Marked Increase in White Adipose Tissue Blood Perfusion in the Type 2 Diabetic GK Rat

Caroline Kampf,1,2 Birgitta Bodin,2 Örjan Källskog,2 Carina Carlsson,2 and Leif Jansson2

The aim of the present study was to evaluate and correlate islet to brown and white adipose tissue (WAT) blood perfusion in one obese rat and one nonobese rat with type 2 diabetes (obese Zucker [OZ] and GK rats, respectively). We measured blood perfusion with a microsphere technique in anesthetized animals and subsequently the blood flow to seven different WAT depots and brown adipose tissue, in addition to the whole pancreas and pancreatic islets. Both GK and OZ rats had higher islet blood perfusion than their respective control strains. Adipose tissue blood flow (ATBF) was similar to or lower than that of controls in the normoglycemic OZ rats. GK rats, however, had 5–10 times higher blood perfusion than control Wistar rats in most WAT depots. Vascular density and macrophage numbers in WAT did not differ between the different strains. The discrepancy in ATBF between the obese-normoglycemic and type 2 diabetic rats opens the intriguing possibility that changes in this blood perfusion may influence and/or modulate the β-cell dysfunction in type 2 diabetes. Diabetes 54:2620–2627, 2005

Obesity is a major worldwide health problem that is statistically associated with increased risk for cardiovascular disease, type 2 diabetes, and stroke (1,2). Obesity closely coexists with insulin resistance and endothelial dysfunction during the natural history of type 2 diabetes (3). It seems established that substances released from white adipose tissue (WAT), including free fatty acids (FFAs), leptin, interleukin 6, tumor necrosis factor-α (TNF-α), and adiponectin, may contribute to both insulin resistance, in muscle and WAT, and β-cell dysfunction (3–6).

Previous studies have demonstrated that pancreatic islet blood flow is increased during glucose intolerance and, at least initially, during type 2 diabetes (7,8). In this context, it is of interest that an impaired WAT blood flow has been linked to obesity and insulin resistance in humans (9–11). Furthermore, in the obese Zucker (OZ) rat, a decrease in WAT blood flow has been observed (12,13), while pancreatic islet blood flow is increased (14). The extraction of plasma triglycerides is probably influenced by WAT blood perfusion, which also facilitates signaling between adipose tissue and other tissues to regulate metabolism (15,16). In addition, the release of FFAs can be modulated by WAT blood flow (16). Thus, change in WAT blood flow can be expected to affect its own metabolic functions. To what extent such changes in adipose tissue function may affect islet blood flow and islet hormone release in general is at present unknown.

In view of the considerations presented above, we hypothesized that adiposity, especially in animal models of type 2 diabetes, may affect the blood perfusion of both WAT and the islets. The aim of the present study was therefore to correlate WAT and islet blood flow in one normoglycemic obese rat strain and one nonobese model of type 2 diabetes (the OZ rat and the GK rat, respectively). Both these rat strains have an increased islet blood flow (14,17,18). Macrophages have been suggested to infiltrate WAT; therefore, we investigated macrophage density.

RESEARCH DESIGN AND METHODS

Male Wistar or GK rats aged 3–4 months were purchased from B&K (Ry, Denmark), and similarly aged OZ or lean Zucker (LZ) rats (crl:ZUC-fjoBR) were purchased from Charles River Laboratories (Hanover, Germany). The animals had free access to food (Type R3; B&K, Sollentuna, Sweden) and water throughout the experiments. All experiments were approved by the local animal ethic committee at Uppsala University (Uppsala, Sweden). Chemicals were purchased from Sigma-Aldrich (Irvine, U.K.) unless otherwise stated.

Blood flow measurements. The rats were anesthetized with an intraperitoneal injection of thiobutabarbital sodium (120 mg per kg body weight) (Inactin; Research Biochemicals, Natick, MA). The animals were then placed on a heated operating table to maintain body temperature at ~38°C. Polyethylene catheters were inserted into the ascending aorta via the right carotid artery and into the left femoral artery and vein. The former catheter was connected to a pressure transducer (PDCR 75/1; Druck; Groby, U.K.), whereas the venous catheter was used to infuse Ringer solution (5 ml per kg body weight per h) to substitute fluid. When the blood pressure had remained stable for at least 20 min, blood flow measurements were performed with a microsphere technique as previously described (19). Briefly, 2.0 × 1010 black nonradioactive microspheres (EZ-Trac; Triton Microspheres, San Diego, CA) with a diameter of 10 or 15 μm were injected through the catheter with its tip in the ascending aorta for 10 s. Starting 5 s before the microsphere injection, and continuing for a total of 60 s, an arterial blood sample was collected by free flow from the catheter in the femoral artery (~0.6 ml/min). The exact withdrawal rate was confirmed in each experiment by weighing the sample. Arterial blood was used for determinations of hematocrit, glucose concentra-
Stained blood vessels or macrophages were quantified in a light microscope. The sections were dehydrated and mounted (Pertex; HistoLab). All reagents developed with diaminobenzidine before counterstaining with hematoxylin. The sections were pretreated with proteinase K for 5 min. The CD68 antibody clone ED-1 (range 6–9). The area of the investigated tissue was determined by using a computerized system for morphometry (Easy Image 300; Tekno Optik, Huddinge, Sweden). Vascular density and macrophage numbers (i.e., the number of stained blood vessels or macrophages per measured adipose area [expressed as mm²] and/or per number of adipose cells) were then calculated.

