Effect of Rapid Normalization of Plasma Glucose Levels on Microvascular Dysfunction and Polyol Metabolism in Diabetic Rats

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Effects of rapid normalization of plasma glucose levels (by insulin infused via Alzet pumps implanted intraperitoneally) on plasma insulin-like growth factor I (IGF-I) levels, granulation tissue polyol levels, and vascular permeation by ^{125}I-labeled albumin were examined in male Sprague-Dawley rats with streptozocin-induced (60–65 mg/kg) diabetes. Two days after implantation of pumps, plasma insulin levels were twice normal levels and remained elevated (1.4–2.5 times normal) throughout the remainder of the study. Plasma glucose levels and granulation tissue polyol levels were normalized within 2 days after initiation of insulin treatment. Plasma IGF-I levels were significantly increased (2 times) by 2 days, but were not normalized until 7 days. In contrast, ^{125}I-albumin permeation normalized at a much slower relatively linear rate and was still not completely normal after 14 days of insulin treatment. In view of *1*) previous studies demonstrating that diabetes-induced increases in ^{125}I-albumin permeation in this tissue are linked to increased metabolism of glucose to sorbitol and 2) the rapid normalization of tissue polyol levels in this study, the relatively linear rate of normalization of vascular permeability over 14 days in these studies suggests that impaired vascular barrier functional integrity in this model is mediated by structural and/or functional vascular alterations associated with sustained increased polyol metabolism rather than by increased polyol levels per se and/or by readily reversible functional and metabolic alterations associated with acute increases in polyol metabolism. The relatively long lag time after normalization of plasma glucose and tissue polyol levels before near normalization of vascular permeability in this model is consistent with corresponding observations on the relationship between improved glycemic control and normalization of microangiopathy and neuropathy in diabetic humans and animals. The slow relatively linear rate of normalization of vascular permeability after normalization of plasma insulin and glucose levels and tissue polyol levels suggests that normalization of impaired vascular barrier function in this model is accomplished by a repair process (probably involving turnover of altered vascular constituents), the precise nature of which remains to be elucidated. *Diabetes* 37:1689–94, 1988

With recently developed methods of intensive insulin therapy, near normoglycemia can be achieved and maintained in many patients with insulin-dependent diabetes mellitus (IDDM) (1). Unfortunately, there have been reports of rapid progression of retinopathy, usually manifested as cotton-wool exudates, with improved glycemic control in some diabetic patients with a history of previous long-standing elevated levels of glycemia (2–6).

The mechanism(s) behind this worsening of retinopathy associated with intensive insulin therapy is unclear. Decreases in retinal blood flow that may occur with rapid decreases in plasma glucose levels have been suggested as one possible mechanism (5). In light of evidence linking growth factors with the development of diabetic microvascular disease (7–9), increased insulin-like growth factor I (IGF-I) levels [observed in some IDDM patients receiving intensive insulin therapy (10–13)] may be another factor contributing to the development of retinopathy.

Several studies have indicated that the late diabetic complications of cataract formation and neuropathy are strongly linked to increased metabolism of glucose to sorbitol by the enzyme aldose reductase (14–16). Recently, increased polyol metabolism in diabetic animals has also been linked to the development of morphological (17–19) and functional (20–25) changes in the microvasculature. The effect of rapid

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normalization of glycemia on polyol metabolism and vascular function in diabetic rats is unknown.

This study was undertaken to determine the effect of rapid normalization of hyperglycemia in streptozocin-induced diabetic rats on plasma IGF-I levels, granulation tissue polyol levels, and microvascular barrier functional integrity in granulation tissue.

The rationale for using granulation tissue as a paradigm for studies of diabetic microangiopathy is based on the clinical observation that new vessels formed in the diabetic milieu by neovascularization of the optic disk and adjoining retina are much more leaky and fragile than neighboring vessels present before the onset of diabetes (26,27). Similarly, new granulation tissue vessels formed by angiogenesis in subcutaneous tissue in diabetic rats are much more leaky (to ^{125}I-labeled albumin) than *1*) neighboring vessels (present before the onset of diabetes) from which the new granulation tissue vessels are derived, i.e., in overlying skin and/or underlying fascia and muscle, and *2*) new granulation tissue vessels in nondiabetic rats (28). Note in this regard that virtually all the biochemical and functional alterations associated with vascular complications of diabetes in human subjects involve constituents present in granulation tissue: e.g., *1*) new vessels (associated with neovascularization of the optic disk and retina), *2*) type IV collagen, *3*) heparan sulfate proteoglycans, and *4*) laminin (29). When granulation tissue is formed in the diabetic milieu and then removed free of contamination by adjoining tissues present before the onset of diabetes, conditions are optimized for assessing the maximal impact of the diabetic milieu on vascular function and on synthesis and metabolism of other granulation tissue constituents.

