Pancreatic islet blood flow during pregnancy in the rat: an increased islet mass is associated with decreased islet blood flow

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Abstract
Increased blood perfusion of pancreatic islets is seen during various conditions of increased demand for insulin secretion. Pregnancy confers an increased need for insulin secretion, met by increased islet mass and volume as well as a decreased threshold for glucose-induced insulin secretion. In the present study, whole pancreatic and islet blood flow were studied with a microsphere technique in Wistar rats on days 15, 18 and 20 of pregnancy and days 2 and 7 post-partum. There were no changes in total pancreatic blood flow during pregnancy and the first post-partum week. Total blood perfusion through islet tissue expressed as flow per weight of whole pancreas was higher at day 15 of pregnancy. When islet blood flow was expressed per gram of islet tissue there was a decrease at day 18 of pregnancy. This decrease of islet blood flow was concomitant to a short-lived increase of the islet mass at the end of pregnancy. We conclude that upregulation of insulin output during late pregnancy does not specifically include increased net blood perfusion through the islets. One possible reason for this might be lack of synchronization between the proliferation of endocrine cells and angiogenesis, resulting in a relative decrease in islet vascular density in the islets.


Introduction
Previous investigations involving experimental models with increased functional stress on the pancreatic β-cells have consistently demonstrated an association with increased blood perfusion of the pancreatic islets (Jansson 1994). We, and others, have suggested that changes in islet blood flow may influence hormone release from the islets (Jansson 1994, Moldovan et al. 1996), providing partial compensation for a relative insufficiency in insulin release. However, experimental support for this notion is mainly based on models involving intrinsic or imposed impairments of β-cell function.

Hyperperfusion may have long-term adverse effects on organ function. The mechanisms behind such damage likely include capillary hypertension with shear-force-induced changes in endothelial function (Zatz & Brenner 1986) including altered production of endothelial factors affecting blood flow, e.g. nitric oxide (Atef et al. 1992, Svensson et al. 1994b). We have previously demonstrated capillary hypertension in models of type 2 diabetes (Carlsson et al. 1997). To further evaluate the effects of increased demands on insulin secretion on islet blood flow, we decided to study islet blood perfusion during gestation. Pregnancy is associated with peripheral insulin resistance and increased insulin secretion in association with normoglycemia or slight hypoglycemia (Freinkel 1980). This leads to a state of increased functional load on the endocrine pancreas. Adaptive mechanisms include growth of the islets and a decreased threshold for glucose-induced insulin secretion. However, in contrast to other models for increased functional demand on the islets, pregnancy is associated with a transient increase in the requirement for insulin secretion, which subsides following delivery. A concomitant rapid decrease in islet β-cell mass mediated mainly by apoptosis has been demonstrated in the early post-partum period (Scaglia et al. 1995). Pregnancy and early lactation thus provide a unique physiologic system for studying changes in islet perfusion during rapid changes in the demand for insulin secretion during normo- or relative hypoglycemia.

Materials and Methods

Animals
Pregnant female Wistar rats were purchased from B&K Universal (Sollentuna, Sweden). The animals had free access to pelleted rat chow and tap water throughout the experiments. Day 0 of pregnancy was defined as the day on which a sperm-containing vaginal smear was observed. The rats were transported to the laboratory in Uppsala on day 8 of pregnancy, and thereafter kept for a minimum of

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7 days, at the animal department, before starting the experiments.

Blood flow measurements were performed at days 15, 18 and 20 of pregnancy and at days 2 and 7 of lactation. Age-matched virgin rats were used as controls. All experiments were approved by the local animal ethical committee and were in accordance with accepted standards of humane animal care.

