# Capillaries of South African Diabetics

## IV. Relation to Retinopathy

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SUMMARY

**The muscle capillaries of diabetic subjects, with and without retinopathy, have been measured. The groups with retinopathy had significantly thicker laminae than those without the fundal changes. No significant difference was found between the group with proliferative retinopathy and the group with nonproliferative retinopathy. The focal and segmental nature of the basal lamina thickening was confirmed by the increasing standard deviation of the measurements within and among capillaries. This study also confirms the fact that, at least for muscle capillaries, an apparent relationship exists between the thickness of the basal lamina of these vessels and the presence of clinical retinopathy. DIABETES *24*:286-90, March, 1975.**

The muscle capillary ultrastructure of South African diabetic patients has been described previously.[1] The average thickness of the basal lamina (basement membrane) of the diabetic group of patients was significantly greater than that of the control group. However, because of considerable overlap between the individual measurements in the two groups, it was concluded that the disease could not be diagnosed by this technic alone. In general, our conclusions were similar to those of Williamson et al.,[2] the essential difference being that, like Siperstein,[3] we could not correlate the capillary thickness with age or duration of the diabetes.

The results suggested, however, that an average capillary basal lamina thickness of 3,000Å or more, measured by our technic, may indicate the presence of widespread capillaropathy. To confirm this impression, we have conducted a further blind study on diabetic patients with and without retinopathy. It was assumed that if the peripheral muscle capillaries reflected the general capillary status, then regardless of ethnic group, sex or age, we would find a significant difference between the capillaries of patients with retinopathy and those without this disease. This has proved to be the case.

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### PATIENTS AND METHODS

Three groups of patients with primary or idiopathic diabetes mellitus were selected for study from the wards and the diabetic clinic of Johannesburg General Hospital (table 1). All had frank clinical diabetes requiring therapy by diet or drugs. Allocation to one of these groups was based on the findings of ophthalmoscopy carried out in a darkroom after capillary dilatation with 2 per cent phenylephrine. The patients were divided into the following groups:

(a) Nine patients without any signs of retinopathy.

(b) Eight patients with mild or moderate, nonproliferative retinopathy, defined by the presence of microaneurysms or small, round hemorrhages with or without hard exudates.

(c) Eight patients with severe or proliferative retinopathy defined by the presence of new vessel formation or fibrous tissue proliferation.

Muscle was obtained from the vastus lateralis by needle biopsy, care being taken to insure that the local anesthetic fluid used did not reach the biopsy site. The tissues were immediately fixed in glutaraldehyde buffered with collidine, rinsed and placed in collidine-buffered sucrose and transported by airmail to the laboratory for electron microscopy. They were then rinsed and processed for electron microscopy by post-fixing for one hour in collidine-buffered osmium tetroxide, dehydrated and embedded in Epon. All sections were cut on a Porter-Blum microtome, and ex-

amined with a Siemens A-1 microscope using a digital readout for magnification setting.

(d) The basal laminae of not less than ten capillaries were measured at ten points as equidistant as possible around the circumference. Frequently parts of the laminae—specifically in the region(s) of the pericyte—were not available for measurement and in these capillaries where ten points could not be clearly read, at least six points were taken. For comparative purposes, the two thinnest points of measurement not less than 1 cm. apart were also noted and treated separately. The basic criterion for accepting a capillary for measurement was the adequacy of the fixation and the ability to clearly determine the inner and outer

TABLE 1

Clinical features and basal lamina thickness in three groups of diabetics with different degrees of retinopathy