MATERIALS AND METHODS

Fifty male Sprague-Dawley rats weighing 243 ± 16 g (mean ± SD) received streptozocin (60–65 mg/kg) (Upjohn, Kalamazoo, MI) by injection via the femoral vein after the induction of ketamine anesthesia (85 mg/kg i.m.). Two sterile pieces of fabric were implanted under the skin of the lower back. The fabric stimulates angiogenesis and ingrowth of fibroblasts, which penetrate the interstices of the fabric and form new granulation tissue (28). Sixteen additional rats weighing 239 ± 17 g also had sterile fabric implanted, again under ketamine anesthesia, and served as nondiabetic control rats.

After 3 wk, Alzet osmotic minipumps (model 2001 or 2002) (Alza, Palo Alto, CA) prepared to deliver 9–10 $U \cdot kg^{-1} \cdot day^{-1}$ human pump insulin (HOE 21 PH/U; generous gift of V. Wagner, Hoechst-Roussel, Somerville, NJ) were implanted in the peritoneal cavity in 32 diabetic rats anesthetized with ketamine. In 16 diabetic and 16 nondiabetic control rats, identical pumps loaded with diluent only (sham pumps) were implanted. Two diabetic rats died before the planned implantation of sham pumps, and two diabetic rats died within 24 h of implanting insulin-containing pumps. All rats had free access to standard laboratory rat chow and water and were kept on a 12-h light-dark cycle throughout the study.

Body weights were measured at least every 3rd day throughout the study. Plasma glucose was also determined at least every 3rd day on blood obtained from the tail vein in the afternoon by the glucose oxidase method (30). Insulin-treated diabetic rats were anesthetized with pentobarbital sodium (35 mg/kg i.p.) and killed at 2 days (n = 6), 4 days (n = 7), 7 days (n = 8), and 14 days (n = 9) after pump implantation. Diabetic and nondiabetic control rats were killed 2 and 14 days (n = 8 in each group) after sham-pump implantation. At death, blood was obtained for measurement of plasma levels of IGF-I, insulin, and glucose.

Plasma insulin levels were determined by the method of Morgan and Lazarow (31) with rat insulin as a standard for sham-operated control and diabetic rats and human insulin for insulin-treated rats. Plasma IGF-I levels were assayed by the double-antibody method of Furlanetto et al. (32) after acid-ethanol extraction (33). The coefficients of variation between and within insulin assays were 10.2 and 9.8%, respectively; coefficients of variation for the IGF-I assay averaged 12.6 and 11.5% between and within assays, respectively.

Vascular permeation by ^{125}I-albumin was determined at the time of death as previously described (24,28). The index of vascular permeation by albumin is referred to as the tissue-to-blood isotope ratio (TBIR-I/Cr). This ratio is obtained by dividing the ratio of $^{125}I_{BSA}/^{51}Cr_{RBC}$ counts in the tissue by the corresponding ratio of counts in a blood sample drawn before terminating the experiment, where BSA represents bovine serum albumin and RBC represents red blood cells; i.e., TBIR-I/Cr = $(^{125}I/^{51}Cr_{tissue})/(^{125}I/^{51}Cr_{blood})$. TBIR-I/Cr > 1 indicates that the volume of distribution of albumin relative to that of RBC is greater in the tissue than in large vessels

TABLE 1
Body weights, plasma levels of insulin, glucose, and insulin-like growth factor I (IGF-I), and granulation tissue polyol levels and albumin permeation (TBIR-I/Cr) in diabetic, insulin-treated diabetic, and nondiabetic rats

