Capillary Basement Membrane Thickness and Capillary Density in Sedentary and Trained Obese Zucker Rats

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The purpose of this study was to determine whether the obese Zucker rat (OZR) develops diabeteslike peripheral vascular disease and evaluate the effects of exercise training (treadmill running, 15 m/min, 17% grade, 60 min/day, 5 days/wk, for 6 or 12 wk) on skeletal muscle vascular disease. Capillary density (CD) and capillary basement membrane (CBM) thickness were measured in the plantar muscle of sedentary and trained OZR and sedentary lean Zucker rats (LZRs). At 11 wk old, when profoundly obese, hyperinsulinemic, and insulin resistant, OZRs had lower CD and thicker CBM than LZRs. These characteristics are consistent with the expression of human diabetic microangiopathy and imply altered diffusion capacity due to increased diffusion distance and changes in the capillary wall. Between 11 and 18 wk of age, OZRs became hyperglycemic. No age-related changes in CD were observed in lean or obese animals, and OZRs had lower CDs than LZRs at 18 wk of age. CBM thickness decreased from 11 to 18 wk of age in both lean and obese animals, but the decline was proportionally greater in OZRs, and the CBM of obese animals was only slightly thicker than in lean 18-wk-old animals. Exercise training did not alter CD or CBM thickness in 11-wk-old animals. In contrast, training for 6 or 12 wk increased both CD and CBM thickness in 18-wk-old animals, normalizing CD but further increasing CBM thickness relative to LZRs. Correlational analysis revealed that CBM thickness is related to basal insulin concentration ($r = .29$, $P < .05$) but not to basal glucose ($r = .12$, $P > .05$). No relationship was evident for CD. OZRs have fewer capillaries and thicker CBM in skeletal muscle at 11 and 18 wk of age. In addition, exercise training may improve skeletal muscle diffusion capacity in OZRs by stimulating capillary proliferation; however, microvascular function may be further compromised by additional thickening of the CBM. The effects of exercise training on CBM thickness may depend on the corresponding changes in the basal insulin concentration. *Diabetes* 38:854–60, 1989

The obese, hyperinsulinemic, hypercholesterolemic, and insulin-resistant obese Zucker rat (OZR) simulates the etiology of human obesity (1). The OZR is also glucose intolerant (1) and moderately hyperglycemic (1,2), similar to the early development of human non-insulin-dependent diabetes mellitus (NIDDM). Although the metabolic characteristics of the OZR have been extensively investigated, evaluation of the cardiovascular system has been limited to cardiac function (3) and the renal circulation (4). The skeletal muscle vasculature of the OZR has not been evaluated.

Peripheral vascular disease is the principal cause of morbidity and mortality in diabetes mellitus (5). The presence of skeletal muscle microvascular disease has been documented in streptozocin-induced diabetic rats and mice and in obese mice (6). In addition, capillary basement membrane (CBM) thickening and decreased capillary density in skeletal muscle have been observed in human diabetes, prediabetes, and NIDDM (5,7–11). The importance of skeletal muscle perfusion and diffusion capacity in the diabetic and prediabetic state is emphasized by recent evidence suggesting that skeletal muscle capillary density and blood flow partially determine the rate of insulin-stimulated glucose uptake, therefore affecting the capacity to maintain glucose homeostasis (12–15).

The mechanisms responsible for the development of peripheral vascular disease in diabetes mellitus are unknown. Glucose and insulin excess and lack of insulin are potential contributors to endothelial and vascular smooth muscle cell pathology (16–18). Significant relationships between peripheral vascular complications and the duration and severity of glucose intolerance support the hypothesis that glucose

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has a role in the development of vascular disease (11,19,20). However, CBM thickening has been observed in normoglycemic prediabetic patients (21). The OZR model uniquely presents obesity and hyperinsulinemia at ~4 wk of age and is profoundly insulin resistant by 11 wk, but it does not develop significant hyperglycemia until 13 wk (1). Therefore, careful selection of age allows for potential partitioning of the effects of hyperinsulinemia, insulin resistance, and hyperglycemia in the development of peripheral microvascular disease in the OZR.