Blood flow measurements

Measurements of blood flow were performed with a microsphere technique as previously described in detail (Jansson & Hellerström 1983). The rats were anesthetized with an intraperitoneal injection of thiobutabarbitral (Inactin; Research Biochemicals, Natick, MA, USA; 120 mg/kg body weight), heparinized and placed on an operating table maintained at body temperature. Polyethylene catheters were inserted into the ascending aorta, via the right carotid artery, and into the left femoral artery and vein. The intravenous catheter was used to continuously infuse Ringer solution (5 ml/kg per hour). Constant monitoring of the mean arterial blood pressure was achieved by connecting the catheter in the ascending aorta to a pressure transducer (PDCR 75/1; Druck Ltd, Groby, Leics, UK). After inserting the catheters the animals were placed in their right side so that pressure from the uterine horns could not affect the venous return from the inferior vena cava. After stable blood pressure had been established (less than 10% variation during 20 min) approximately 1·5–2·0 × 10⁵ non-radioactive microspheres (E-Z Trac Ultraspheres; IMT, Stason Labs, Irvine, CA, USA), with a mean diameter of 10 µm, were injected into the carotid catheter during 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 immediately before the injection of spheres. The exact flow rate was confirmed in each experiment by recording the weight of the sample. After securing the reference sample, another blood sample was drawn for measurements of blood glucose and serum insulin concentrations (see below). Thereafter the animals were killed, the uterine horns were removed from the pregnant rats, and the fetuses were counted and examined (see below). Then the pancreas and adrenal glands were removed in toto, blotted, weighed and subjected to a freeze–thawing technique to visualize the microspheres and allow separate counting of intra-islet microspheres (Jansson & Hellerström 1981). Furthermore, samples of the duodenum and descending colon (approximately 150 mg each) were removed and treated by the same freeze–thawing technique. The microspheres present in the organs were counted in a microscope equipped with both bright and dark field illumination (Jansson & Hellerström 1981). The microspheres in the blood samples were directly counted in a light microscope after transferring the blood to glass microfiber filters (pore size < 0·2 µm).

The blood flow values were calculated according to the formula

\[ Q_{\text{org}} = \frac{Q_{\text{ref}} \times N_{\text{org}}}{N_{\text{ref}}} \]

where \( Q_{\text{org}} \) is organ blood flow (ml/min), \( Q_{\text{ref}} \) is withdrawal rate of reference sample (ml/min), \( N_{\text{org}} \) is number of microspheres present in the organ and \( N_{\text{ref}} \) is number of microspheres in the reference sample. A difference < 10% in blood flow values between the adrenal glands indicates adequate dispersal of microspheres with the circulation.

Evaluation of fetal outcome

Viable and non-viable fetuses were counted, inspected for gross external malformations, blotted and weighed together with the corresponding placenta. The number of resorptions was recorded. The offspring of the lactating rats were likewise weighed and inspected for external malformations, both at birth and at the time of the blood flow measurements.

Measurements of pancreatic islet volume per pancreas

In conjunction with the counting of the microspheres in the pancreas, the islet volume per pancreas was determined using a point-counting method adapted for use in freeze–thawed samples (Carlsson et al. 1996). Islets with a diameter less than 50 µm can not be analyzed with this method. The estimated islet mass was obtained by multiplying the islet volume per pancreas with the total pancreatic weight. The observer performing these measurements was unaware of the origin of the samples.

Measurements of blood glucose and serum insulin concentration

Arterial blood samples were obtained after securing the reference blood sample and analyzed for blood glucose concentrations with a blood glucose meter (Medisense; Baxter Travenol Laboratories Inc., Deerfield, IL, USA). Serum insulin concentrations were measured with ELISA technique (Rat Insulin ELISA; Mercodia AB, Uppsala, Sweden) with rat insulin (Novo Nordic, Bagsværd, Denmark) as a standard.

Statistical calculations

All values were expressed as means ± S.E.M. Calculations of statistical significance were performed with Student’s t-test for unpaired observations or one-way repeated measurement ANOVA in conjunction with Bonferroni’s test by use of SigmaStat (SSPD; Erfart, Frankfurt, Germany). \( P < 0·05 \) was considered to be statistically significant.

Results

Expected variations in maternal and fetal body weights and placental weights were recorded (Table 1). The number of
fetuses/pups in the different study groups examined did not differ (Table 1). No fetuses with external gross malformations were seen.

The mean arterial blood pressure and hematocrit values were decreased during pregnancy and on day 2 post-partum when compared with virgin rats (Table 2). However, on day 7 post-partum the hematocrit value was similar to that of virgin rats (Table 2). Blood glucose concentrations were higher in the virgin rats (Fig. 1a), whereas serum insulin concentrations were decreased in the two groups studied post-partum (Fig. 1b).

Pancreas weight was similar in all rats with the exception of rats examined at day 7 post-partum, where a higher pancreas weight was recorded (Table 1). A marked increase in both pancreatic islet volume (Fig. 2a) and islet mass (Fig. 2b) was seen on day 18 of pregnancy. After partus, these values returned down to values similar to those of virgin rats.

