Diet-induced obesity and pancreatic islet blood flow in the rat: a preferential increase in islet blood perfusion persists after withdrawal of the diet and normalization of body weight

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Abstract
The aim of the present study was to evaluate the effects of diet-induced obesity on pancreatic islet blood perfusion in normal Wistar rats. Furthermore, we investigated to what extent any obesity-associated changes in islet blood flow could be reversed after reversion to a normal diet with normalization of body weight. Young adult female Wistar rats were offered a palatable mixed high-caloric diet (cafeteria diet) in addition to standard pelleted chow. Age-matched control rats received standard pelleted chow only. After 4 weeks the diet-treated rats had a body weight of approximately 15% more than that of the controls. All diet-treated rats had decreased glucose tolerance and increased serum insulin concentrations, but basal blood glucose concentrations were similar in anesthetized diet-treated and control rats. Whole pancreatic and islet blood flow rates were measured with a microsphere technique. The islet blood flow as well as fractional islet blood flow were increased (P<0.01) in rats fed the cafeteria diet, while blood perfusion of the whole pancreas was similar to that of the control rats. In a second experiment, rats received the cafeteria diet for 4 weeks and were then fed standard pelleted food alone for another 3 weeks, while controls received standard diet for 7 weeks. After this period total body weight, retroperitoneal fat pad weight and glucose tolerance were similar to those of the controls. Whole pancreatic blood flow was unchanged as compared with that of control rats. However, both islet blood flow (P<0.01) and fractional blood flow (P<0.01) were increased. We conclude that diet-induced obesity in rats is associated with decreased glucose tolerance, hyperinsulinemia and a specific increase in absolute and fractional islet blood perfusion. This increase persists for at least 3 weeks after the diet is withdrawn despite normalization of body weight and glucose tolerance.

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Introduction
Previous studies have shown that an increased functional demand on the endocrine pancreas is associated with changes in islet blood perfusion (Jansson 1994). Thus augmented islet blood flow has been demonstrated in Sprague-Dawley rats both after partial pancreatectomy (Jansson & Sandler 1989) and after a 48 h glucose infusion (Styrud et al. 1992), as well as in F1 hybrids of the GK (Goto-Kakizaki) rat, an animal model resembling mild non-insulin-dependent diabetes (Svensson et al. 1994). Furthermore, experiments by Atef and co-workers (1992) demonstrated an increased islet blood perfusion in genetically fat hyperinsulinemic Zucker rats and in normal rats subjected to lesions of the hypothalamus causing hyperphagia and hyperglycemia. Whether changes in islet blood flow are involved in the development of non-insulin-dependent diabetes or merely represent an adaptive response remains to be established. This study aimed to investigate pancreatic islet blood flow in an animal model with an increased functional demand on the pancreatic islets, caused by moderate body weight increase. Obesity was induced in normal rats by a non-invasive method, namely voluntary overeating. We also investigated to what extent an obesity-induced change in islet blood flow would be reversed after the body weight had returned to normal values.

Materials and Methods

Animals and diet
Female Wistar rats aged 7 weeks were purchased from B&K Universal AB (Sollentuna, Sweden) or Møllegaard (Bomholtgaard, Ry, Denmark). The rats were randomly assigned to either a cafeteria diet group or a control group
and were kept two in each cage under a 12 h/12 h light/darkness cycle. All animals had unlimited access to standard pelleted food (Type R34, AB AnalyCen, Lidköping, Sweden) and tap water during the entire course of the experiment. The rats in the diet group were offered, in addition to the standard diet, a mixed palatable continuously changing so-called cafeteria diet (Scalfani & Springer 1976, Stock & Rothwell 1979, Naim et al. 1985, Gianotti et al. 1988, Prats et al. 1989) consisting of 'supermarket food', including butter, salted biscuits, cookies, digestive biscuits, peanuts, peanut butter, brawn, meatballs, sausages, liver paste, cereals, bread and snacks, of which four items were presented daily. After 4 weeks the cafeteria diet treatment was discontinued. Some of the diet-treated rats and control rats were immediately subjected to blood flow measurements. All remaining animals were fed standard pelleted chow for another 3 weeks before blood flow measurements were performed. The body weight of all animals was recorded weekly. All experiments were approved by the local animal ethics committee.