Aerobic exercise training is often prescribed in the treatment of insulin resistance resulting from obesity or diabetes mellitus (12,22,23). Exercise training potentially enhances skeletal muscle perfusion by the acute increase in blood flow to active skeletal muscle and by the stimulation of capillary growth. The capacity for capillary proliferation in the diabetic and prediabetic state is unclear because of conflicting results (22,24,25), and the effects of exercise training on CBM thickening are likewise unclear (26).

The purpose of this study was to evaluate CBM thickness and capillary density (CD) in sedentary OZRs (S-OZRs) and their lean littermates (LZRs) at 11 and 18 wk of age to determine the metabolic conditions accompanying skeletal muscle microvascular disease in the OZR. In addition, CBM thickness and CD were measured in OZRs that began exercise training early (6 wk) and later (11 wk) in life to determine whether training prevented or reversed skeletal muscle vascular disease.

RESEARCH DESIGN AND METHODS

Animals. Five-week-old male OZRs (*fa/fa*) and their heterozygote lean littermates (*FA/fa*) were obtained from the Obesity Core Center (Vassar College, Poughkeepsie, NY). Obese animals were randomly assigned to S-OZR or exercise groups, and LZR animals served as age-matched controls. Exercise training was conducted from 6 to 11 (T6–11), 11 to 18 (T11–18), or 6 to 18 (T6–18) wk of age. Rat chow and water were provided ad libitum, and the animals experienced a 12-h light-dark cycle.

Exercise training was treadmill running, with the speed, grade, and duration of exercise gradually increased over the first 10 days of training, which were then maintained at 15 m/min, 17% grade, 60 min/day, and 5 days/wk, respectively. An avoidable shock grid was attached to the rear of the treadmill. Maximal current was <5 mA, and animals receiving an excessive number of shocks or experiencing difficulty maintaining treadmill speed were removed from the treadmill and allowed to rest. All animals completed ≥98% of the training sessions, and all exercise sessions were between 0600 and 1100.

Blood and tissue analysis. A fasting (12-h) tail vein blood sample was obtained before the start of surgery. Plasma was stored at −80°C and used in the subsequent analysis of glucose (Beckman Glucose Analyzer II, Irvine, CA; between- and within-run coefficient of variation [C.V.] <1%) and insulin (Radioassay Systems, Carson, CA; within-run C.V. = 4.3%, between-run C.V. = 9.7%) concentrations. Insulin concentrations were determined from a binding curve generated from rat insulin standards (Lilly, Indianapolis, IN).

To verify that the rats adapted to aerobic training, a 5% homogenate solution of the left plantar muscle was prepared, and citrate synthase activity was measured at 30°C (within-run C.V. = 2.5%) (27).

Tissue fixation. Forty-eight hours after the last training session and after a 12-h fast, the animals were prepared for perfusion fixation of the hind limb. Animals were anesthetized with pentobarbital sodium (75–100 mg/kg i.p.), and the left plantar muscle was removed and preserved for subsequent analysis of citrate synthase activity. The descending aorta and vena cava were cannulated. The left iliac artery was ligated, and the right hind limb was flushed with 30 ml of Krebs-Henseleit buffer containing 8 mmol/ml glucose and 1 U/ml heparin, and the animal was killed.

The knee joint was secured in the extended position, and the hind limb was fixed by perfusion for 5–10 min at 4–6 ml/min with 2.5% glutaraldehyde and 2.0% paraformaldehyde containing 75 mM sucrose in a 0.1 M phosphate buffer (pH 7.4). The plantar muscle was removed and divided into 1 × 1 × 3-mm blocks. To maintain muscle cell orientation, the long axis of the muscle fibers were parallel to the long axis of the block. The tissues were fixed for an additional 1.5 h in glutaraldehyde/paraformaldehyde and were post-fixed in 1% osmium for 1 h, dehydrated in 50, 70, 80, 90, and 100% acetone, and embedded in Spurr's resin.