| | Age (yrs.) | Sex | Ethnic group | Ht. (ins.) | Wt. (lbs.) | %Ideal body wt. | Diabetes duration (yrs.) | B.P. (mm. Hg) | Other clinical features | Treatment in last 10 years | Basal lamina thickness in Å average | average at thinnest point |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Group A | | | | | | | | | | | | |
| | 70 | M | Black | 64 | 134 | 103 | 1 | 130/80 | | Diet | 2,117 | 1,694 |
| | 61 | F | Black | 65½ | 135 | 105 | 1/12 | 140/80 | | Tolbutamide | 1,957 | 1,488 |
| No | 23 | F | Black | 61 | 113 | 103 | 1/12 | 120/80 | | Insulin | 2,393 | 1,711 |
| Clin- | 47 | M | Black | 68½ | 162 | 109 | 3 | 220/134 | Large left ventricle; narrow retinal arterioles | Diet | 1,756 | 1,342 |
| ical Retin- opathy | 31 | M | Black | 66 | 135 | 99 | 1/3 | 115/75 | Axillary vein thrombosis | Insulin | 1,764 | 1,305 |
| | 18 | M | White | 73 | 180 | 108 | 8 | 140/85 | Absent knee jerks | Insulin | 3,153 | 1,851 |
| | 54 | M | White | 64½ | 163 | 123 | 15 | 140/90 | 4 previous myocardial infarctions | Insulin | 1,738 | 1,292 |
| | 60 | M | White | 68 | 177 | 123 | 10 | 160/100 | Angina pectoris; narrow retinal arterioles | Chlorpropamide and phenformin | 2,856 | 1,992 |
| | 23 | F | Indian | 62 | 123 | 109 | 2 | 120/80 | | Insulin | 2,106 | 1,560 |
| Mean ± | 43 | - | - | 65.8 | 146.9 | 109 | 4.4 | | - | - | 2,204 | 1,581 |
| S.D. | ±19.5 | | | 3.6 | 24.1 | ±8.5 | 5.4 | | | | ±506 | ±249 |
| S.E. | 6.5 | | | 1.2 | 8.0 | 2.8 | 1.8 | | | | 168 | 83 |

| | Age (yrs.) | Sex | Ethnic group | Ht. (ins.) | Wt. (lbs.) | %Ideal body wt. | Diabetes duration (yrs.) | B.P. (mm. Hg) | Other clinical features | Treatment in last 10 years | Basal lamina thickness in Å average | average at thinnest point |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Group B | | | | | | | | | | | | |
| | 46 | F | Black | 62 | 145 | 128 | 5 | 170/110 | Narrow retinal arterioles; moderate proteinuria | Chlorpropamide | 3,228 | 2,420 |
| Mild or | 50 | F | Black | 64 | 138 | 115 | 9 | 150/88 | Mild proteinuria | Insulin | 4,592 | 2,940 |
| moderate | 64 | F | Black | 59 | 139 | 134 | 15 | 110/70 | Previous myocardial infarction | Tolbutamide | 4,225 | 2,813 |
| non- | 50 | F | White | 60 | 104 | 97 | 10 | 170/90 | Moderate proteinuria | Insulin | 5,067 | 3,861 |
| prolif- | 44 | M | White | 61 | 128 | 108 | 10 | 120/80 | Previous myocardial infarction | Chlorpropamide | 3,311 | 2,025 |
| erative retin- opathy | 64 | M | White | 73 | 220 | 132 | 7 | 185/125 | Peripheral vascular disease and neuropathy; narrow retinal arterioles | Chlorpropamide and phenformin | 2,314 | 1,345 |
| | 55 | F | Indian | 56 | 119 | 132 | 15 | 160/100 | Moderate proteinuria | Chlorpropamide | 4,571 | 2,924 |
| | 22 | F | Indian | 64 | 109 | 90.8 | 5 | 118/72 | Idiopathic edema | Insulin | 5,124 | 3,478 |
| Mean ± | 49 | - | - | 62.4 | 137.8 | 117 | 9.5 | - | - | - | 4,054 | 2,846 |
| S.D. | ±13.3 | | | 5.0 | 36.3 | ±17.0 | ±3.9 | | | | ±1,001 | ±828 |
| S.E. | 4.7 | | | 1.7 | 12.1 | 6.0 | 1.3 | | | | 354 | 276 |

*(Continued on next page)*

TABLE 1 (*Continued*)