Blood flow measurements

Measurements of the blood flow to the pancreas, duodenum, adrenals and liver were performed with a microsphere technique (Jansson & Hellerström 1983). Briefly, non-fasted rats were anesthetized with pentobarbital sodium (50 mg/kg body weight; Mebumal vet; Nordvacc AB, Solna, Sweden), heparinized and placed on a heated operating table. Polyethylene catheters were inserted into the ascending aorta, via the right carotid artery, and into the left femoral artery. Constant monitoring of the mean arterial blood pressure was achieved by connecting the catheter in the ascending aorta to a pressure transducer (PDPCR 75/1; Druck Ltd, Groby, UK). When a stable blood pressure had been established (less than 5% variation over 20 min) approximately 1.5–2.0 × 10⁶ non-radioactive microspheres (NEN-Trac; DuPont Pharmaceuticals Inc., Wilmington, DE, USA) with a diameter of 11 µm were injected into the ascending aorta for 10 s. An arterial blood sample was collected by free flow from the catheter in the femoral artery at a rate of approximately 0.50 ml/min for a total of 60 s, starting 5 s before the injection of the microspheres. The exact withdrawal rate was confirmed in each experiment by recording the weight of the blood sample. Arterial blood samples were then collected for measurement of blood glucose and serum insulin. Thereafter the animals were killed and the pancreas, adrenal glands, samples from the duodenum and liver, and the entire right retroperitoneal fat pad were removed, blotted and weighed. To visualize the microspheres all organ samples, except for the retroperitoneal fat pads, were then treated by a previously described freeze-thawing technique (Jansson & Hellerström 1981). Briefly, the organs are compressed between object slides and frozen at −18 °C for at least 24 h. The microsphere contents of the organs, including the islets, are then counted in a microscope equipped with both bright- and dark-field illumination. Calculations of the blood flow values were made according to the formula $Q_{org} = Q_{i.o} \times (N_{org} / N_{ref})$, where $Q_{org}$ is organ blood flow (ml/min), $Q_{i.o}$ is withdrawal rate of reference sample (ml/min), $N_{org}$ is number of microspheres present in the organ and $N_{ref}$ is number of microspheres in the reference sample. A difference of less than 10% in blood flow values of the adrenal glands denoted an adequate mixing of microspheres in the circulation.

Glucose tolerance test and blood glucose measurements

An i.p. glucose tolerance test (2 g/kg body weight β-glucose, 300 mg/ml) was performed on animals in the postabsorptive state at about 1000 h on the day before the blood flow measurements. Blood samples were obtained from the cut tip of the tail and analyzed with test reagent strips (Exac Tech; Baxter Travenol Inc., Deerfield, IL, USA).

Measurement of insulin in pancreas and serum

Approximately 25–30 mg of the pancreas from each animal was removed and homogenized in 100 µl distilled water. A sample of 50 µl was removed from the homogenate and transferred to a tube containing 125 µl acid/ethanol (0.18 M HCl in 95% (v/v) ethanol). The samples were extracted overnight at +4 °C, and insulin was determined by RIA. The content of insulin in serum samples was also measured by RIA (Heding 1972).

Statistical analysis

All values are expressed as means ± s.e.m. Data were evaluated by Student’s two-tailed t-test for unpaired observations or ANOVA.

Results

At the beginning of the experiment mean body weight did not differ between the group assigned to cafeteria or control diet (data not shown). After 4 weeks of cafeteria diet the body weight of the diet-fed rats was approximately 15% higher than that of the control animals (Table 1). After the cafeteria-diet-fed and control animals had received standard pelleted chow for another 3 weeks there were no significant differences in body weight between the formerly diet-treated group and control rats (Table 1).