	Days postoperation							
	Diabetic (sham operated)		Diabetic with insulin pump				Nondiabetic (sham operated)	
	2	14	2	4	7	14	2	14
n	8	8	6	7	8	9	8	8
Weight (g)	234 ± 24	253 ± 34	289 ± 30	268 ± 46	317 ± 36	367 ± 31	334 ± 39	359 ± 26
Glucose (mg/dl)	479 ± 49	489 ± 68	179 ± 104	237 ± 80	257 ± 124	110 ± 91	166 ± 34	140 ± 18
Polyols (nmol/g)	12.6 ± 2.7	11.0 ± 1.8	5.8 ± 1.8	5.7 ± 1.9	6.5 ± 1.5	5.4 ± 0.7	4.5 ± 1.7	4.5 ± 1.5
Insulin (ng/ml)	7.5 (5.0–42.5)	7.5 (2.5–45.0)	50 (47–180)	62 (41–136)	36 (13–64)	36 (14–160)	22.5 (5.0–45.0)	27.5 (15–42.5)
IGF-I (ng/ml)	182 (31–388)	144 (6–835)	569(408–644)	495 (89–962)	1413 (228–4611)	940 (651–1714)	779 (477–3364)	1547 (852–2615)
TBIR-I/Cr	3.00 ± 0.17	3.08 ± 0.14	2.82 ± 0.21	2.69 ± 0.13	2.46 ± 0.12	2.08 ± 0.05	2.01 ± 0.09	1.99 ± 0.08

Values are means ± SD or medians with ranges in parentheses.

and is an index of permeation of the vasculature by albumin into the extravascular space (24,28).

Assessment of polyol levels. Chemical ionization gas chromatography–mass spectrometry with ammonia as reagent and helium as carrier was used to measure the tissue levels of the trimethylsilyl (TMS) derivatives of polyols (34). Because TMS sorbitol does not separate from TMS dulcitol or TMS mannitol, we have reported all levels as polyol. Details of these techniques are described in previous publications (21,22).

Statistics. All data except plasma insulin and IGF-I values are reported as means ± SD. Because of the very large variance in plasma IGF-I and insulin levels, the median and range are reported for these parameters. A 3 × 2 factorial design analysis of variance (ANOVA) was performed on 2- and 14-day diabetic rats given insulin pumps (4- and 7-day insulin-pump rats were excluded) and the 2- and 14-day sham-operated diabetic and nondiabetic rats with the SAS General Linear Models Procedure.

Because standard deviations for most parameters assessed varied considerably between groups, the data were transformed to their natural logarithms before the ANOVA analysis. Because natural logarithm–transformed IGF-I data still were not normally distributed, a nonparametric (rank-order) Blom transformation of IGF-I data was made before the ANOVA analysis.

Although a relatively large number of comparisons can be made on the data shown in Table 1, only 12 of them address the hypotheses tested in this investigation; the remaining comparisons merely confirm previous observations or are descriptive in nature and not central to the hypotheses investigated. For these reasons, we have reported uncorrected *P* values for comparisons of data in Table 1. However, note *1*) the *P* values reported are based on two-tailed tests of significance, and *2*) the conclusions of the study do not rest on marginally significant tests of statistical significance, which might be confounded by multiple comparisons.

RESULTS

Sham-operated diabetic rats gained only 6% in body weight over the 5-wk study period. Nondiabetic rats gained 50% over the same 5-wk period. Insulin-pump diabetic rats gained weight rapidly and caught up with control rats by the end of the experiment (Table 1). Median plasma insulin levels of sham-operated diabetic rats were ~0.25–0.33 times the values in nondiabetic rats (P = .03 for 2-day and .003 for 14-day sham-operated nondiabetic rats; Table 1).

Sham-operated diabetic rats, compared with nondiabetic rats, showed marked elevations (at 2 and 14 days) in plasma glucose (~3.1 times, P = .0001), granulation tissue polyol levels (~2.6 times, P = .0001), and the TBIR-1/Cr index of ^{125}I-albumin permeation (~1.5 times, P = .0001). In contrast, plasma IGF-I levels were markedly suppressed (0.23 times, P = .0003 for 2-day sham-operated diabetic vs. nondiabetic rats; ~0.09 times, P = .0001 for 14-day sham-operated diabetic vs. nondiabetic rats).

