Insulin Release from the Pancreas and Fuel Metabolism during Late Gestation in Chemically Diabetic Rats

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Abstract

The effects of chemical diabetes and fasting on fuel metabolism and insulin secretory activity in late pregnancy were investigated. Female Wistar rats were made chemically diabetic (CD) by intravenous injection of streptozotocine (30 mg/kg) 2 weeks before conception. When CD pregnant rats were fed, plasma glucose and insulin levels were not significantly different from those of normal pregnant rats. Ketone body levels, however, were higher in CD pregnant rats than in normal pregnant rats, indicating insulin resistance in CD rats. Insulin secretion from the perfused pancreas caused by arginine or glucose was markedly decreased in CD pregnant rats. The pregnant rats were fasted for 2 days, from day 19 to 21 of gestation. Plasma glucose and insulin concentrations decreased similarly in the two groups, whereas ketone body concentrations in CD pregnant rats were significantly higher than those in normal pregnant rats. Glucose-induced insulin secretion by the perfused pancreas was markedly attenuated by fasting and was not significantly different in normal and CD pregnant rats. These observations suggest that diabetes mellitus accelerates starvation in late gestation, due to increased insulin resistance and poor insulin secretion, and that fasting in diabetic pregnancy amplifies ketogenesis.

It is well known that pregnancy strikingly augments catabolism in fuel metabolism (Herrera et al., 1969; Felig and Lynch 1970; Freinkel, 1980). Freinkel has called this state “accelerated starvation” (Freinkel et al., 1972). In healthy pregnant women, heightened insulin secretion associated with food intake prevents metabolic deterioration such as hyperglycemia and hyperketonemia. However, since insulin secretion from the pancreas may not be sufficient to maintain euglycemia in diabetic pregnant women, hyperglycemia and hyperketonemia tend to occur, especially in late pregnancy (Metzger et al., 1982).

We have previously shown that ketonemia is a prominent feature of pregnancy in both humans and rats (Tanigawa et al.,
Forty-eight-hour fasting in the rat during late pregnancy strikingly increases ketone body production which is accompanied by markedly attenuated insulin secretion from the perfused pancreas (Tanigawa et al., 1989). In the present study, we investigated whether diabetes mellitus further accelerates metabolic deterioration caused by starvation during late pregnancy in rats. The secretory activity of the diabetic pancreas was also determined by using the perfused pancreas from fed and fasted pregnant rats.

**Materials and Methods**

Female Wistar rats weighing 200–250 g were used in this study. They were made diabetic by a single intravenous injection of streptozotocin (STZ, 30 mg/kg body weight, Sigma) 2 weeks before conception. A vehicle solution was injected into control animals. One week after the injection, both groups of nonfasted rats received an intraperitoneal injection of glucose (2 g/kg body weight). Blood samples for glucose and insulin measurement were withdrawn every 30 min for 120 min from the tail vein without anesthesia.

Male and female animals were housed together overnight; the presence of a vaginal plug the following morning indicated that copulation had occurred, and that day was designated pregnancy day 1. In pregnant rats, experiments were performed on day 21 of gestation. Some of them were starved from days 19 to 21 of gestation. Only dams had more than 8 fetuses were used. All experimental manipulations were performed between 9:00 a.m. and 12:00 a.m.

On day 21 of gestation, blood samples for glucose and insulin measurement were taken from the tail vein. Under pentobarbital anesthesia (50 mg/kg body weight, intraperitoneal) blood was withdrawn from the jugular vein for the determination of ketone body concentrations. Blood samples were immediately centrifuged at 4°C and plasma was stored at −70°C until assayed. Then the pancreas was isolated and perfused by the procedure described by Goto et al. (1978). The pancreas was perfused with a medium consisting of Krebs-Ringer bicarbonate buffer containing 0.25% bovine serum albumin (Seikagaku Kogyo Co., Japan), 4.6% dextran (Midori Juji, Japan) and glucose as required.

![Graph](image_url)

**Fig. 1.** Plasma glucose and insulin responses to intraperitoneal glucose loading in control rats (○—○, n=18) and STZ-treated rats (●—●, n=25) in the fed state in the morning. Means±SEM are shown. **P < 0.005 vs. control group.
by means of a pump at a flow rate of 1.9 ml/min. The medium was gassed with 95% O2—5% CO2 and kept at pH 7.4 and 37°C. Effluent from the portal vein was collected in tubes every minute, frozen immediately, and stored at −70°C until assayed.

The insulin content (Ic) of the pancreas was determined after termination of the perfusion. The pancreas was removed, minced, sonicated in 20 volumes of cold acid-ethanol (0.18 M HCl in 75% (V/V) ethanol) and extracted overnight at 4°C. After centrifugation at 2,000 g for 30 min at 4°C, samples of the supernatants were stored at −70°C until assayed. The total insulin secretion (Is) during a stimulation period of 20 min was estimated from the insulin concentrations in the perfusate (µU/ml) multiplied by the flow rate (1.9 ml/min). The insulin secretion ratio (Is/Is+Ic) was calculated to estimate the insulin secretory capacity of the β-cells (Curry, 1986).