Total pancreatic blood flow was similar in all groups of animals studied (Table 2). When islet blood flow was expressed as flow per weight of whole pancreas, the blood perfusion was higher on day 15 of pregnancy, but was otherwise similar in all groups studied (Fig. 3a). However, when islet blood flow was corrected for changes in islet volume and mass, and was expressed per gram of islet tissue there was a marked decrease in islet blood flow on day 18 of pregnancy (Fig. 3b). Islet blood flow per gram of islet tissue in pregnant and post-partum rats did not differ from that of virgin rats. Blood flow values in the duodenum, colon and adrenal glands did not differ between the experimental groups (Table 2).

**Discussion**

The major finding of the present study is that pregnancy in the rat is associated with a decreased islet blood perfusion calculated per islet weight at day 18 in gestation. Previous investigations involving experimental models with increased functional stress on the pancreatic β-cells have demonstrated a consistent association with increased blood perfusion of the pancreatic islets (Jansson 1994). Such models include chronic and acute hyperglycemia (Jansson & Hellerström 1986, Atef et al. 1992, Styrud et al. 1992), partial pancreatectomy with a diminished number of β-cells (Jansson & Sandler 1989), as well as animals with induced moderate weight gain, i.e. increased peripheral insulin resistance (Atef et al. 1994). Islet blood flow is also

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**Table 1** Body weight, number of fetuses, fetal weights and placental weights in virgin female Wistar rats, on days 15, 18 and 20 of pregnancy and days 2 and 7 post-partum (pp)

<table>
<thead>
<tr>
<th>Day: No. of animals:</th>
<th>Virgin 9</th>
<th>15 8</th>
<th>18 9</th>
<th>20 7</th>
<th>2 pp 7</th>
<th>7 pp 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>247 ± 5</td>
<td>259 ± 11</td>
<td>305 ± 7***</td>
<td>342 ± 13***</td>
<td>260 ± 10</td>
<td>275 ± 12</td>
</tr>
<tr>
<td>Pancreas weight (mg)</td>
<td>928 ± 39</td>
<td>868 ± 32</td>
<td>872 ± 29</td>
<td>972 ± 63</td>
<td>928 ± 64</td>
<td>1158 ± 62**</td>
</tr>
<tr>
<td>Number of fetuses/pups</td>
<td>NA</td>
<td>9.1 ± 0.4</td>
<td>9.5 ± 0.5</td>
<td>8.4 ± 0.9</td>
<td>8.0 ± 0.8</td>
<td>9.0 ± 0.7</td>
</tr>
<tr>
<td>Weight/fetus or pup (g)</td>
<td>NA</td>
<td>0.33 ± 0.02</td>
<td>1.53 ± 0.09</td>
<td>4.28 ± 0.20</td>
<td>7.9 ± 0.2</td>
<td>14.8 ± 0.6</td>
</tr>
<tr>
<td>Placental weight (g)</td>
<td>NA</td>
<td>0.21 ± 0.01</td>
<td>0.36 ± 0.01</td>
<td>0.47 ± 0.02</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Values are means ± s.e.m. **Denotes P<0.01 and ***P<0.001 when compared with virgin rats with one-way repeated measurements ANOVA with Bonferroni’s correction. NA, not applicable.

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**Table 2** Hematocrit, mean arterial blood pressure and blood flow values in virgin female Wistar rats, on days 15, 18 and 20 of pregnancy and days 2 and 7 post-partum (pp)

<table>
<thead>
<tr>
<th>Day: No. of animals:</th>
<th>Virgin 9</th>
<th>15 8</th>
<th>18 9</th>
<th>20 7</th>
<th>2 pp 7</th>
<th>7 pp 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit (%)</td>
<td>46.5 ± 0.7</td>
<td>41.4 ± 0.6***</td>
<td>38.9 ± 0.7***</td>
<td>36.7 ± 0.7***</td>
<td>40.0 ± 1.0***</td>
<td>44.6 ± 0.8</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mm Hg)</td>
<td>84 ± 2</td>
<td>73 ± 2**</td>
<td>78 ± 2</td>
<td>66 ± 4***</td>
<td>69 ± 1***</td>
<td>71 ± 2***</td>
</tr>
<tr>
<td>Pancreatic blood flow (ml/min per g)</td>
<td>1.10 ± 0.11</td>
<td>1.38 ± 0.21</td>
<td>0.99 ± 0.09</td>
<td>1.14 ± 0.28</td>
<td>1.22 ± 0.18</td>
<td>1.45 ± 0.25</td>
</tr>
<tr>
<td>Duodenal blood flow (ml/min per g)</td>
<td>1.98 ± 0.28</td>
<td>1.31 ± 0.24</td>
<td>1.58 ± 0.22</td>
<td>1.63 ± 0.41</td>
<td>2.03 ± 0.32</td>
<td>2.77 ± 0.60</td>
</tr>
<tr>
<td>Colonic blood flow (ml/min per g)</td>
<td>0.61 ± 0.20</td>
<td>0.28 ± 0.06</td>
<td>0.36 ± 0.07</td>
<td>0.39 ± 0.10</td>
<td>0.78 ± 0.28</td>
<td>0.84 ± 0.20</td>
</tr>
<tr>
<td>Adrenal blood flow (ml/min per g)</td>
<td>3.37 ± 0.40</td>
<td>4.19 ± 0.81</td>
<td>4.98 ± 0.60</td>
<td>5.31 ± 0.97</td>
<td>5.39 ± 0.77</td>
<td>5.32 ± 0.90</td>
</tr>
</tbody>
</table>