Two days after implantation of insulin pumps, plasma insulin levels were twice those of 2-day sham-operated nondiabetic rats (P = .0001) and remained markedly elevated (~1.4–2.5 times normal) throughout the study (Table 1). Insulin-pump diabetic rats showed a dramatic decrease in plasma glucose levels (Fig. 1), with near normalization by 2 days, complete normalization by 6 days, and then relatively stable but hypoglycemic levels from day 9 until the termination of the experiment. Differences in mean glucose values shown in Fig. 1 and Table 1 are probably due to the fact that glucose values in Fig. 1 were obtained in the afternoon, whereas those in Table 1 were obtained in the morning on the day of death.

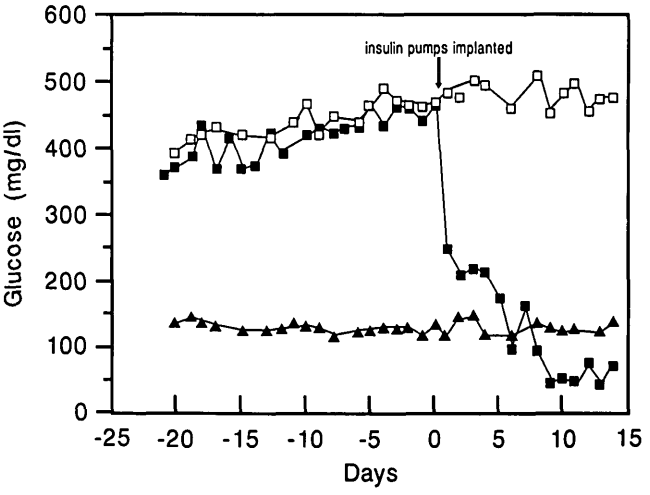

FIG. 1. Change in mean plasma glucose levels (mg/dl) with time in diabetic (□), insulin-treated diabetic (■), and nondiabetic (▲) rats. Insulin treatment began on day 0 (see MATERIALS AND METHODS for details).

Plasma glucose values and granulation tissue polyol levels were virtually normalized in 2-day insulin-pump diabetic rats (Table 1 and Fig. 2); corresponding values in 14-day insulin-pump diabetic rats did not differ significantly from those in 2-day insulin-pump diabetic rats or 14-day nondiabetic rats.

Plasma IGF-I values in 2- and 14-day insulin-pump diabetic rats were significantly higher than those in corresponding sham-operated diabetic rats (P = .044 and .0001, respectively) and were significantly higher in 14-day than in 2-day insulin-pump diabetic rats (P = .018).

^{125}I-albumin permeation in 2-day insulin-pump diabetic rats was significantly decreased (P = .016 vs. 2-day sham-operated diabetic rats) and was much lower in 14-day than in 2-day insulin-pump diabetic rats (P = .0001). ^{125}I-albumin permeation was still slightly elevated (P = .047) in 14-day insulin-pump diabetic rats (vs. 14-day nondiabetic rats).

In view of the appearance of an inverse relationship between IGF-I values and TBIR-I/Cr values across all groups (Table 1), individual values were plotted as shown in Fig. 3. The trend for I/Cr values to decrease with increasing IGF-I values is evident for I/Cr values between ln 0.9 and 1.2; however, IGF-I values are invariate for I/Cr values between 0.6 and 0.9.

DISCUSSION

The increases in granulation tissue polyol levels and ^{125}I-albumin permeation of granulation tissue vessels, as well as the decreased plasma levels of IGF-I, in sham-operated diabetic rats (vs. sham-operated nondiabetic rats) are consistent with previous observations in this animal model (20–24,28). The finding that granulation tissue polyol levels fell

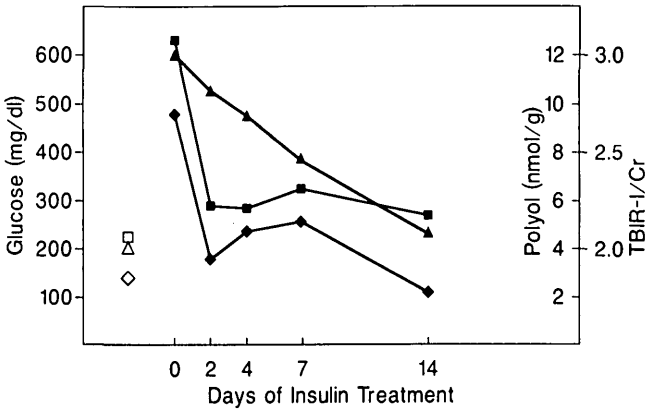

FIG. 2. Changes in mean plasma glucose (mg/dl) and insulin-like growth factor I (ng/dl) levels and in granulation tissue polyol levels (nmol/g wet wt) and ^{125}I-albumin permeation (TBIR-I/Cr) in insulin-treated diabetic rats. Values shown at time 0 are for 2-day sham-operated diabetic rats (■, polyols; ▲, I/Cr; ♦, glucose). Control values are from 14-day sham-operated control rats (□, polyols; △, I/Cr; ◇, plasma glucose).