The weight of the retroperitoneal fat pads of the cafeteria-diet-fed rats had increased after 4 weeks on the diet compared with control rats (Table 1). After 3 weeks
Table 1 Effects of a cafeteria diet on body weight, retroperitoneal fat pad weight, blood glucose and serum insulin concentrations and pancreatic insulin content. Values are means ± S.E.M. for the number of rats shown in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>Pellet diet for 4 weeks (7)</th>
<th>Cafeteria diet for 4 weeks (7)</th>
<th>Pellet diet for 7 weeks (7)</th>
<th>Cafeteria diet for 4-7 weeks (8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>244 ± 4</td>
<td>277 ± 7**</td>
<td>245 ± 3</td>
<td>256 ± 6</td>
</tr>
<tr>
<td>Retroperitoneal fat on right side (g)</td>
<td>2.25 ± 0.56</td>
<td>5.52 ± 0.34**</td>
<td>1.69 ± 0.48</td>
<td>1.89 ± 0.24</td>
</tr>
<tr>
<td>B-glucose (mmol/l)</td>
<td>4.4 ± 0.3</td>
<td>46 ± 0.2</td>
<td>44 ± 0.4</td>
<td>4.7 ± 0.3</td>
</tr>
<tr>
<td>S-insulin (ng/ml)</td>
<td>3.24 ± 1.12</td>
<td>6.54 ± 2.38*</td>
<td>2.60 ± 0.42</td>
<td>2.69 ± 0.85</td>
</tr>
<tr>
<td>Insulin content (µg/g pancreas)</td>
<td>100.2 ± 26.0</td>
<td>117.5 ± 17.4</td>
<td>133.1 ± 20.2</td>
<td>192.5 ± 45.4</td>
</tr>
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</table>

*P<0.05; **P<0.01 when compared with the corresponding rats given pellets.

Figure 1 Blood glucose levels after an i.p. injection of d-glucose (2 g/kg body weight) in female Wistar rats offered a cafeteria diet for 4 weeks (•; n=8), in the same rats after a further 3-week period on standard pelleted chow (●; n=8) and in control rats maintained on standard diet for 4 weeks (■; n=5) or 7 weeks (○; n=7). Values are means ± S.E.M. ***P<0.001 when compared with control rats.

off the diet the fat pad weight of these rats was similar to those of animals given the control diet for 7 weeks (Table 1).

All rats were normoglycemic at the start of the experiment (data not shown). However, the rats fed the cafeteria diet for 4 weeks had an impaired glucose tolerance compared with their control group (Fig. 1). Rats which were initially fed a cafeteria diet and then standard pelleted food for an additional 3 weeks demonstrated no discernible differences in glucose tolerance from rats given control diet for 7 weeks (Fig. 1). Rats fed cafeteria diet for 4 weeks had increased serum insulin concentrations compared with control rats, whereas no significant difference in the pancreatic content of insulin was seen. No differences in either serum insulin levels or pancreatic content of insulin were seen between control rats and rats which had been fed cafeteria diet and later standard pelleted chow only (Table 1).

The mean arterial blood pressure as measured in anesthetized rats was similar in all groups of animals (data not shown). The blood flow to the whole pancreas did not differ between diet-treated and control rats 4 or 7 weeks after commencing the diet (Table 2). The rats that had received cafeteria diet for 4 weeks had both an increased absolute and fractional islet blood flow as compared with the controls (Table 2). These changes persisted in the group of rats that had been off the cafeteria diet for 3 weeks before the blood flow measurements when compared with animals given only control diet for 7 weeks (Table 2). No differences in the blood flow to the liver or the duodenum were detected between any of the experimental groups (Table 2). A negative correlation between islet blood flow and serum insulin levels was found within the cafeteria-diet-treated group after 4 weeks (r=-0.9147; P=0.004). No such correlation was seen in the 4-week control group or any of the 7-week treated animals.

