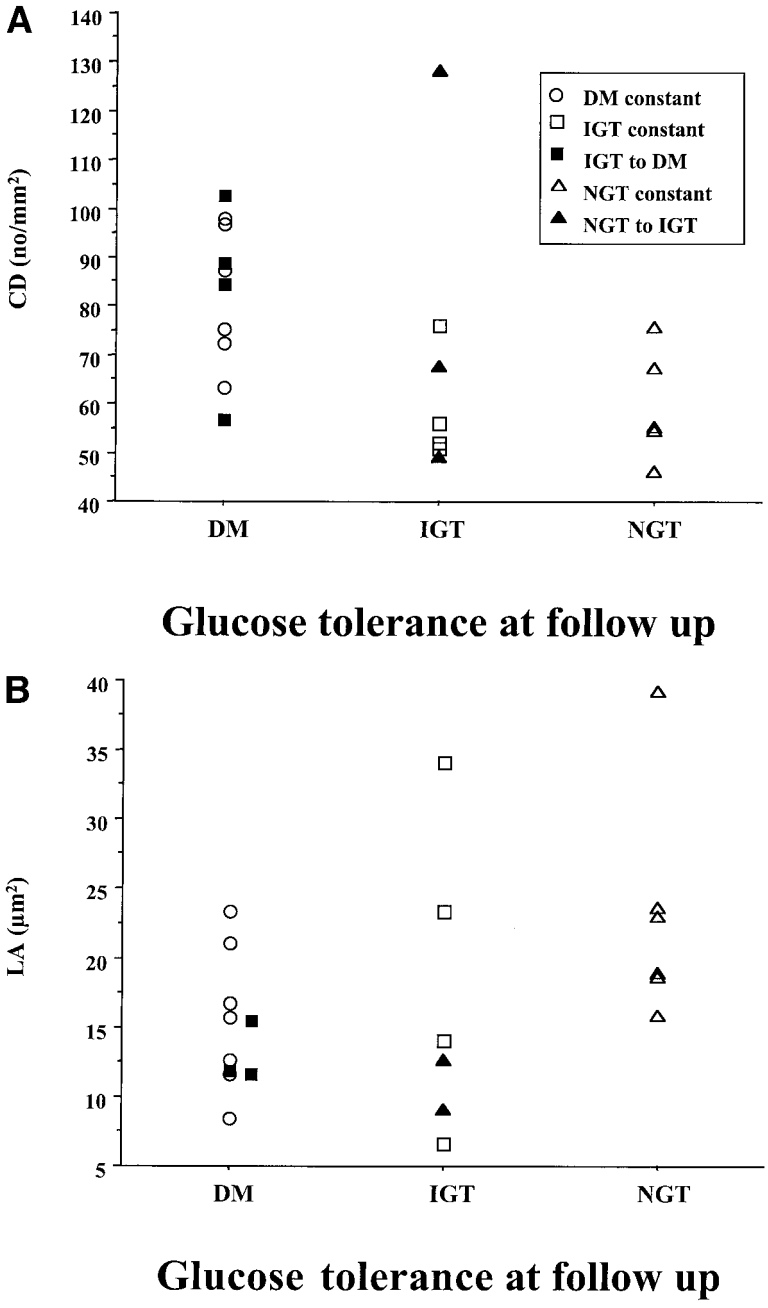


FIG. 1. Capillary density (CD) (*A*) and luminal area (LA) (*B*) at baseline versus glucose tolerance at follow-up. *A*: Capillary density at baseline was higher in subjects with diabetes at follow-up compared with those with persistent NGT ($P = 0.0200$) and in those converting from IGT to diabetes compared with those with persistent IGT ($P = 0.0433$). *B*: Luminal area at baseline did not differ between the three groups with different glucose tolerances at follow-up. However, subjects whose glucose tolerance deteriorated from IGT to diabetes and from NGT to IGT showed significantly ($P = 0.0200$) lower luminal areas at baseline compared with those without deteriorating glucose tolerance. ○, constant diabetes (DM); □, constant IGT; ■, IGT converted to diabetes at follow-up; △, constant NGT; ▲, NGT converted to IGT at follow-up.

height, or BMI between groups with or without peripheral neuropathy at baseline (Table 3). Patients with clinical peripheral neuropathy (all with diabetes or IGT) showed significantly lower SNAP ($P = 0.0029$) and MNFD ($P = 0.0005$) compared with subjects with diabetes or IGT and without neuropathy (Table 3). Hence, axonal loss and low MNFD were associated with clinical peripheral neuropathy. Subjects with clinical peripheral neuropathy at baseline showed a significantly higher symptom score (Neuropathy Symptom Score) at follow-up than those without (1.5 [3.0] vs. 0.0 [1.0]; $P = 0.0078$), indicating that the symptom score used may be relevant in setting criteria for peripheral neuropathy.