Sampling and measurement. Analyses of CD and CBM thickness were performed on randomly sequenced micrographs by an investigator who did not know the nature of the samples. Muscle cross sections 1-μm thick from two random blocks per animal were stained with methylene blue. CD was determined by counting the total number of capillaries and complete muscle fibers in each block and calculating the capillary-to–muscle fiber ratio. Two hundred fibers were observed per animal.

Thin sections on 300-mesh grids were stained with uranyl acetate and lead citrate and examined in a Philips-300 electron microscope. Micrographs of 5 capillaries from each block were obtained, resulting in evaluation of 10 capillaries per animal. Six or seven animals were evaluated in each group, resulting in evaluation of 60–70 capillaries per group, for a total of 430 capillaries for all groups. Capillaries cut in cross section were chosen on the basis of a circular profile and presence of a continuous basement membrane and continuity in shape.

The CBM area (nm^2) and outer capillary circumference (nm) were determined with a Zeiss Videoplan (New York). Capillary outside diameter was calculated from the measured circumference, and CBM area was evaluated by subtracting the area described by the CBM-to–endothelial cell junction from the area described by the outer CBM boundary. The CBM thickness was calculated by dividing the CBM area by the circumference. Endothelial cell thickness was determined from the average of 10 radial measurements spaced evenly around the capillary circumference. Portions of the capillary wall containing endothelial nuclei were not evaluated. Total capillary wall thickness was taken to be the sum of the average endothelial and CBM thicknesses for each capillary. Capillary inside diameter was determined by subtracting the wall thickness from the outside diameter.

Statistical analysis. Differences between groups were determined by analysis of variance and Student's protected *t* methods, with the significance level set at $P < .05$. The 95% confidence intervals (CIs) for capillary wall dimensions of

TABLE 1
Fasting insulin and glucose and plantar citrate synthase activity

	Insulin (μU/ml)		Glucose (mM)		Insulin/glucose		Citrate synthase (μmol · min^{-1} · g^{-1})	
	Mean ± SE	*n*	Mean ± SE	*n*	Mean ± SE	*n*	Mean ± SE	*n*
LZR-11	256 ± 11	3	7.14 ± 0.94	5	42.8 ± 10.1	3	31.10 ± 2.64	4
S-OZR-11	271 ± 7	4	9.24 ± 0.27	5	30.1 ± 1.6	4	36.86 ± 5.10	4
T6–11	272 ± 11	4	7.98 ± 0.56	6	35.2 ± 3.8	4	51.18 ± 5.00*†	4
LZR-18	251 ± 5	6	6.92 ± 0.33	6	36.7 ± 2.2	6	31.33 ± 2.32	3
S-OZR-18	290 ± 7*‡	6	9.35 ± 0.61	5	32.0 ± 2.4*	5	42.62 ± 2.57*	5
T11–18	279 ± 8*	5	10.91 ± 0.44*†‡	6	25.6 ± 1.0*†‡	5	51.34 ± 5.62*	4
T6–18	310 ± 7*†‡§	6	8.94 ± 0.42*§	6	35.0 ± 1.4§	6	54.96 ± 3.05*†	6

LZR lean Zucker rats; S-OZR, sedentary obese Zucker rats; T, trained rats; numbers with abbreviations indicate weeks of age.
*Significantly different from age-matched LZR.
†Significantly different from age-matched S-OZR.
‡Significantly different from group-matched 11-wk-old rats.
§Significantly different from T11–18.

LZRs were calculated and used to identify age-matched animals that demonstrated significant wall thickening. Tests for significant differences in proportions were used to determine group differences in the incidence of CBM, endothelial cell, and total wall thickening. Relationships between variables were analyzed by calculation of product-moment correlation coefficients.

RESULTS

Citrate synthase. Oxidative capacity of the plantar muscle was increased by 40% in T6–11, 20% in T11–18, and 30% in T6–18 rats compared with S-OZRs (Table 1). There was no difference between T11–18 and T6–18 citrate synthase activities. Thus, the training program elicited the expected training response in OZRs.