### Morphometrical evaluation
Twelve or more randomly chosen tissue sections from each adipose tissue depot and animal were stained with either BS-1 (to visualize blood vessels) or ED-1 (to visualize macrophages). The numbers of stained blood vessels or macrophages were quantified in a light microscope (40× magnification). In each type of adipose tissue, ~8 fields were counted (range 6–9). The area of the investigated tissue was determined by using a computerized system for morphometry (Easy Image 300; Tekno Optik, Huddinge, Sweden). Vascular density and macrophage numbers (i.e., the number of stained blood vessels or macrophages per measured adipose area [expressed as mm²] and/or per number of adipose cells) were then calculated.

### RESULTS

#### General values (Table 1)

The OZ rats were markedly heavier than all other rats. The pancreas weight was similar in all groups with the exception of LZ, which had larger pancreata compared with the other groups. When pancreas weight was expressed as a fraction of body weight, this ratio was lower in OZ rats and higher in LZ rats when compared with the Wistar and GK rats.

The weight of the psoas fat pads was markedly higher in OZ rats when compared with all the other rats, whereas the fat pad weight in GK rats was higher than in Wistar rats. When the psoas fat pad weight was given as a fraction of the body weight, this ratio was lower in OZ rats and higher in LZ rats when compared with the Wistar and GK rats.

#### Statistical calculations

All values are given as means ± SE. Probabilities (P) of chance differences were calculated with Student’s unpaired t test, or with one-way repeated-measurement ANOVA with Tukey’s correction (SigmaStat; SSPD, Erfurt, Germany). A value of P < 0.05 was considered to be statistically significant. Unless otherwise stated, comparisons were made between GK and Wistar rats or OZ and Lz rats, respectively.

#### Blood flow measurements

Total pancreatic blood flow was similar in all groups of animals when the measurements were performed with 15-μm microspheres (Table 1). Islet blood flow expressed per gram pancreas was markedly higher in the OZ rats (Fig. 1A). When islet blood
perfusion in all depots when compared with WF (Fig. 2B and C and Fig. 2E and F), OZ, and LZ rats (P < 0.01 for all comparisons), with the exception of epididymal fat (Fig. 2D). Also, the blood flow to brown adipose tissue was higher in GK rats when compared with the other strains (Fig. 2H). In epididymal and dorsal subcutaneous fat, the blood perfusion was lower in OZ compared with LZ rats (Fig. 2C and D).

Duodenal and colonic blood flow values were of the same order of magnitude in the different strains. Adrenal blood flow was lower in OZ rats when compared with LZ rats (Table 1).

Morphological studies. Staining with BS-1 made it easy to identify blood vessels in both pancreatic islets (Fig. 3A) and WAT (Fig. 3B). Islet vascular density was similar in Wistar and GK rats, while islets of OZ rats contained slightly more capillaries than LZ rats (Fig. 4A). When OZ rats were compared with WF or GK rats, no differences were seen (P > 0.7; ANOVA).

The vascular density was similar in WAT in all depots when Wistar and GK rats were compared (Fig. 4B). When LZ and OZ rats were compared, the density was markedly lower in the psoas fat pad of OZ rats (Fig. 4B). LZ and OZ rats had similar vascular density of pancreatic (P = 0.097), abdominal subcutaneous (P = 0.056), and dorsal subcutaneous fat (P > 0.25).

The size of individual fat cells in the different WAT depots varied. The number of fat cells per millimeter squared was similar in Wistar and GK rats (Fig. 5A), whereas the number of fat cells was much lower in OZ rats than in LZ rats in all depots investigated (Fig. 5A).

When calculating the number of microvessels per fat cell to compensate for strain-dependent differences in size of adipocytes, a somewhat different picture appeared (Fig. 5B). Thus, there were once again no differences between Wistar and GK rats. However, the blood perfusion per fat cell was much higher in all depots of OZ rats when compared with LZ rats except for dorsal subcutaneous WAT (Fig. 5B).