Endoneurial capillary morphometry and glucose tolerance

Capillary density. When classified by metabolic status at follow-up 6 years after nerve biopsy, patients with diabetes ($n = 10$) had a significantly higher capillary density compared with subjects with NGT ($n = 5$) (86.0 [24.3] vs. 54.9 [17.1] number/mm^2; $P = 0.0200$) (Fig. 1*A* and Table 2). However, Fig. 1*A* also shows that subjects with IGT at baseline who progressed to diabetes ($n = 4$) 6 years later exhibited a significantly higher capillary density in their baseline biopsies compared with those with persistent IGT ($n = 4$; 86.7 [25.2] vs. 54.1 [14.6] number/mm^2; $P = 0.0433$). One baseline subject with NGT, who progressed to IGT, had a very high capillary density (128.1 number/mm^2) at baseline. If this subject was excluded from the analysis, capillary density was significantly increased among patients with diabetes ($n = 9$) compared with subjects with NGT at baseline ($n = 8$; 75.3 [22.3] vs. 55.1 [15.8] number/mm^2; $P = 0.0343$). Hence, high capillary density was positively associated with future and current diabetes.

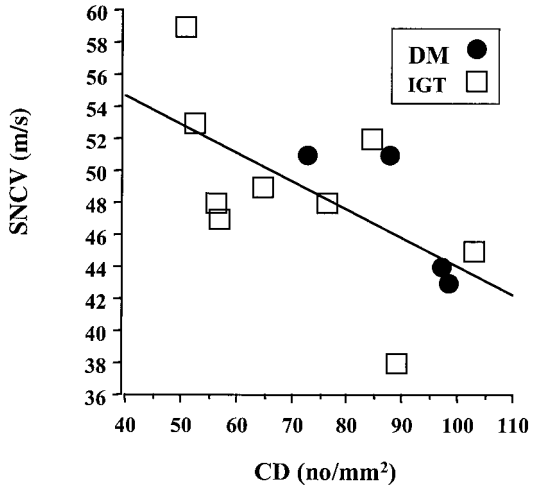


FIG. 2. Capillary density (CD) at baseline versus SNCV at follow-up among subjects with diabetes (●) or IGT (□). CD at baseline correlated significantly with SNCV at follow-up ($r_s = -0.691$; $P = 0.0166$).

Luminal area. Tables 1 and 2 show that luminal area did not differ between the three groups with different glucose tolerances. However, Fig. 1*B* shows that subjects whose glucose tolerance deteriorated at follow-up (three from IGT to diabetes and two from NGT to IGT) had significantly lower luminal area than subjects whose glucose tolerance did not deteriorate (constant NGT [$n = 6$] and constant IGT [$n = 4$; 11.9 {2.4} vs. 20.8 {7.8}; $P = 0.0200$]). Hence, reduction in luminal area was associated with deterioration in glucose tolerance in IGT and NGT.

Endothelial/pericyte nuclear ratio and basement membrane area. The endothelial/pericyte nuclear ratio was significantly increased in both subjects with diabetes ($P = 0.0141$) and IGT ($P = 0.0299$) compared with subjects with NGT at baseline but not at follow-up (Tables 1 and 2). The basement membrane area did not differ among the three glucose tolerance groups (Tables 1 and 2).

Endoneurial capillary morphometry and plasma insulin concentrations. In subjects with diabetes at baseline, serum insulin levels at fasting, 40 min, and 120 min correlated negatively with the basement membrane area ($r_s = -0.748$, $P = 0.0249$; $r_s = -0.729$, $P = 0.0286$; and $r_s = -0.602$, $P = 0.0710$, respectively), whereas there were no significant correlations with capillary density or MNFD.