Blood parameters. OZR animals were slightly hyperinsulinemic at 11 wk and profoundly hyperinsulinemic at 18 wk of age (Table 1). The progressive increase in basal insulin with age was not attenuated by training. In fact, T6–18 rats had significantly higher fasting blood insulin concentrations than S-OZRs at 18 wk of age. OZRs were mildly hyperglycemic at 11 wk and had surpassed clinical hyperglycemia (>7.2 μmol/ml) at 18 wk of age (28). Training did not affect fasting blood glucose concentrations in 11-wk-old OZRs (T6–11), and OZRs trained from 11 to 18 wk of age had significantly higher fasting glucose concentrations than LZRs or S-OZRs at 18 wk of age. On the other hand, animals trained for 12 wk (T6–18 rats) had significantly lower fasting glucose concentrations than S-OZRs or T11–18 rats at 18 wk of age. The insulin-to-glucose ratio tended to be lower in obese animals. Training had a variable effect on insulin/glucose: the higher insulin concentrations found in T6–18 rats produced a ratio equivalent to those of LZRs, but T11–18 rats had ratios even more depressed than their sedentary counterparts.

Capillary density. OZRs had fewer capillaries per muscle fiber than LZRs at 11 and 18 wk of age (Fig. 1). Training did not stimulate capillary growth in 11-wk-old OZRs (T6–11 rats); however, the capillary density of T11–18 and T6–18 rats was not significantly different from 18-wk-old LZRs. There were no age-related changes in CD for sedentary LZRs or S-OZRs.

CBM thickness. OZRs had thicker CBM than LZRs at 11 and 18 wk of age (Fig. 2). An age-related decline in CBM thickness occurred in sedentary lean and obese animals. Aerobic training did not affect CBM thickness in T6–11 rats

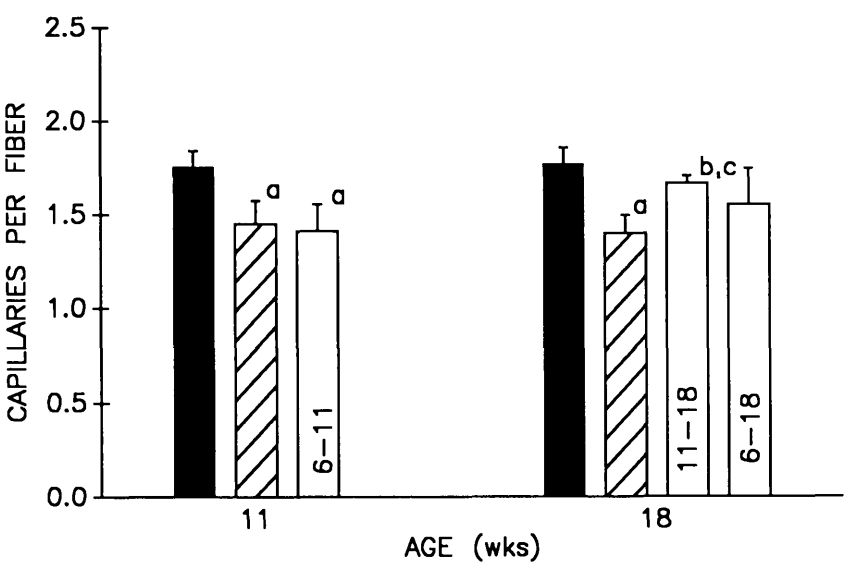

FIG. 1. Capillary/muscle fiber ratio in plantar muscle of sedentary lean (*solid bars*) and obese (*hatched bars*) Zucker rats at 11 and 18 wk of age, respectively, and obese rats trained over various ages (*open bars*). Significance level $P < .05$: a, vs. age-matched lean; b, vs. age-matched sedentary obese; c, vs. group matched at 11 wk old.

