Age-induced changes in pancreatic islet blood flow: evidence for an impaired regulation in diabetic GK rats

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Svensson, Annika M., Claes-Göran Östenson, and Leif Jansson. Age-induced changes in pancreatic islet blood flow: evidence for an impaired regulation in diabetic GK rats. Am J Physiol Endocrinol Metab 279: E1139–E1144, 2000.—The present study aimed to compare longitudinal variations in islet blood perfusion in rats with different degrees of impairment of glucose metabolism. For this purpose, mildly diabetic Goto-Kakizaki (GK) rats, glucose intolerant F1 hybrids of GK and Wistar (W) rats (H), and control W rats were examined at 5 wk, 12 wk, or 1 yr of age, using the microsphere technique for blood flow measurements. W rats showed progressively increasing islet blood flow (IBF) throughout the experiment. Both GK and H rats demonstrated increasing IBF between 5 and 12 wk. However, H rats showed no further increment in IBF at 1 yr, whereas GK rats displayed a pronounced decrease in IBF between 12 wk and 1 yr of age. The augmented IBF seen in older W rats may constitute an adaptation to the increasing demand for insulin secretion by upregulation of islet blood flow. This would lead to morphological and/or functional changes in the vasculature, eventually resulting in deterioration of glucose metabolism seen in the aging GK rat.

It is not known to what extent a long-term increase in glucose load may affect the regulation of pancreatic vasculature in glucose-intolerant and diabetic subjects. However, it has been hypothesized that the hyperperfusion associated with increased blood glucose levels would impose protracted stress on the blood vessels. This would lead to morphological and/or functional changes in the vasculature, eventually resulting in deterioration in the function of various organs (4, 18, 30, 35, 37).

It was therefore of interest to investigate a possible relationship between high glucose exposure and dysfunction of islet vasculature. Previous studies have shown increased islet blood flow (IBF) together with slightly augmented whole pancreatic blood flow in young adult mildly diabetic Goto-Kakizaki (GK) rats, and in glucose intolerant F1 hybrids between male GK and female Wistar (W) rats (H) from the Stockholm colony (2, 33, 34).

The following experiments evaluated the hypothesis that rats with abnormal glucose homeostasis would show altered vascular function with time and, furthermore, that the magnitude of such changes would correlate with the severity of the glucose intolerance. Longitudinal changes in glucose tolerance and pancreatic and islet blood flow were evaluated in GK, H, and normoglycemic W rats. In addition, the ability to respond to an acute increase in blood glucose with an augmentation of pancreatic IBF was investigated in diabetic GK rats.

MATERIALS AND METHODS

Animals. Female W rats were purchased from B & K Universal (Sollentuna, Sweden). Female GK-Wistar rats (GK) and F1 hybrids (H), which were offspring of female W and male GK rats, were obtained from the Stockholm GK colony (Department of Endocrinology, Karolinska Hospital, Stockholm, Sweden), established with breeding couples from Tohoku University School of Medicine, Sendai, Japan in 1989. All animals had free access to tap water and pelleted food, with the exception of a 12-h fast preceding the glucose tolerance tests (see below). All experiments were approved by the animal ethics committee at Uppsala University, Uppsala.

Intraperitoneal glucose tolerance test. An intraperitoneal glucose tolerance test (ipGTT) was performed 2 days before the blood flow measurements. After an overnight fast, the animals were injected intraperitoneally with a 30% (wt/vol) D-glucose solution (2 g glucose/kg body wt). Blood samples were drawn from the tail vein immediately before and 10, 30, 60, and 120 min after glucose administration. Blood glucose concentrations were measured with test reagent strips (ExaTech, Baxter Travenol, Deerfield, IL). Area under the curve for the ipGTT was determined by computerized image analysis (MOP-Videoplan, Zeiss Svenska, Stockholm, Sweden).

Blood flow measurements. Details of this procedure have been given in previous publications (20). Rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (60 mg/kg body wt), heparinized, and placed on an operating table maintained at body temperature (38°C). Polyethylene catheters were inserted into the left femoral...
artery and into the ascending aorta via the right carotid artery. The latter catheter was connected to a pressure transducer (PDCR 75/1, Druck, Groby, UK) to monitor mean arterial blood pressure (MAP). After MAP had been stable for 15 min, $1.5-3.0 \times 10^9$ nonradioactive microspheres (diameter 11 μm; NEN-Trac; Du Pont Pharmaceuticals, Wilmington, DE) were injected into the ascending aorta over 10 s. An arterial blood sample was collected by free flow from the catheter in the femoral artery starting 5 s before the microsphere injection and continuing for a total of 60 s. The withdrawal rate was determined by weighing the sample.