Clinical features and basal lamina thickness in three groups of diabetics with different degrees of retinopathy

| | Age (yrs.) | Sex | Ethnic group | Ht. (ins.) | Wt. (lbs.) | %Ideal body wt. | Diabetes duration (yrs.) | B.P. (mm. Hg) | Other clinical features | Treatment in last 10 yrs. | Basal lamina thickness in Å average | Basal lamina thickness in Å average at thinnest point |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Group C | | | | | | | | | | | | |
| | 52 | M | White | 65 | 143 | 108 | 7 | 150/90 | Mild proteinuria | Chlorpropamide | 5,562 | 3,910 |
| Severe or | 24 | F | White | 67 | 146 | 111 | 20 | 160/95 | Nephrotic; blood urea 104 mg. per 100 ml. | Insulin | 3,971 | 2,980 |
| Proliferative Retinopathy | 58 | M | Indian | 64 | 101 | 78 | 12 | 190/100 | Previous infarction; peripheral vascular disease and neuropathy | Chlorpropamide | 3,876 | 2,261 |
| | 47 | M | Indian | 61 | 115 | 99 | 28 | 125/86 | Mild proteinuria | Insulin | 4,776 | 3,193 |
| | 70 | F | Indian | 59 | 117 | 113 | 20 | 210/100 | Large left ventricle; peripheral vascular disease and neuropathy | Chlorpropamide and metformin | 5,095 | 3,462 |
| | 63 | M | Indian | 65 | 152 | 114 | 29 | 160/100 | Previous infarction; nephrotic | Chlorpropamide | 5,300 | 3,598 |
| | 44 | M | Indian | 69 | 148 | 99 | 10 | 114/86 | Marked proteinuria; peripheral neuropathy | Tolbutamide | 5,426 | 3,767 |
| | 46 | M | Chinese | 60 | 131 | 109 | 15 | 160/80 | Marked proteinuria; peripheral neuropathy | Insulin | 2,428 | 1,669 |
| Mean ± | 50 | | | 63.8 | 131.6 | 103.8 | 17.6 | | | | 4,554 | 3,105 |
| S.D. | ±14.0 | | | 3.3 | 18.7 | ±11.9 | ±8.0 | | | | ±1,068 | ±779 |
| S.E. | 4.9 | | | 1.164 | 6.23 | 4.2 | 2.8 | | | | 377 | 275 |

limits of the lamina. The significance tests used were the Student's *t*-test and the nonparametric Mann-Whitney *U*-test.

## RESULTS

(1) Mean ages and weights (expressed as percentage of standard body weight) and blood pressure levels were similar in the three groups. Severity of diabetes and type of therapy were also similar. Degree of control appeared to be satisfactory in most patients, and race and sex composition were variable.

(2) As expected, the mean (±S.D.) duration of diabetes was shortest in the group without signs of retinopathy, longer in the group with moderate nonproliferative retinopathy and longest in that with severe or proliferative changes. Also diabetic nephropathy and other complications tended to be more common or severe in the groups with retinopathy (table 1).

(3) (a) The mean (±S.D.) basal lamina thickness of the diabetics without retinal signs was significantly less than that of the group with nonproliferative and proliferative signs. The mean (±S.D.) width of the basal lamina measured at its thinnest point was also significantly less in the patients without signs of clinical retinopathy as opposed to those with nonproliferative and proliferative retinopathy. However, the differences between the nonproliferative and proliferative groups were not significant (table 2).

(b) To test if this was a focal and segmental capillary lesion, as is believed to be the case,[1,2] the Standard Deviation was measured against the basal lamina thickness: first, using the deviation *within* capillaries that is, taking all measurements of each capillary to determine focal thickening and second, by comparing the deviation *between* capillaries to the thickness of the lamina to measure segmental thickening. It was found that as the basal lamina thickness increased so did the S.D. within each capillary and between capillaries (figures 1 and 2).

## DISCUSSION

Although the number of patients examined was small, the results of this study are clear-cut. Among the diabetics without retinal signs, the average basal lamina thickness was 2,204 Å, which compares favorably with our previous studies on diabetics—2,857 Å for essential diabetics[1] and 2,782 Å for patients with pancreatic diabetes.[4] The groups of diabetics with clinical signs of retinopathy had basal laminae which were significantly thicker than those of patients without such signs. In the latter group, only one patient had an average thickness of