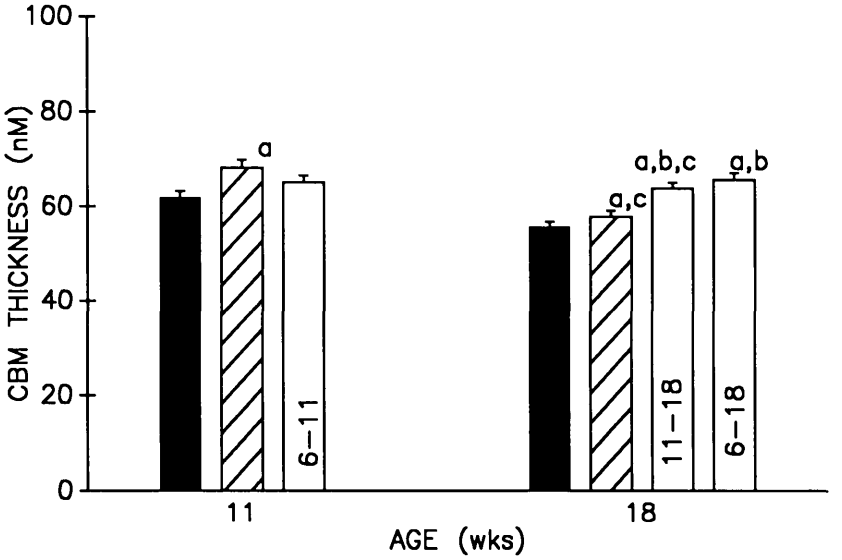

FIG. 2. Capillary basement membrane (CBM) thickness in plantar muscle of sedentary lean (*solid bars*) and obese (*hatched bars*) Zucker rats at 11 and 18 wk of age, respectively, and obese rats trained over various ages (*open bars*). $n = 6$ for all groups except 18-wk-old sedentary obese group, in which $n = 7$. Significance level $P < .05$: a, vs. age-matched lean; b, vs. age-matched sedentary obese; c, vs. group matched at 11 wk old.

but increased CBM thickness in T11–18 and T6–18 rats. Logarithmic transformation of the data did not alter the results.

Endothelial cell and total wall thickness. Obese animals had larger endothelial and wall thicknesses at 11 wk of age, but these differences disappeared by 18 wk of age (Table 2), because age-related declines occurred in obese animals. Training did not affect wall thickness in T6–11 rats, but, as observed for CBM thickness, endothelial and total wall thicknesses increased in T11–18 and T6–18 rats.

Capillary diameter. At 11 and 18 wk of age, S-OZRs and LZRs had similar capillary diameters (Table 2). Age-related differences were not present. Training appeared to slightly increase outside but not inside lumen diameters, reflecting the increased endothelial and CBM thickness.

Incidence of thickening. The 95% CIs (2.31SD)for age-matched LZRs were calculated to determine the incidence of CBM, endothelial, and total wall thickening. Obese animals had a higher incidence of CBM, endothelial, and total wall thickening than their lean counterparts (Table 3). Training did not alter the incidence of CBM thickening at 11 wk of age, but it decreased the incidence of endothelial and total wall thickening. Training generally increased the incidence of thickening in 18-wk-old animals.

Correlational analysis. There were no apparent relationships between capillary density and average CBM thickness, endothelial thickness, total wall thickness, or capillary diameters (Table 4). Endothelial and CBM thicknesses were positively related ($r = .44$, $P < .05$). A modest relationship was observed between CBM thickness and inside diameter ($r = .28$, $P < .05$).

Endothelial and total wall thicknesses were not related to the fasting glucose or insulin concentrations or insulin/glucose ratio ($P > .05$; Table 5). However, CBM thickness was positively related to the fasting insulin concentration ($r = .29$, $P < .05$).

DISCUSSION

We demonstrated the presence of moderate diabetes like microvascular abnormalities in skeletal muscle of OZRs. S-OZRs have fewer capillaries and thicker CBMs than their lean littermates. The age-related decline in CBM thickness is greater in obese animals, so CBM thickening is more pronounced at 11 wk of age. Exercise training has no effect on CD or CBM thickness in 11-wk-old OZRs but tends to increase both in older (18-wk-old) OZRs.