Blood flow was measured in an arterial blood sample from the carotid catheter with test reagent sticks as given above. The animals were killed, and the pancreas and adrenal glands were taken out, blotted, weighed, and treated with a freeze-thawing technique to visualize the pancreatic islets and the microspheres (19). The number of microspheres was determined in the endocrine and exocrine pancreas, in the adrenal glands, and in the reference blood sample. The organ blood flow values were calculated according to the formula $Q_{org} = Q_{ref} \times N_{org}/N_{ref}$ where $Q_{org}$ is organ blood flow (ml/min), $Q_{ref}$ is the withdrawal rate of the reference sample (ml/min), $N_{org}$ is the number of microspheres present in the organ, and $N_{ref}$ represents the number of microspheres in the reference sample. The blood flow values of the adrenals confirmed adequate mixing of the microspheres into the circulation.

Estimation of pancreatic islet volume. The pancreatic islet volume in 5- and 12-wk-old rats was determined by an independent observer using a point counting method (36) adapted for use in freeze-thawed samples (5). Islets with a diameter <50 μm could not be analyzed with this method.

Measurement of pancreatic insulin content. Pancreatic samples (15–25 mg) were homogenized in 1 ml of redistilled water. A 50-μl aliquot of the homogenate was extracted with 125 μl of acid-ethanol [0.18 M HCl in 95% (vol/vol) ethanol] overnight at 4°C. The insulin content was determined with radioimmunoassay (13) and correlated with wet pancreatic weight.

Statistical analysis. All values were expressed as means ± SE. Statistical comparisons were made with Student’s two-tailed unpaired t-test or by ANOVA (Sigmasat, SSPS, Erfurt, Germany) in conjunction with Bonferroni’s correction.

### RESULTS

GK rats showed significantly lower body weights compared with control W rats at all ages and to H rats at 5 wk of age (Table 1). H rats had lower body weights than controls at 12 wk and at 1 yr of age (Table 1).

Blood glucose concentrations at the time of the blood flow measurements, i.e., under anesthesia, were higher in GK than in H and control rats at all time points (Table 1), whereas those of the H rats were higher than those of control animals at 5 and 12 wk of age (Table 1).

Both H and GK rats showed impaired ipGTT (Fig. 1, A-C) at all time points, as evidenced by an increased area under the curve (Table 1). Both 12- and 52-wk-old GK rats showed a lingering augmentation of blood glucose at 120 min compared with the corresponding age-matched H rats (Fig. 1, B and C).

Pancreatic islet volumes were similar in W, H, and GK rats at 5 and 12 wk (Table 1). Also, there were no detectable differences in total pancreatic insulin content correlated with pancreatic wet weight between W, H, and GK rats at any time point investigated (data not shown).

MAP was similar in all groups of animals at all ages, except for an increase in 5-wk-old H rats (Table 1).

Whole pancreatic blood flow decreased between 5 and 12 wk of age in W rats ($P < 0.001$, ANOVA) and was then maintained at a similar low level at 52 wk (Fig. 2). In both H and GK rats, pancreatic blood flow remained virtually constant between 5 and 12 wk, whereas a reduction had occurred at 52 wk (Fig. 2; $P < 0.05$, ANOVA).

Pancreatic blood flow values were similar in W, H, and GK rats at 5 wk and again at 1 yr of age. However, when 12-wk-old rats were compared, both H and GK rats displayed higher blood flow than controls (Fig. 2).

W rats displayed an increase in IBF with increasing age (Fig. 3; 5 wk vs. 12 wk: $P < 0.01$; 12 wk vs. 1 yr: $P < 0.05$, ANOVA). In H rats, a similar augmentation of IBF was seen between 5 and 12 wk (Fig. 3; $P < 0.05$, ANOVA), whereas no further increase could be seen between 12 wk and 1 yr. Likewise, GK rats showed an increase in IBF between 5 and 12 wk (Fig. 3; $P < 0.001$,