TABLE 2

| Group | | Duration (years) | B.L. Average (Å) (10 points) | B.L. Average (Å) (2 thinnest pts.) |
|---|---|---|---|---|
| A | Mean | 4.389 | 2,204.4 | 1,581.667 |
| N=9 | S.D. | 5.359 | 506.34 | 249.348 |
| | S.E. | 1.786 | 168.78 | 83.116 |
| B | Mean | 9.500 | 4,054.00 | 2,725.75 |
| N=8 | S.D. | 3.928 | 1,001.354 | 796.286 |
| | S.E. | 1.389 | 353.910 | 281.532 |
| C | Mean | 17.625 | 4,554.25 | 3,105.000 |
| N=8 | S.D. | 8.088 | 1,068.669 | 779.612 |
| | S.E. | 2.860 | 377.835 | 275.637 |
| *t*-tests | A vs B | P<.05 | P<.01 | P<.01 |
| | A vs C | P<.01 | P<.01 | P<.01 |
| | B vs C | P<.05 | NS | NS |
| Mann-Whitney or two sample rank test | A vs B | P<.05 | P<.01 | P<.01 |
| | A vs C | P<.01 | P<.01 | P<.01 |
| | B vs C | NS | NS | NS |

A = No Clinical Retinopathy
B = Mild or Moderate (nonproliferative) Retinopathy
C = Severe (proliferative) Retinopathy

over 3,000 Å, while among the patients with clinical retinopathy, only two patients had an average thickness less than 3,000 Å; no significant difference between the two groups of patients with retinopathy was demonstrated. Diabetics with retinopathy have been observed to have thick capillary basal laminae,[2] but

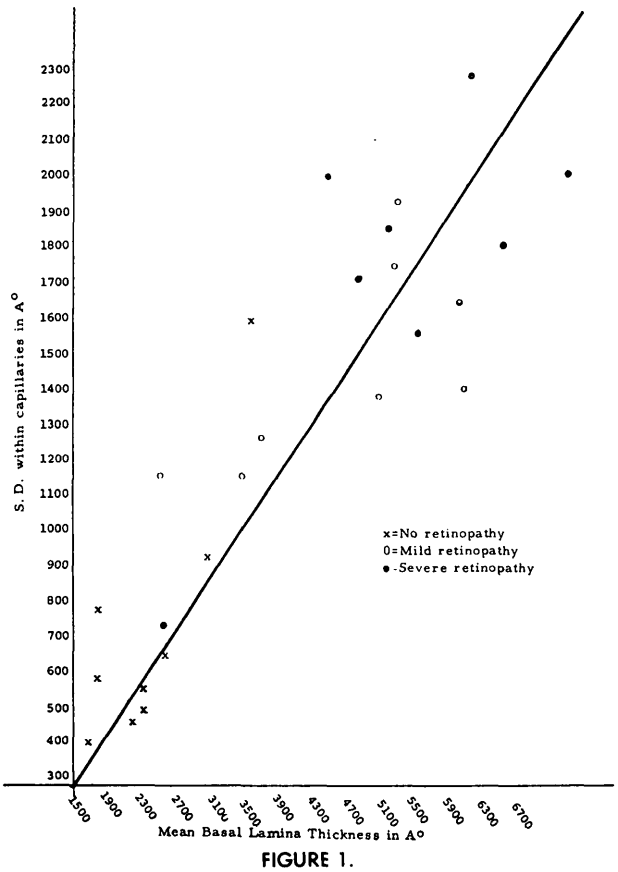


FIGURE 1.