dramatically in 2-day insulin-pump diabetic rats and did not differ significantly from those of 2-day sham-operated control rats is consistent with the normalization of plasma glucose levels in these rats as well as with numerous reports demonstrating that the polyol content of cells and tissues is related to ambient glucose levels in vivo and that polyol levels increase during incubation of cells and tissues in vitro in media containing high concentrations of glucose (14–16,35–37).

In contrast to the rapid normalization of granulation tissue polyol levels and plasma glucose levels in insulin-pump diabetic rats, a slower rate of normalization was observed for plasma IGF-I levels and vascular permeability (as reflected in TBIR-I/Cr values). The delayed normalization of vascular permeability and IGF-I levels (relative to plasma glucose and granulation tissue polyol levels) suggests that they are not as closely linked to hyperglycemia and increased polyol metabolism as the latter two phenomena are to each other. The apparent inverse relationship between plasma IGF-I levels and granulation tissue I/Cr values in Table 1 is probably merely associative rather than casual in view of *1*) the plots of the individual data points (Fig. 3) showing that the trend is evident only in the 50% of rats with I/Cr values above the mean and *2*) the relatively linear rate of normalization of I/Cr with time over the entire range of I/Cr values. This interpretation is consistent with previous observations that castration prevents the increase in vascular permeability in granulation tissue of diabetic rats but has no effect on plasma levels of IGF-I and glucose (22).

These observations, together with evidence that *1*) aldose reductase inhibitors prevent increases in albumin permeation without affecting plasma glucose levels (20,21,24) and *2*) increased albumin permeation in rats with mild diabetes is intermediate (24) between that observed in sham-operated severely diabetic rats and sham-operated control rats in this study, argue against the likelihood that the relatively linear rate of normalization of albumin permeation by insulin treatment is attributable to a simple "threshold" phenomenon or relationship between vascular permeability increases and metabolic and hormonal imbalances associated with insulinopenia and hyperglycemia.

The finding that the index of ^{125}I-albumin permeation was still only 50% normalized 7 days after implanting insulin pumps (5 days after polyol levels were 84% normalized) indicates that the vascular changes (mediating increased albumin permeation) associated with increased polyol metabolism are not nearly as readily reversed as glucose-mediated increases in polyol metabolism per se. These observations suggest that impaired endothelial barrier function in this model is not simply a readily reversible functional manifestation or consequence of transient imbalances in glucose and energy metabolism associated with insulinopenia and hyperglycemia. In other words, it is not caused by a readily reversible impairment of endothelial cell energy metabolism and function (i.e., contractile) analogous to the immediate loss of myocardial contractility and altered coronary vascular resistance induced by global no-flow myocardial ischemia [both of which are rapidly normalized after restoration of blood flow (38,39)]. It is also not analogous to the rapidly reversible loss of glomerular capillary barrier function (to endogenous albumin) in rats produced by ligation of the renal artery or vein (40).

The relatively slow linear rate of normalization of vascular permeability during the 14-day period after implantation of insulin pumps suggests that normalization of vascular permeability by insulin is accomplished by a repair process, the rate of which is relatively independent of fluctuations in plasma glucose, IGF-I, insulin levels, and tissue polyol levels in the insulin-treated diabetic rats in these experiments. Although the precise nature of this repair process remains to be elucidated, the likelihood that it may involve correction of structural-compositional alterations in vascular constitu-