The lower CD observed in skeletal muscle of diabetic animals (29,30) and humans (31) is thought to reflect capillary

TABLE 2
Capillary density and dimensions

	Capillary density (capillary/fiber)	CBM (nm^2)	Endothelial cell thickness (nm)	Total wall thickness (nm)	ID (μm)	OD (μm)
LZR-11	1.754 ± 0.085	61.87 ± 1.33	174 ± 4	236 ± 2.64 (4)	3.841 ± 0.107	4.313 ± 0.110
S-OZR-11	1.449 ± 0.124*	68.13 ± 1.66*	203 ± 7*	271 ± 5.10 (4)	4.023 ± 0.118	4.566 ± 0.115
T6–11	1.412 ± 0.145*	65.10 ± 1.43	200 ± 12*	265 ± 5.00*† (4)	4.310 ± 0.127	4.839 ± 0.119*†
LZR-18	1.770 ± 0.088	55.67 ± 1.04	147 ± 5‡	203 ± 2.32 (3)	3.730 ± 0.096	4.177 ± 0.102
S-OZR-18	1.402 ± 0.097*	57.82 ± 1.24*‡	136 ± 4‡	194 ± 2.57 (5)*	3.947 ± 0.081	4.345 ± 0.083
T11–18	1.665 ± 0.040†‡	63.70 ± 1.29*†‡	160 ± 6*†‡	224 ± 5.62 (4)*	4.939 ± 0.160*†	5.386 ± 0.157*†‡
T6–18	1.558 ± 0.189	65.57 ± 1.48*†	164 ± 5*†‡	230 ± 3.05 (6)*†	4.038 ± 0.106	4.497 ± 0.109*‡

CBM, capillary basement membrane; ID, capillary lumen inside diameter; OD, capillary outside diameter; other abbreviations as in Table 1.
*Significantly different from age-matched LZR.
†Significantly different from age-matched S-OZR.
‡Significantly different from T11–18.

TABLE 3
Incidence of capillary wall thickening

	Capillary basement membrane	Endothelial cell thickness	Total wall thickness
LZR-11	1:6	0:6	0:6
S-OZR-11	3:6*	5:6*	5:6*
T6–11	3:6*	2:6*†	2:6*†
LZR-18	0:6	1:6	0:6
S-OZR-18	2:7*	0:7	1:7*
T11–18	5:6*†	2:6†	2:6*
T6–18	5:6*†	1:6	2:6*

Values are ratios of animals with incidence of thickening to total animals in group. Abbreviations as in Table 1.
*$P < .05$ vs. age-matched LZR.
†$P < .05$ vs. age-matched S-OZR.

degeneration and inhibition of capillary proliferation in the diabetic state (32). The decrease in CD results in increased diffusion distances that may impede insulin action, including the stimulation of glucose uptake, thereby increasing the severity of hyperglycemia (12–15,33). This hypothesis is supported by significant inverse relationships between circulating concentrations of glucose and insulin and skeletal muscle CD in obese and diabetic patients (12,13,23,34). Furthermore, Lithell et al. (34) and Krotkiewski et al. (12) concluded that skeletal muscle morphology (fiber diameter and CD, hence diffusion distances) could account for ~80% of the total variation in fasting serum insulin in glucose-tolerant individuals. However, other investigators have been unable to demonstrate such relationships in the early stages of glucose intolerance (10). Similarly, we found no significant correlations between CD and fasting serum glucose or insulin concentrations in the mildly hyperglycemic OZRs. Alterations in insulin-receptor interactions or postreceptor binding events may account for the apparent dissociation of skeletal muscle capillarity and circulating insulin in the early stages of glucose intolerance. However, in the early stages of the metabolic syndrome, we observed a significantly lower CD in the S-OZRs.

In general, both normo- and hyperglycemic animals have thinner CBM than humans of similar maturational age, and the absolute and proportional thickening of the CBM in diabetic animals is more variable and seems minor compared with that observed in humans (Table 6). Although hyperglycemic Chinese hamsters, KK mice, sand rats, and streptozocin-induced diabetic rhesus monkeys have normal CBM thicknesses (5,35), monkeys with prolonged hyperglycemia (19,36) and hyperglycemic South African hamsters (37) have thickened CBMs.