<table>
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<th>Age of animals, wk</th>
<th>5</th>
<th>5</th>
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<td>Strain of animals</td>
<td>W</td>
<td>H</td>
<td>GK</td>
<td>W</td>
<td>H</td>
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<tr>
<td>Body weight, g</td>
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<td>83±2</td>
<td>74±2*</td>
<td>209±4</td>
<td>186±2‡</td>
<td>193±5*</td>
<td>381±13</td>
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<td>114±4*</td>
<td>108±6</td>
<td>111±3</td>
<td>121±7</td>
<td>102±6</td>
<td>103±5</td>
<td>103±5</td>
<td>97±7</td>
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<td>Islet volume, % of pancreas</td>
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<td>1.39±0.15</td>
<td>1.34±0.14</td>
<td>1.87±0.16</td>
<td>1.69±0.24</td>
<td>1.76±0.19</td>
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<td>Blood glucose concentration, mM</td>
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<td>6.9±0.4‡</td>
<td>8.3±0.4‡</td>
<td>5.1±0.2</td>
<td>6.3±0.3‡</td>
<td>10.8±0.8‡</td>
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<td>Area under curve, mM/min</td>
<td>875±70</td>
<td>1,123±134‡</td>
<td>1,275±102‡</td>
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<td>1,610±100‡</td>
<td>1,725±180‡</td>
<td>950±98</td>
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Values are means ± SE. MAP, mean arterial blood pressure; W, Wistar rats; GK, Goto-Kakizaki rats; H, F1 hybrid of GK and W rats; ND, not determined. *$P < 0.05$, ‡$P < 0.01$ and ‡$P < 0.001$ by ANOVA compared with W rats of corresponding age.
ANOVA). However, at 1 yr, a marked decline in IBF was seen in the GK rats compared with 12-wk-old animals of the same strain (Fig. 3; \( P < 0.01 \), ANOVA).

H rats showed increased IBF at 5 and 12 wk compared with controls, whereas at 1 yr of age, no such difference was seen (Fig. 3). IBF was higher in 12-wk-old GK rats than in W rats of the same age. However, at both 5 wk and 1 yr of age, IBF was markedly decreased in diabetic GK rats compared with control and with H rats (Fig. 3; \( P < 0.001 \) for both comparisons, ANOVA).

**DISCUSSION**

The regulation of IBF is distinct from that of the exocrine pancreatic blood flow. A number of possibly interdependent neural and local factors have been suggested to influence islet blood perfusion (18). Our earlier investigations have pointed to an association between augmented functional load on the islets and increased IBF in different experimental systems, i.e., 48-h continuous high-glucose load, pregnancy, and diet-induced and inherited obesity (18, 31, 33, 34). An increment in the demand for insulin secretion with advancing age has been demonstrated previously in normal rats (7). The increase in IBF observed in W rats between 5 wk and 1 yr of age, confirming earlier findings (23), may thus be related to augmented functional load on the \( \beta \)-cell mass as well as to a moderate in-

Fig. 1. Intraperitoneal glucose tolerance test in Wistar (W) rats (○), \( F_1 \) hybrid (H) rats (●) and Goto-Kakizaki (GK) rats (■) at 5 (A), 12 (B), or 52 (C) wk of age. Blood glucose values for both GK and H rats were higher than those of W rats at all time points after glucose injection in both 5-, 12-, and 52-wk-old animals (\( P < 0.05 \) or \( P < 0.01 \), ANOVA). Both 12- and 52-wk-old GK rats showed increased 120-min values vs. corresponding age-matched H rats (\( P < 0.05 \) and \( P < 0.01 \), respectively, ANOVA).

Fig. 2. Whole pancreatic blood flow in W rats (filled bars), H rats (open bars) and GK rats (gray bars) at 5 wk, 12 wk, or 1 yr of age. Values represent means ± SE for 7–10 experiments. *\( P < 0.05 \) and ***\( P < 0.001 \) vs. W rats of the same age with ANOVA.
crease in islet volume (14, 15) and could constitute a compensatory mechanism whereby aging animals maintain glucose homeostasis. The aim of the present study was to make comparisons of IBF between normal, glucose-intolerant, and overtly diabetic rats at different ages, to investigate longitudinal changes within the groups, and, finally, to elucidate a possible relationship between IBF and the degree of glucose intolerance.

The GK rat is a nonobese animal model of hereditary non-insulin-dependent diabetes mellitus characterized by impaired glucose tolerance and mild hyperglycemia (10, 24, 32). Insulin release is decreased already during the 1st wk of life, and this disturbance seems to be related to aberrations in glucose metabolism and stimulus-secretion coupling within the β-cells (8, 25, 26, 28, 29). Impairment of β-cell function, although less pronounced, is also present in H rats (1, 11).