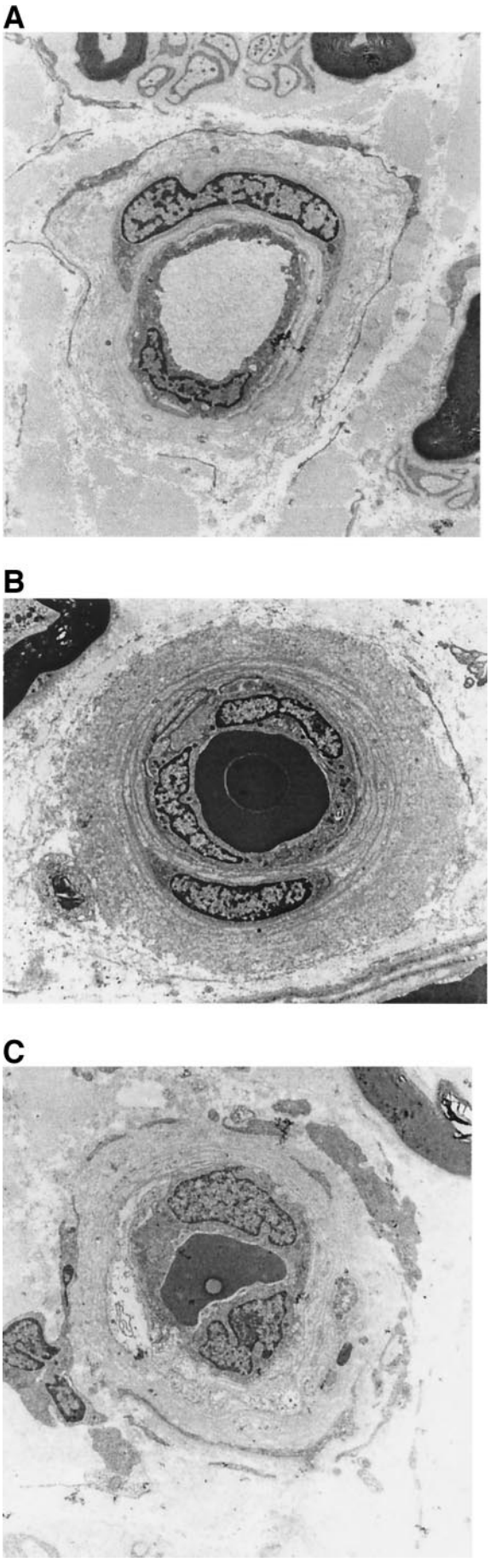
Endoneurial capillary morphometry and neuropathy. In subjects with diabetes or IGT at baseline, high capillary density was associated with disturbed nerve conduction velocity at follow-up (low SNCV; $r_s = -0.691$, $P = 0.0166$) (Fig. 2). Among subjects with diabetes or IGT, those with clinical peripheral neuropathy at baseline ($n = 10$) had a significantly increased basement membrane area compared with that of those without peripheral neuropathy ($n = 7$; 114.6 [68.8] vs. 75.3 [28.7] μm^2; $P = 0.0084$) (Figs. 3 and 4). Furthermore, there was a significant and inverse correlation between the basement membrane area and MNFD in those with diabetes or IGT at baseline ($r_s = -0.559$; $P = 0.0304$) (Fig. 5*A*). Moreover, among subjects with diabetes at baseline, there was a close correlation between basement membrane area and axonal loss (low SNAP) at baseline ($r_s = -0.894$; $P = 0.0073$) (Fig. 5*B*).

DISCUSSION

This prospective study has quantified the key alterations in the endoneurial vasculature in relation to the development and progression of glucose intolerance and neuropathy. Firstly, we have demonstrated that sural nerve endoneurial capillary density is increased in subjects with IGT who develop diabetes and in patients with manifest diabetes compared with subjects with NGT. Secondly, we have demonstrated a reduction in capillary luminal area in subjects progressing from NGT to IGT and from IGT to diabetes. If it is indicative of reduced tissue blood flow, this finding links tissue hypoperfusion to the deterioration of glucose tolerance and suggests that increased endoneurial capillary density may be compensatory for endoneurial hypoperfusion. This is consistent with our previous observation (19) that poor aerobic capacity correlates with increased skeletal muscle capillary density. With regard to nerve, sural nerve oxygen tension is reduced in diabetic patients with established neuropathy (29) but without a significant alteration in either luminal area or endoneurial capillary density (26–28,30,31). However, our recent study of patients with subclinical neuropathy (32) demonstrates that increased epineurial nerve blood flow may reflect arterio-venous shunting. This supports the concept that increased endoneurial capillary density may be considered a compensatory reaction to hypoperfusion and hypoxia.