TABLE 4
Relationships between capillary density (CD) and dimensions

	n	CBM	Endothelial cell thickness	Total wall thickness	ID	OD
CD	43	−.1013	−.0742	−.1122	.1463	.1559
CBM	430		.4378*	.5790*	.2752*	.2001*

Values are *r* from correlation matrix. CBM, capillary basement membrane; ID, capillary lumen inside diameter; OD, capillary outside diameter.
*$P < .05$.

TABLE 5
Relationships between capillary dimensions and fasting serum insulin and glucose

	Insulin (I)	Glucose (G)	I/G
Capillary density	−.0584	−.1923	.0940
Capillary basement membrane	.2875*	.1223	.0758
Endothelial cell thickness	−.0347	−.0553	−.1091
Wall thickness	.0189	−.0304	−.1092
ID	.0506	.1968	.1306
OD	.0520	.1957	−.1374

Values are *r* from correlation matrix. ID, capillary lumen inside diameter; OD, capillary outside diameter.
*$P < .05$.

The skeletal muscle CBM thickness of animal models of NIDDM has not been thoroughly evaluated. Profoundly ketotic and diabetic spiny mice, which mimic insulin-dependent diabetes mellitus (IDDM), have pronounced CBM thickening (44%), whereas mildly diabetic spiny mice, which more closely resemble NIDDM, have only a slight degree (3–9%) of CBM thickening (38). Similarly, we found a small (≤10%) but statistically significant degree of skeletal muscle CBM thickening in both normo- and hyperglycemic OZRs. Creutzfeldt et al. (38) dismissed the 3–9% increase in CBM thickness observed in mildly diabetic spiny mice as functionally insignificant, and reports of increased permeability of the diabetic CBM indicate that the thickened membrane does not present a significant diffusion barrier to small solutes (8,39,40). However, if skeletal muscle CBM thickening is considered an early expression of the metabolic and vascular diabetic syndrome (5,7,19,21), even modest degrees of thickening could be diagnostically significant and would substantiate the link between the metabolic syndrome and peripheral vascular disease.

One study suggests that endothelial degeneration and capillary regrowth result in sequential deposition of uniformly thick basal lamina and overall increase in total CBM width (41). If the study is correct, CBM thickness is determined by the number of degeneration-regeneration cycles in a given capillary. This hypothesis is supported by the natural increase in CBM width observed with age (7,8,20,41). The diabetic milieu apparently accelerates the aging process by inducing endothelial damage and degeneration and increasing capillary turnover (41). The relatively thin CBM observed in animals may be explained by this hypothesis. The CBM thicknesses observed in animals are similar to the 0- to 10-

TABLE 6
Human and animal skeletal muscle capillary basement membrane (CBM) thickness

	CBM thickness (nm)	
	Human	Animals
Normal	108–115	50–80
Insulin-dependent diabetic	150–240 (100)	70–120 (10–50)
Non-insulin-dependent diabetic	140 (25)	70–80 (3–9)

Data were compiled from refs. 5, 7, 8, 11, 19, 35–38. Values in parentheses are percentages of thickening.

yr-old estimates extrapolated from values obtained in normal and diabetic patients (8,20). If the rate of capillary turnover is similar between animals and humans, CBM thickness may be more dependent on absolute age than maturational age, and pathological thickening of the CBM may not be detected until later in life in the animal model. Furthermore, a significant decline in CBM thickness was observed in 11- to 18-wk-old sedentary lean and obese animals. This observation is in direct contrast to the previously reported increase in CBM thickness with age (7,8,20). We hesitate to rule out an age-related increase in CBM thickness in OZRs because of the narrow age range studied and the relatively early maturational state of 11- and 18-wk-old OZRs, but the absence of such an observable relationship would not be unexpected if the capillaries were undergoing their first degeneration-regeneration cycle.