An increase in MAP was noted in the H rats at 5 wk of age, whereas the pressures were similar in all other age groups. The reason for this single outlying value is unknown, and we interpret it as random. The body weights were lower in the GK rats at 5 wk of age compared with W and H rats. However, in 12- and 52-wk-old GK and H rats, the body weights were similar to or lower than those of W rats. This means that there is no correlation between the degree of glucose intolerance and body weight development in GK and H rats. The reasons for this are unknown but may reflect the fact that the GK rat in itself is a smaller rat strain than W rats and that the H rats inherit this trait.

We and others (2, 33, 34) have demonstrated increased islet perfusion in adult GK and H rats. The results of the present study showing augmented IBF in 12-wk-old GK and H rats thus agree with previous data. GK rats show progressive proliferation of fibrous tissue in the islets, eventually leading to the formation of irregularly shaped, so-called “starfish islets” (9, 16). Although a previous paper described decreased islet volume and pancreatic insulin content in 4-mo-old GK rats from the Paris colony (27), a study on GK rats from the London colony (17), as well as investigations involving W, GK, and H rats from the Stockholm colony (1, 12), failed to demonstrate differences in islet β-cell density or insulin content between GK rats and controls at 12 wk of age. Comparable results were obtained in the present study, where no differences in either parameter were seen between the W, H, and GK rats, suggesting that all groups of rats examined had similar functional β-cells mass. However, the highly irregular islet outline (9, 12, 16) prevented exact measurements of islet volume in the 1-yr-old rats using freeze-thawed specimens. Hence, it cannot be excluded that islet mass was diminished to some extent in 1-yr-old GK rats. However, it seems unlikely that islet and capillary volume would be decreased to such an extent that this alone would account for alterations in the blood perfusion observed in these rats.

H rats showed increased IBF at 5 and 12 wk compared with controls. Interestingly, 1-yr-old H rats did not show augmented IBF despite glucose intolerance similar to that observed in younger H rats. Even more intriguing are the low levels of IBF observed in 5-wk-old and 1-yr-old GK rats, which are significantly decreased compared with both W and H rats, in the face of increased functional load on the islets. The relative hypoperfusion of the islets could be explained without rejecting the suggested hypothesis implicating a correlation between IBF and functional load if one assumes that a specific functional impairment of the islet blood vessels is present in the GK rat. For instance it could be speculated that the adaptive ability of islet vessels is less developed in the 5-wk-old GK rats than in age-matched controls, due to impaired or retarded maturation of the vasculature.

The finding that 1-yr-old hyperglycemic GK rats have lower IBF compared with age-matched H and W rats, as well as with 12-wk-old GK rats, could be explained by deteriorated capillary structure and/or function in aging GK rats. Whether a long-standing increase in glucose load resulting in increased capillary hyperperfusion and an associated tangential capillary pressure affects the vasculature of the islets is not known. However, it could be hypothesized that hyperperfusion leads to structural changes in the islet vasculature analogous to what has been suggested for the development of diabetic complications in kidney and retina (30, 37, 38). Hyperglycemic pseudohypoxemia with increased formation of superoxide anions and nitric oxide could be of importance in this context (37).

To further explore the nature of the functional impairment of islet vasculature in the GK rat, the effect of an acute glucose load on the pancreatic islet blood flow was previously (34) investigated in 12-wk-old animals, i.e., at a time when basal IBF is still increased compared with control rats. In normal rats, an acute glucose load augmented IBF, whereas GK rats failed to
respond with increased IBF. These results suggested that a functional impairment of the vasculature is present in the GK rat already at 12 wk of age.

In conclusion, the relationship between the degree of glucose intolerance and islet blood perfusion varied among GK, H, and W rats of different ages. Taken together, the results suggest defects in islet vascular regulation in GK and, possibly to a lesser degree, in H rats. Such aberrations could be related to the exposure to increased levels of glucose. Conversely, the inability to adjust to the increased demand for insulin secretion by increasing the blood flow through the islets could contribute to the progressive worsening of glucose intolerance in older GK rats. Underlying morphological changes in islet vasculature remain to be investigated. Similarly, because previous studies demonstrated that short-term normalization of blood glucose with pharmacological treatment decreased islet capillary pressure in adult GK rats (6), it would be of interest to investigate whether long-term treatment with agents that lower blood glucose could prevent the deterioration in glucose metabolism and/or the decrease in IBF seen in 1-yr-old rats in the present study.

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