The changes described thus far are of relevance to the pathogenesis of diabetic neuropathy. Endoneurial vessels maintain the endoneurial microenvironment and are therefore important for nerve fiber function. A reduction in endoneurial capillary density and hence increased intercapillary distance is considered one of the key alterations regulating endoneurial perfusion (33). A recent detailed study (34) in a variety of advanced neuropathies, including diabetic neuropathy, has demonstrated a significant increase in epineurial but not endoneurial capillaries. In the current study, we demonstrated a negative correlation between endoneurial capillary density and disturbed nerve function, which is suggestive of a compensatory increase in vascular density in early neuropathy. However, in diabetic patients with established neuropathy, no alteration in endoneurial capillary density has been demonstrated (26–28,30,31). Hence, there is a reduction in endoneurial capillary density with progression of neuropathy.

A variety of other pathological alterations have been associated with the severity of human diabetic neuropathy. The hallmark of diabetic microangiopathy is basement membrane thickening. Many studies (26–28,30,31) have shown a strong correlation between basement membrane thickening versus neuropathic severity in diabetic and nondiabetic human neuropathies. The current study has confirmed that this is an early process and that it relates specifically to neuropathy. Our patients with neuropathy had a significant increase in basement membrane area compared with those without neuropathy. Furthermore, we demonstrated a significant correlation with axonal loss and increased basement membrane area at this early stage of nerve damage. This not only confirms the findings in patients with established (27,28,30,31) and mild (26) neu-



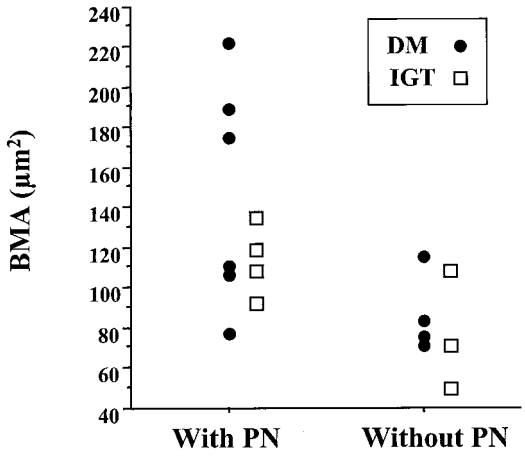


FIG. 4. Basement membrane area (BMA) in relation to clinical peripheral neuropathy (PN) at baseline among subjects with diabetes (●) or IGT (□). Basement membrane area was significantly increased among subjects with peripheral neuropathy compared with those without (P = 0.0084).

ropathy but also shows that basement membrane thickening may precede more severe nerve damage (16).

The lack of correlation between the basement membrane area and the degree of glucose tolerance in our study suggests that increased basement membrane thickness is not simply an epiphenomenon related to hyperglycemia but is related specifically to the development and progression of peripheral neuropathy. In addition, increased basement membrane area correlated with decreased plasma insulin concentration in our diabetic subjects. Hence, as suggested, hypoinsulinemia may promote the development of polyneuropathy, regardless of the degree of hyperglycemia (35). The lack of correlation between plasma insulin concentration and MNFD supports our hypothesis that hypoinsulinemia primarily affects the basement membrane area, thereby promoting the development of polyneuropathy. Luminal occlusion has been demonstrated in some (36) but not all (26–28,30,31) studies. In the current study, there was no difference in luminal area between those with and without neuropathy, and there was no relation between luminal area with nerve function or axonal loss, which supports the majority of these studies (26–28,30,31).

Because insulin resistance and hyperinsulinemia with associated alteration in capillary function are hallmarks of IGT and early type 2 diabetes, it is tempting to speculate that early endoneurial capillary microangiopathy in these groups could be attributed to insulin resistance and/or hyperinsulinemia. While there is an established relationship between insulin resistance and alterations in the function and structure of blood vessels in classically insulin-responsive tissues such as muscle and fat, little is known of this relationship in other tissues (37). Thus, among its many actions, insulin is a vasoactive hormone, which at physiological concentrations increases skeletal muscle tissue perfusion by recruiting microvascular beds (38). By definition, insulin-resistant states exhibit dimin-

FIG. 3. Electron micrographs (magnification ×2,600) of endoneurial capillaries from a control subject (*A*) compared with a diabetic patient with (*B*) and a diabetic patient without (*C*) peripheral neuropathy, demonstrating basement membrane thickening in those with neuropathy.