Values are means ± s.e.m. **Denotes P<0.01 and ***P<0.001 compared with virgin rats when calculated with one-way repeated measurements ANOVA with Bonferroni’s correction.
increased in animal models of type 2 diabetes, such as GK rats (Svensson et al. 1994a, 1996), obese Zucker rats (Atef et al. 1992) and obese-hyperglycemic mice (Carlsson et al. 1996). We, and others, have previously suggested that changes in islet blood flow may influence hormone release from the islets (Jansson 1994, Moldovan et al. 1996), and provide partial compensation for insufficient insulin release.

Adaptation to pregnancy involves profound metabolic and hemodynamic changes. Basal plasma insulin and glucagon concentrations are elevated (Metzger et al. 1974, Saudek et al. 1975), while blood glucose remains constant or decreases slightly (Girard et al. 1984). The insulin secretion is increased partially due to an increased insulin resistance (Flint et al. 1979, Freinkel 1980, Sutter-Dub et al. 1984, Leturque et al. 1984, 1986). A crucial feature of the adaptation to the pregnant state is lowering of the threshold for glucose-stimulated insulin release (Green & Taylor 1972, Sorenson & Brelje 1997). No significant changes in serum insulin concentrations were noted in the present study, whereas blood glucose concentrations were decreased during pregnancy and lactation. The lack of effect on serum insulin concentration probably reflects the fact that the samples were taken from anesthetized rats, where the increased sympathetic tonus may decrease insulin secretion.
Increased need for insulin secretion tends to be accompanied by an increase in islet mass in various experimental settings. Such effects have been observed, for example, after partial pancreatectomy (Bonner-Weir et al. 1983) and following glucose infusions (Bonner-Weir et al. 1989). It should be noted in this context that in most of the islet blood flow studies referred to above, values were given as the perfusion of the whole organ, with no correction for possible changes in islet mass. Indeed, when such corrections were made, such as in obese-hyperglycemic mice, there were no differences in islet blood flow when compared with normal controls (Carlsson et al. 1996). However, in GK rats (Svensson et al. 2000), a type 2 diabetes model, as well as in NOD mice (Carlsson et al. 1998), a true hyperperfusion of blood was seen.

Pregnancy entails increased β-cell proliferation with an associated increase in β-cell mass, which attains a maximum at the end of pregnancy (Hellman 1960, Hellerström 1963, Van Assche 1974, Green et al. 1981, Marynissen et al. 1983, Parsons et al. 1992, 1994). Placental lactogen, growth hormone and prolactin have emerged as the main candidates for mediating pregnancy-specific changes in the β-cells (for a review, see Sorenson & Brejde 1997). Post-partum the increased islet mass rapidly involutes and returns to normal pre-gestational values within 7–10 days (Marynissen et al. 1983, Scaglia et al. 1995). Of particular interest is the finding that this involution seems to occur mainly via apoptosis (Scaglia et al. 1995), that is without evoking any inflammatory reaction. Our study involves animals at days 2 and 7 post-partum, i.e. before and after the major peak of apoptosis. Our findings confirmed a significant increase in islet volume and islet mass on day 18 of pregnancy, and a rapid return to pre-gestational values post-partum. It should be noted that the method used for these determinations, encompassing direct observations of freeze-thawed preparations in dark field microscopy, precludes the detection of small differences in islet volume (Carlsson et al. 1996).