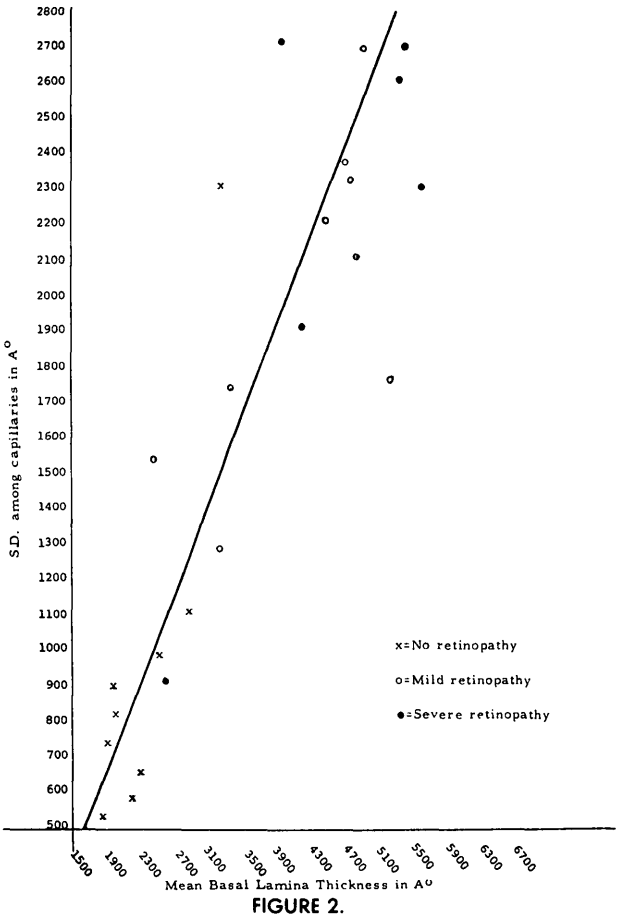


FIGURE 2.

the difference between patients with clinical retinopathy and those without, has not previously been reported. Neither our present findings nor previous clinical observations by others have demonstrated that any significant ethnic factors contribute to the prevalence, severity, or behavior of diabetic retinopathy, nor can the results be related to other variables examined, including sex, weight, age, duration of diabetes, blood pressure, or treatment employed.

Because of the number of cases in each group and the obvious skew, it was felt that nonparametric statistics should be used. In any event since the distribution of this population (as perhaps in other populations used for this type of investigation) is not strictly Gaussian, the ranks test is more appropriate than the *t*-test. For the "duration of the disease" the B vs C is significant ($p < 0.05$) using the *t*-test, but for the nonparametric test B vs C is not significant. In all other cases, the two statistical tests give comparable results. It is quite possible that the normality and equal variance assumptions required in the *t*-test are not adequately met by these data—hence the "contradictory" inference. In any event, since duration can only be dated from the time the doctor confirms the diagnosis, and this date does not necessarily coincide with the duration of the metabolic abnormality, the relationship between duration and basal lamina thickness remains equivocal for our series of patients. (The correlation coefficients between duration of diabetes and basal lamina thickness are not significant for groups A or B, if this is computed for all twenty-five subjects. However, then the correlation coefficient (0.528 or 0.596 if logarithms are used) is significant at the $P < 0.01$ or $P < 0.005$ levels, respectively.)

We have not found in this or in previous studies, any relationship between age and thickness of the basal lamina. This, too, is consistent with our knowledge of the disease since microangiopathy can be severe at an early age. Although it seems highly probable that capillaries do increase in thickness with age,[2] the over-riding pathological change in diabetic patients is a function of the disease and not the age of the patient. Therefore, if the capillary lesion is widespread, as we believe to be the case, then the thickness of the lamina, like the retinopathy, will reflect the severity of the complications, and not the age of the patient. Finally, the focal and segmental nature of the lesion grows more marked as the severity increases. This is demonstrated by the increasing standard deviation in the measurements within the capillaries, and between the capillaries.

This study has several important implications. It demonstrates that there is a clear-cut difference in the capillaries of patients with and without clinical retinal signs, but the clinically nonproliferative phase of the retinopathy cannot be differentiated from the proliferative phase on the basis of the muscle capillaries. We have previously suggested that an average thickness of 3,000 Å suggests the probability of significant diabetic capillaropathy. Now we believe that a mean of 4,000 Å, as measured by our technic, is compatible with the presence of definite eye signs, whether or not these have been detected by routine ophthalmoscopy. Thus, for any diabetic patient, regardless of age or known duration of the disease, a mean measurement of 3,000-4,000 Å on muscle capillaries merits a thorough retinal examination by an ophthalmologist; and since the triad of neuropathy, retinopathy and nephropathy is widely accepted,[5] a renal work-up is also indicated. By using this technic and following patients at regular intervals, it may be possible to monitor capillary pathology and evaluate various therapeutic regimes.

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