The CD68 antibody clone ED-1 made macrophages within the WAT easily distinguishable (Fig. 3C). The number of macrophages per millimeter squared was similar when the WAT depots were compared between Wistar and GK rats and OZ and LZ rats, respectively, with the exception of the psoas fat pad in GK rats, which contained fewer macrophages than Wistar rats (Fig. 6A). This value in GK rats was also lower than that observed in LZ rats (P = 0.01; ANOVA).

When the number of macrophages were calculated per adipocyte, the general finding was that there were minor differences when Wistar and GK rats were compared (Fig. 6B). However, the WAT depots of OZ rats contained more macrophages than those of LZ rats (Fig. 6B).

DISCUSSION

The major finding in the present study was that GK rats, a type 2 diabetes model, have higher adipose tissue blood perfusion in most depots, both when compared with control rats and normoglycemic markedly OZ rats. This opens the intriguing possibility that changes in adipose tissue blood flow (ATBF) may influence and/or modulate...
the β-cell dysfunction in type 2 diabetes, perhaps by altering the storage and release of lipids.

In the present study we could, as expected, verify the massive obesity of the OZ rat. These animals are leptin resistant due to a receptor point mutation (23). The insulin resistance in OZ rats can, at least initially, be compensated for by hyperinsulinemia, so the animals remain normoglycemic. This was reflected in a higher islet mass in the OZ rats when compared with the other strains. Thus, OZ rats are an obese animal model, which through adaptive processes is able to maintain normoglycemia.

The GK rat, on the other hand, was hyperglycemic, had normal serum insulin concentrations, and had an islet mass which was similar to that of WF rats, even though...
there is a tendency ($P = 0.08$) toward a decrease in the latter. Somewhat surprisingly, the weight of the psoas fat pad was almost doubled in the GK rats when compared with WF rats. This suggests, since the weight of this depot fairly accurately mirrors total body fat (24), that the GK rat is also an obese rat model, albeit not to the same degree as OZ rats. Thus, the GK rat mirrors several traits of type 2 diabetes in humans, including obesity and increased insulin resistance in both liver (25) and WAT (26), but lack hyperinsulinemia. In view of this it should be possible to elucidate the roles of insulin resistance associated with obesity and hyperglycemia on islet and WAT blood perfusion.

White ATBF was $\sim 3 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$ in the control strains, thereby confirming previous results in humans and rodents (15). One striking difference, however, was the five- to tenfold higher blood perfusion found in the subcutaneous fat on the anterior throat in all studied rat strains. The reason for this marked difference is unknown. ATBF distribution in different sites did not differ either when different strains or fat depots were compared. One exception from this general finding was the subcutaneous fat on the anterior throat just described above. This is in accord with a previous study in female Wistar rats, where no regional differences were detected when retroperitoneal, parametral, mesenteric, and subcutaneous fat blood flow was compared in either basal or trained rats (27). A heterogeneity has also been seen in humans, where subcutaneous ATBF was higher in the upper than the lower parts of the abdominal wall. It should be noted, however, that the blood perfusion is very low in the present study,
since the animals have not been recently fed (16), and minor differences are likely to be difficult to detect.

The ATBF in the obese, normoglycemic OZ rat was similar to or lower than that in the control LZ animals. This supports previous findings in this model (12,13,28,29). We also found that the individual WAT cells were larger in OZ rats, whereas the vascular density was approximately similar. Thus, when the number of microvessels was expressed per fat cell, the vascular density was higher in OZ rats when compared with LZ rats. This means that the unchanged or slightly impaired ATBF most likely reflects changes in the regulation of the blood perfusion rather than a decreased vascularity. It may be that the decreased ATBF reflects the hypercellular-hypertrophic obesity in this animal model, which has been suggested to also encompass a functionally important blood flow–mediated decrease in delivery and removal of nutrients and substrates to and from WAT (13).

The most surprising finding in the present study was that the GK rat had markedly increased ATBF values (ranging from 5 to 10 times higher) in all examined depots besides the epididymal fat pad. This flow increase was not due to any increase in vascularity, since the vascular density was similar to that seen in WF rats. Thus, the regulation of ATBF is likely to be affected. Before trying to interpret possible responsible mechanisms, we would like to shortly recapitulate how normal ATBF regulation occurs. Major factors affecting the blood perfusion of WAT include feeding, which in humans can increase subcutaneous ATBF up to fourfold (30). This increases substrate delivery for triglyceride clearance (15,31), and when ATBF was increased by adrenaline it was reported that triglyceride extraction increased in parallel with increased blood flow (31). Also, exercise and other types of stress increase ATBF in humans, although less markedly (32,33). Furthermore, ATBF increases during fasting overnight (34). During both these conditions, WAT releases nonesterified fatty acids and thereby requires a supply of albumin for transportation purposes.