Discussion

Obesity and an associated increase in peripheral insulin resistance is a common finding in patients with non-insulin-dependent diabetes mellitus. To investigate the effects of increased food intake and moderate obesity on the pancreatic circulation, we studied Wistar rats treated for 4 weeks with a cafeteria diet. This diet, consisting of an assortment of highly palatable foods, induces overeating in the rat without the need for force-feeding or other interventions (Sclafani & Springer 1976, Stock & Rothwell 1979, Naim et al. 1985, Gianotti et al. 1988, Prats et al. 1989). However, the intake of nutrients, vitamins and trace elements is difficult to estimate with this diet (Moore 1987). Since, in the present study, the diet was used for a short time only merely to induce obesity, the relative contribution of different nutrients to the diet was deemed to be of minor importance. Previous studies in cafeteria-diet-fed rats have shown that this diet does not affect lean body mass (Sclafani & Springer 1976, Stock & Rothwell
Table 2 Effects of a cafeteria diet on pancreatic, islet, fractional islet, duodenal and hepatic arterial blood flow. Values are means ± S.E.M. for the number of rats shown in parentheses

<table>
<thead>
<tr>
<th></th>
<th>Pellet diet for 4 weeks (7)</th>
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<th>Pellet diet for 7 weeks (7)</th>
<th>Cafeteria+pellet diet for 4+3 weeks (8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic blood flow (PBF) (ml/min per g pancreas)</td>
<td>0.56 ± 0.03</td>
<td>0.55 ± 0.07</td>
<td>0.58 ± 0.07</td>
<td>0.60 ± 0.06</td>
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<tr>
<td>Islet blood flow (μl/min per g pancreas)</td>
<td>55 ± 3</td>
<td>80 ± 8**</td>
<td>59 ± 6</td>
<td>86 ± 8**</td>
</tr>
<tr>
<td>Islet blood flow (% of PBF)</td>
<td>9.9 ± 0.4</td>
<td>15.0 ± 0.9**</td>
<td>10.3 ± 0.6</td>
<td>14.4 ± 0.6**</td>
</tr>
<tr>
<td>Duodenal blood flow (ml/min per g duodenum)</td>
<td>0.49 ± 0.09</td>
<td>0.74 ± 0.16</td>
<td>0.52 ± 0.15</td>
<td>0.53 ± 0.14</td>
</tr>
<tr>
<td>Arterial liver blood flow (μl/min per g liver)</td>
<td>70 ± 10</td>
<td>40 ± 19</td>
<td>50 ± 20</td>
<td>60 ± 10</td>
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**P<0.01 when compared with the corresponding rats given pellets.

1979). In the present study, the wet weight of the retroperitoneal fat pads was measured as an index of the increase in total body fat mass. The marked increase in the weight of the fat pads after 4 weeks of cafeteria diet was readily abolished after an additional 3 weeks off the diet. The moderate obesity of the diet-treated rats was associated with a deteriorated glucose tolerance and increased serum insulin concentration. However, 3 weeks after discontinuation of the cafeteria diet the body weight, the glucose tolerance and the serum insulin concentration were normalized compared with age-matched controls, confirming that the disturbed glucose homeostasis was secondary to obesity and/or the increased food intake.