Pregnancy is characterized by modifications in maternal blood circulation including a reduction in total peripheral vascular resistance, declining mean arterial blood pressure and decreased pressor responsiveness to vasodilator agents (Blizard & Folk 1990). As expected, we observed a decreased mean arterial blood pressure, as well as a decrease in hematocrit in the present study. It has previously been shown that pancreatic blood perfusion is subjected to autoregulation when the blood pressure exceeds 65–70 mm Hg (Kvietys et al. 1982). Thus, it is unlikely that the observed minor decrease in arterial blood pressure would per se influence pancreatic or islet blood flow. Neither is the small decrease in hematocrit likely to have significantly confounded the blood perfusion measurements, since we have previously observed that such decreases induced by isovolemic hemodilution have only a minor influence on islet blood flow (Hindlycke & Jansson 1992).

No differences in the blood perfusion of the whole pancreas, duodenum, colon or adrenal glands were observed at any of the time points studied. Although the volume of the vascular compartment in the islets was not specifically measured in the present study, it is likely that the vascular density is relatively decreased in late-pregnant islets, since induction of angiogenesis usually requires several days, up to 1 week. Conversely, the rapid return of the islet blood flow per islet mass to pre-gestational values post-partum could be explained by the post-partum reduction in β-cell mass. This notion awaits experimental confirmation. However, preliminary experiments involving administration of an angiogenesis inhibitor to pregnant rats have demonstrated islet blood flow values similar to...
those in the present study (P Leino, L Jansson & UJ Eriksson unpublished observation). This suggests that stimulation of angiogenesis is not a prerequisite for the adaptation of islets to pregnancy.

Expression and activity of nitric oxide synthases are generally increased during gestation, resulting in augmented synthesis of nitric oxide, which would act in the direction of increased blood flow (Salas 1998). The renin–angiotensin system has an established role in the control of blood pressure and local blood flow. There are indications of an intrinsic renin–angiotensin system in the pancreas that affects β-cells as well as endothelial cells in both endocrine and exocrine parenchyma (Carlsson 2001). Angiotensin II sensitivity in uterine and mesenteric resistance arteries diminishes during the later part of pregnancy (Hermsteiner et al. 2001). Inhibition of the renin–angiotensin system at the level of angiotensin-converting enzyme (ACE) or at the level of the angiotensin II receptor was recently shown to result in increased islet blood flow (Carlsson et al. 1998a). However, the sensitivity to angiotensin II is known to vary between organs during gestation, and it could be that the pancreatic islet vessels are more sensitive in this respect, thereby contributing to the decreased islet blood flow seen on day 18 of gestation. The sensitivity to endothelin, a vasoconstrictor, is changed during pregnancy. During early gestation the sensitivity is increased in mesenteric resistance arteries, whereas it decreases later during gestation (Hermsteiner et al. 2001). It should be noted that endothelin increases in response to endothelial damage per se, and has been suggested to contribute to the pathophysiology of pre-eclampsia (Granger et al. 2001). The islet circulation has been shown to be markedly sensitive to endothelin (L Jansson, unpublished observation) and it may thus play a role in the observed decrease in islet blood flow.

Other factors that may contribute to the modifications of the general blood circulation during pregnancy include increasing levels of sex steroids (Hyttén & Chamberlain 1980). Certain estrogens and progesterone have a potential to act as vasodilators and have been shown to counteract the effects of a nitric oxide inhibitor in late-pregnant rats (Liao et al. 1996). Furthermore, sex steroids and pregnancy were recently suggested to increase the vascular sensitivity to the vasodilator effects of calcitonin gene-related peptide (CGRP) (Gangula et al. 1999). Relaxin, an insulin-like peptide produced by the corpus luteum and the placenta, is a vasodilator whose cellular mechanisms of action include stimulation of nitric oxide generation (Bani 1997, Skött & Carter 2002). A number of gastrointestinal hormones have been reported to increase during gestation (Linden et al. 1987, Holst et al. 1992, Goldstein et al. 1995, Guillozet et al. 1998). There is a possibility that altered concentrations of some of these substances could contribute to the changes in islet blood flow observed during gestation.

In conclusion, the present study demonstrated a relative hyperperfusion of the hypertrophic pancreatic islets on day 18 of pregnancy, when blood flow was calculated per islet mass. The mechanisms underlying these alterations are at present unknown. We propose that during late pregnancy, decreased islet blood flow is a physiological mechanism to prevent an excessive increase in islet blood perfusion with possible shear stress induced deleterious effects on islet function.

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