In humans, adrenergic influences on ATBF are predominant with β-mediated vasodilation and α₂-mediated vasoconstriction (15,16). This may explain the influences of exercise or fasting. It seems as if circulating cat-
echolamines are more important in exercise-induced hyperemia as seen after studies in patients with spinal cord lesions (35). The increased ATBF seen after feeding is not fully explained. It correlates with insulin secretion and suppression of nonesterified fatty acids. Even though insulin is not the local signal responsible it can stimulate ATBF through sympathetic activation (9). It can, however, be blocked by propranolol, completely in some depots and partially in others (36), suggesting that adrenergic receptors are involved. Thus, increased sympathetic nervous activity could be one explanation for the increased ATBF in GK rats (see further below).

Like in many other vascular beds in the body, that of WAT is also sensitive to nitric oxide (NO). This substance determines the absolute level of ATBF, but a major proportion of the postprandial enhancement of ATBF is under β-adrenergic control (37). This means that NO has a permissive effect for other blood flow regulatory systems to exercise their effects, similar to what has been observed in pancreatic islets (38). In humans, one previous study demonstrated no effects on ATBF of the NO synthase inhibitor monomethyl-arginine (39), but they used ethanol outflow-to-inflow ratio in a microdialysis system to evaluate blood flow, which has a low sensitivity (40). It should be noted that the increased islet blood flow seen in GK rats can be normalized by NO synthase inhibition (38). Furthermore, an increased NO synthase activity has been observed in the retina of GK rats (41). Thus, there is some evidence that NO levels may be increased in GK rats, and if this is the case this may well explain the hyperperfusion of blood in GK WAT.

The degree of insulin sensitivity in humans and experimental animals is closely related to ATBF responsiveness (9,15). Thus, impaired regulation of ATBF seems to be another facet of the insulin resistance syndrome (9). Substances released from WAT may contribute to these changes in blood perfusion as well as insulin resistance and β-cell dysfunction. Such factors include TNF-α, FFAs, adiponectin, resistin, and leptin (3,42). In OZ rats, TNF-α has been suggested to be important, mainly by inducing hepatic insulin resistance (43), whereas the role of this substance in GK rats is unknown. Both resistin and adiponectin expression are decreased in OZ rats (44), and once again, their concentrations in GK rats are unknown. Since both resistin and adiponectin increase blood flow (45), their participation in the increased ATBF remains a possibility. Resistin, however, is a general vasodilator acting on receptors of endothelial cells, which then probably mediate the effects through NO (46). In OZ rats the leptin receptor is deficient, whereas no such changes are known or likely to exist in GK rats. Thus, a reasonable working hypothesis for further studies is that hyperleptinemia in GK rats may mediate an increased ATBF through increased local synthesis of NO. However, the findings on islet blood flow referred to below shed some doubt also on this possibility.

Pancreatic islet blood perfusion is also regulated by the factors referred to above. When 10-μm microspheres were used for blood flow measurements, islet blood perfusion of both GK and OZ rats was increased when compared with the control strains (online appendix), thereby confirming previous studies (17,18,47). For a further discussion on islet blood perfusion in these animals, see the online appendix.

It seems as if the GK rat is unique in the sense that both islet blood perfusion and ATBF are increased simultaneously, whereas only islet blood flow is increased in the OZ rat. It has been suggested that macrophages may infiltrate WAT in obesity (48,49). These cells can, in addition to adipocytes, also express cytokines and may by themselves affect the production of adipocytokines (50). However, there were no major differences in macrophage content of WAT when comparing GK or OZ rats when they were expressed per area. At the moment, the coupling between type 2 diabetes in GK rats and their increased ATBF is unknown. It can be speculated that the ability to increase ATBF to such a large extent may enable GK rats to continuously mobilize FFAs and thereby prevent accumulation of WAT depots (i.e., prevent any marked degree of obesity). The increased blood perfusion of brown adipose tissue may also increase caloric expenditure. Some support from that stems from the increased serum FFAs and cholesterol of GK rats. In support of that, we have recently observed that both fatty acids and triglycerides may by themselves increase islet blood flow (L.J., Ö.K., A. Delgado Verdugo, T. Alsgård, C.K., unpublished observation).

ACKNOWLEDGMENTS

Financial support was received from the Swedish Research Council (72X-109), the Swedish Diabetes Association, the Juvenile Diabetes Research Foundation, the EFSD/Novo Nordisk for Type 2 Diabetes Research Grant, and the Family Ernfors Fund.

The skilled technical assistance of Astrid Nordin and Eva Törnells is gratefully acknowledged.

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