CBM thickening in the renal and retinal vascular beds is thought to be a consequence of hyperglycemia (5,21). Furthermore, reports of a relationship between CBM thickness and duration of diabetes has been considered evidence that the diabetic milieu stimulates skeletal muscle CBM deposition (19). Although a significant relationship has been observed between skeletal muscle CBM thickness and glucose excess and insulin lack in IDDM (19,28), other investigators have been unable to verify this relationship (5,8,10), and CBM thickening has been found in normoglycemic prediabetic patients (5,7) and asymptomatic chemical diabetes (42). In addition, Fajans et al. (43,44) reported a 25% incidence of CBM thickening in NIDDM patients who were either hyper- or hypoinsulinemic. We identified significant CBM thickening in hyperinsulinemic OZRs under both normo- and hyperglycemic conditions, demonstrating that glucose excess and insulin lack are not prerequisites of CBM thickening. Furthermore, CBM thickening was most pronounced in the normoglycemic younger animals. These results are comparable with those of Mauer et al. (8), in which the degree of CBM thickening was shown to diminish with age in NIDDM patients, and contradict the findings in hypoinsulinemic IDDM patients (11,19,20). A modest but significant positive relationship between CBM thickness and serum insulin was evident in the OZRs ($r = .29$, $P < .05$) and was most pronounced at 18 wk of age ($r = .38$, $P < .05$), although there was no such relationship between CBM thickness and blood glucose at either age. Therefore, as demonstrated by the OZR model, hyperinsulinemia may be more important than mild hyperglycemia in determining skeletal muscle CBM thickening associated with obesity and NIDDM.

Exercise training is often prescribed in the treatment of obesity and diabetes mellitus (22,23). Obese and diabetic patients are capable of increases in functional and oxidative capacity equivalent to those observed in control subjects (12,22,23,25), and a significant increase in skeletal muscle oxidative capacity was observed in the trained OZRs. However, normal subjects demonstrate increases in both capillary/muscle fiber and capillary/mm^2 of skeletal muscle, whereas diabetic patients have only moderate increases in capillary/muscle fiber and no increase in capillary/mm^2 (22–24). Investigators have proposed that less potential for capillary growth exists in the diabetic state (22,24). Our results indicate that the potential for capillary growth persists in the hyperglycemic OZRs, as reflected by normal capillary/muscle fiber in 18-wk-old trained OZRs. However, a similar training protocol and increase in oxidative capacity did not stimulate capillary growth in 11-wk-old OZRs. The implications of this apparent age-related phenomenon are unclear.

Few reports of the effects of exercise training on CBM thickness are available; however, isolated reports indicate that both aerobic exercise (26) and improved glycemic control (42,45) can decrease CBM thickness in diabetic patients. We observed similar trends in 11-wk-old OZRs. In contrast, we found more pronounced CBM thickening in trained OZRs at 18 wk of age, and the age-related decline in CBM thickness observed in sedentary lean and obese animals was not observed in trained animals.

The OZRs that demonstrated increases in CD (T11–18 and T6–18 rats) also exhibited thickening of the CBM. There are several possible explanations for this relationship: new capillaries formed in response to the training stimulus may have formed thicker CBM; training may have decreased capillary degeneration rather than increased capillary proliferation, and older capillaries may continue to deposit basal laminae and produce thicker CBM; and training may increase the rate of capillary turnover, resulting in sequential deposition of basal laminae and a net increase in capillary number. Groups T11–18 and T6–18 also demonstrated an increase in basal serum insulin, which supports a role for insulin excess in CBM thickening in NIDDM.

These results indicate that early signs of microvascular disease are present in the skeletal muscle of OZRs. Capillary loss and CBM thickening are present before the development of hyperglycemia and are apparently independent of fasting glucose concentrations. Therefore, genetic or metabolic factors other than hyperglycemia must influence the development of diabetic microvascular disease, and a modest relationship between insulin excess and CBM thickness was observed. Aerobic exercise training does not significantly alter the CBM in 11-wk-old OZRs, but it enhances thickening in 18-wk-old OZRs. The thickening of CBM in trained animals parallels capillary proliferation and may be related to an increase in circulating insulin.

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