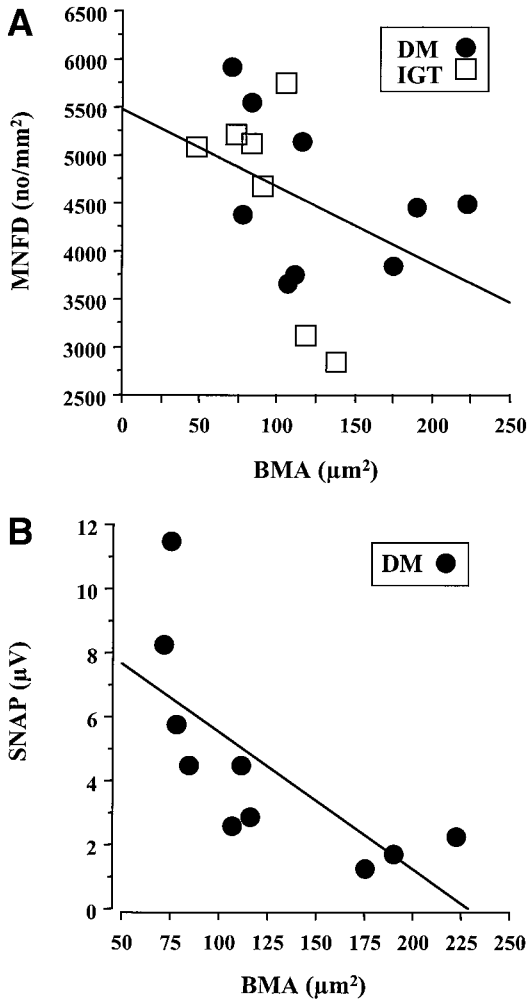


FIG. 5. Basement membrane area (BMA) in relation to MNFD (*A*) and SNAPs (*B*) at baseline in subjects with diabetes (●) or IGT (□). *A*: Basement membrane area correlated significantly with MNFD in diabetes or IGT ($r_s = -0.559$; $P = 0.0304$). *B*: Basement membrane area correlated significantly with SNAP in diabetes ($r_s = -0.894$; $P = 0.0073$).

ished insulin-mediated glucose uptake into peripheral tissues but also display impaired insulin-mediated vasodilatation (39). We have previously shown (19) that increased skeletal muscle capillary density correlates with insulin levels and that it precedes the development of diabetes in subjects with IGT. Our observations of increased capillary density in both nerve and muscle suggest a compensatory response to insulin resistance. However, the complex effects of insulin are exemplified in rats with insulinoma, in which hyperinsulinemia without obvious insulin resistance is associated with increased endoneurial capillary density (40). Moreover, hyperinsulinemia in diabetic animals (41) and in diabetic patients with insulin neuritis (32) leads to arterio-venous shunting and a reduction in endoneurial oxygen tension (41). Although insulin concentrations were increased in subjects with diabetes or IGT in the current study, no correlation with insulin concentrations and capillary density was noted. This suggests that insulin resistance rather than hyperinsulinemia per se is associated with increased capillary density.

Although we are limited by a small sample size, we still cautiously conclude that increased capillary density in the sural nerve precedes the development of diabetes in subjects with IGT. Furthermore, decreased capillary luminal area in the sural nerve precedes deterioration in glucose tolerance in both IGT and NGT. In addition, endothelial cell hyperplasia and/or pericyte degeneration in sural nerve occur in IGT and diabetes. Moreover, endoneurial microangiopathy, and in particular basement membrane thickening, is related to clinical, neurophysiological, and morphologic measures of neuropathy in subjects with diabetes and IGT. These data are consistent with the hypothesis that endoneurial capillary microangiopathy presages deterioration in glucose tolerance and is an early and persistent feature in the processes underlying diabetic peripheral neuropathy. Additionally, whereas insulin resistance/hyperinsulinemia is generally invoked as a cause of increased macrovascular risk in subjects with IGT or type 2 diabetes, these results would suggest that a microvascular complication, i.e., diabetic neuropathy, might also be associated with these elements of what is generally referred to as "the metabolic syndrome."

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