The finding of a specific increase in islet and fractional islet blood flow after 4 weeks of cafeteria diet is in accordance with earlier studies in which an increased functional load on the pancreatic islets was demonstrated to be associated with augmented islet blood flow (Jansson & Sandler 1989, Stryrud et al. 1992, Atef et al. 1992, Svensson et al. 1994). In this study no increase in pancreatic insulin content was observed in cafeteria-diet-treated animals after either 4 or 7 weeks. This finding argues against the possibility that an enlargement of the islet mass significantly contributed to the increased values of absolute and fractional islet blood flow. However, it cannot be excluded that diminished insulin storage in individual B-cells may have confounded this observation in the diet-treated rats. A rough estimation of islet morphology when the content of microspheres in the islets was evaluated by the freeze–thawing technique (Jansson & Hellerström 1981) did not reveal any marked changes in the size, number or configuration of the islets. Previous experiments have shown that an acute bolus dose of glucose in rats augments pancreatic islet blood flow for a period of about 30 min after administration (Jansson 1984). The diet-treated rats in this study did not have a significantly increased basal blood glucose at the time of the blood flow measurements. However, an impaired intraperitoneal glucose tolerance test was demonstrated. It is therefore possible that postabsorptive blood glucose values in cafeteria-diet-treated rats were higher than in controls. Whether repeated episodes of hyperglycemia influence the regulation of islet blood flow is not known. In this context it can be noted that both glucose-intolerant F1 hybrids of the GK rat and obese Zucker rats display a specific increase in islet blood flow (Atef et al. 1992, Svensson et al. 1994).

The negative correlation between islet blood flow rate and serum insulin concentrations within the group of diet-treated rats is intriguing. No previous investigations have been performed to evaluate this issue in individual animals subjected to increased functional load on the islets. In contrast, hyperinsulinemia has been shown to be associated with augmented islet blood flow in normal rats, e.g. after acute glucose administration or after a 48 h glucose infusion. In addition, in the obese Zucker rat and in rats subjected to VMH lesions, elevated insulin concentrations have also been demonstrated together with enhanced islet blood flow (Atef et al. 1992). The results of these investigations represented mean values for groups of rats and no attempts were made to correlate serum insulin concentrations with islet blood flow in individual animals. Pharmacologically induced hyperinsulinemia, caused by terbutaline or exogenous insulin, is associated with a decreased islet blood flow (Jansson et al. 1989, Jansson & Berne 1993). Starved rats which exhibit impaired insulin release nevertheless display increased islet blood flow in response to glucose (Jansson 1985). Also injection of mamoheptolose increased islet blood flow, while serum insulin levels were decreased (Jansson 1985). These findings substantiate the suggestion that islet blood flow is not necessarily correlated with serum insulin levels per se, but may instead be regulated by the need for insulin secretion.

It has been suggested that capillary hypertension may induce vascular damage, ultimately leading to organ failure (Parving et al. 1983, Tooke 1989). The present study has shown an increased islet blood flow in diet-induced obese rats, which may be associated with an increased capillary pressure within the islets. Whether such an increased islet blood flow contributes to, or is an effect of, an increased insulin demand remains unknown. It is therefore of interest to evaluate whether induced changes in islet blood flow persist or are reversed when the causative factor, such as hyperglycemia or an increased functional demand, is withdrawn. In an earlier study, in which glucose infusion...
for 48 h induced increased islet blood perfusion, the redistribution of the blood flow within the pancreas was demonstrated to remain 10 days after the infusion. However after this, the absolute value of islet blood flow was reduced to that of control rats (Styrud et al. 1992). Another study showed a persisting increase in islet blood flow in rats 8 weeks after a 70% pancreatectomy (Jansson & Sandler 1989). Putative factors behind the persistently changed regulation of islet blood flow in these studies are a shift in the relation between sympathetic and parasympathetic nervous activity (Jeanrenaud 1985, Jeanrenaud et al. 1985), an altered secretion of gastrointestinal hormones and changes in the balance between locally produced vasoconstrictors, such as endothelins and angiotensin II, and vasodilating substances, such as nitric oxide.

In conclusion this study has demonstrated that diet-induced obese rats display a specific increase in islet blood flow, which persists after a period on a normal diet, when body weight and glucose metabolism have returned to normal. An inverse correlation was also found between serum insulin levels and islet blood flow in the obese animals. We propose that increased islet blood perfusion is a mechanism by which adequate hormonal release from the islets can be maintained or increased during augmented functional load on the islets. Furthermore, the regulation of islet blood flow under these circumstances may be viewed as an adaptive response to the need for insulin secretion.

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