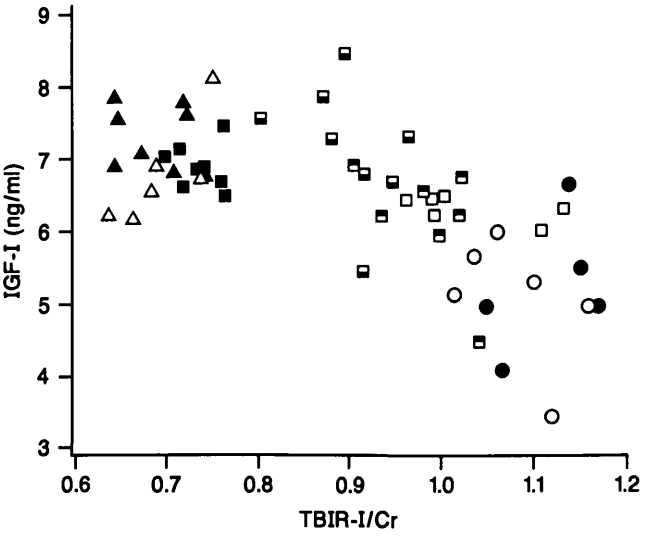

FIG. 3. Relationship between ^{125}I-albumin permeation (TBIR-I/Cr) and plasma insulin-like growth factor I (IGF-I) values for individual rats from all groups (data transformed to natural logarithms). ○, 2-day sham-operated diabetic rats; ●, 14-day sham-operated diabetic rats; □, 2-day insulin-pump diabetic rats; ⬒, 4-day insulin-pump diabetic rats; ⬓, 7-day insulin-pump diabetic rats; ■, 14-day insulin-pump diabetic rats; △, 2-day sham-operated nondiabetic rats; ▲, 14-day sham-operated nondiabetic rats.

ents critical to endothelial barrier functional integrity is supported by numerous reports documenting structural and compositional changes in vascular constituents in diabetic humans and rats and in galactose-fed rats (29,41). Alterations in some of these constituents, i.e., heparan sulfate proteoglycans and sialoproteins, have been linked to loss of vascular barrier function in glomerular and retinal capillaries (42–45).

The observation that vascular permeation by ^{125}I-albumin is almost normalized 14 days after insulin treatment is consistent with previous observations that albumin permeation is completely normalized in granulation tissue 3 wk after islet transplantation (46). In the latter study, no information was available regarding the rate of normalization of vascular permeability. These observations also are consistent with the time course of normalization of fluorescein leakage into the eyes of diabetic rats after islet transplantation reported by Krupin et al. (47) and the somewhat slower normalization of albuminuria and glomerular mesangial changes after islet transplantation in diabetic rats reported by Mauer et al. (48,49). In the study by Krupin et al. (47), normalization of fluorescein leakage did not occur until 13 days after islet transplantation. Because insulin secretion in response to an intravenous glucose tolerance test (IGTT) was still abnormal 5 days after islet transplantation but was normal at 13 days, although plasma glucose levels had normalized by 5 days and were normal during IGTTs at both 5 and 13 days, Krupin et al. concluded that "ocular vascular permeability was more closely correlated with insulin than blood glucose abnormalities." In this study, on the other hand, the linearity of the rate of normalization of vascular permeability for 14 days after rapid correction of insulinopenia and hyperglycemia suggests that the apparent correlation between ocular vascular permeability and insulin abnormalities reported by Krupin et al. was associative rather than causal.

It is well known that insulin has a direct effect on the secretion of IGF-I from the liver in rats (50,51). It is of interest that the marked increase in IGF-I levels seen 2 days after the initiation of insulin treatment, the plateau of IGF-I levels between 2 and 4 days, and their subsequent normalization by 7 days in this study are consistent with the triphasic changes in plasma somatomedin C values in poorly controlled diabetic humans after initiation of intensive insulin treatment (52).

In summary, we have shown that some metabolic (decreased plasma IGF-I levels) and vascular functional (^{125}I-albumin permeation) abnormalities caused by diabetes correct relatively slowly compared with the rapid normalization of glycemia and granulation tissue polyol levels after initiating insulin therapy. These new findings, together with previously published observations, suggest *1*) increased vascular permeability in this model is the consequence of slowly reversible biochemical and/or structural alterations in the vasculature, and *2*) normalization of vascular permeability associated with insulin treatment is accomplished by a repair process, the nature of which remains to be elucidated. Although we did not observe a worsening of microvascular dysfunction in granulation tissue by rapid normalization of glycemia with insulin therapy in diabetic rats, retinal vasculature clearly may respond differently.

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