Plasma glucose was measured by the glucose oxidase method. Plasma ketone bodies were determined by the diazonium salt method (Harano et al., 1983). Insulin concentrations in the plasma and the perfusate were determined by radioimmunoassay (Desbuquois and Aurbach, 1971) with rat insulin used as the standard.

Statistical analysis was performed by unpaired Student’s t-test when two mean values were compared. When more than two mean values were compared, Scheffe’s method was used.

Table 1. Effects of pregnancy and starvation on body weight, plasma glucose, plasma insulin and plasma ketone bodies in control and CD rats.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>BW (g)</th>
<th>Glucose (mmol/l)</th>
<th>Insulin (µU/ml)</th>
<th>Ketone bodies (umol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonpregnant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>11</td>
<td>250.0±1.6</td>
<td>7.6±0.2</td>
<td>56.2±4.4</td>
<td>205.2±16.0</td>
</tr>
<tr>
<td>CD</td>
<td>7</td>
<td>244.3±6.2</td>
<td>8.8±0.4</td>
<td>53.1±3.5</td>
<td>231.3±30.6</td>
</tr>
<tr>
<td>P</td>
<td>n.s.</td>
<td></td>
<td>&lt;0.05</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Pregnant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>8</td>
<td>337.5±5.9</td>
<td>5.2±0.2</td>
<td>82.0±5.8</td>
<td>402.1±38.0</td>
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<tr>
<td>CD</td>
<td>10</td>
<td>328.0±4.4</td>
<td>5.4±0.1</td>
<td>72.8±2.2</td>
<td>552.0±44.9</td>
</tr>
<tr>
<td>P</td>
<td>n.s.</td>
<td></td>
<td>n.s.</td>
<td>n.s.</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Starved</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>8</td>
<td>309.4±5.0</td>
<td>4.1±0.2</td>
<td>35.4±5.6</td>
<td>6496.4±358.5</td>
</tr>
<tr>
<td>CD</td>
<td>8</td>
<td>303.1±4.7</td>
<td>4.3±0.2</td>
<td>29.3±4.5</td>
<td>7217.6±239.7</td>
</tr>
<tr>
<td>P</td>
<td>n.s.</td>
<td></td>
<td>n.s.</td>
<td>n.s.</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM. n.s. = not significant

Results

Basal plasma glucose concentrations were slightly higher in nonpregnant STZ-treated rats than in nonpregnant control rats in the fed state in the morning (Fig. 1). Plasma glucose concentration after intraperitoneal glucose administration in the STZ group were significantly higher than those in the control group. Therefore these STZ-treated rats were considered as chemically diabetic (CD) animals before mating. There was marked deterioration of insulin secretory activity in STZ-treated rats. The insulino-genic index (0–30 min, Δinsulin/Δglucose) was significantly lower in STZ-treated rats (1.51±0.40 vs. 0.04±0.01, P<0.005).

As shown in Table 1, plasma glucose concentrations in CD rats were slightly but significantly higher than in control rats when they were not pregnant. Plasma glucose concentrations in fed pregnant rats were markedly decreased near term in both groups, while plasma insulin concentrations were significantly (P<0.005) higher than those in the nonpregnant state. Body weight, plasma glucose levels and plasma
insulin concentrations in the fed state did not differ significantly between control and CD pregnant rats. The amount of plasma ketone bodies in CD rats, however, was significantly greater than in control pregnant rats, suggesting decreased sensitivity of the liver to insulin in CD rats.

Starvation for 48 hr, from day 19 to 21 of gestation, resulted in marked weight loss and decreases in plasma glucose concentrations in both groups of pregnant rats. Plasma insulin in both groups was decreased after 48 hr of fasting, although insulin concentrations in the two groups changed along parallel lines. Ketone body concentrations in CD pregnant rats, however, were significantly higher than in control pregnant rats. Thus, ketone body concentrations appeared to be very sensitive indices for determining the degree of metabolic deterioration not only in the starved state but also in the fed state during late gestation.

Dynamic measurement of in vitro insulin secretion was performed in the perfused pancreas from control and CD animals. Fig. 2

![Diagram](image-url)

Fig. 2, Insulin response to 19 mM arginine in the presence of 4.4 mM glucose from the perfused pancreas of control (○-○) and diabetic (●-●) pregnant rats. Data were derived from 5 separate experiments. *P < 0.05, **P < 0.01 vs. diabetic pregnant group.
illustrates the dynamic insulin secretory response during 20 min of arginine stimulation at 19 mM in the presence of 4.4 mM glucose. The release of insulin from the pancreas was markedly potentiated by pregnancy as compared with the nonpregnant values in our previous study (Seino et al., 1980). The release of insulin from the CD pancreas was attenuated in both the first and second phases. It should be noted that the rise in the first phase of insulin release was significantly delayed in the β-cells of diabetic pancreas.

Attenuated secretory activity in CD mother rats was also observed when the pancreases were exposed to 16.7 mM glucose as shown in Fig. 3. The magnitude of insulin release in both the first and second phases was significantly lower in CD pregnant rats than in control pregnant rats. It was again noticed that the rise in the first phase of insulin release was significantly delayed in the β-cells of diabetic pancreas.

Forty-eight-hour fasting in late pregnancy markedly attenuated glucose-induced insulin release. The release of insulin from the perfused pancreas was greatly inhibited in both control and CD pregnant rats. The β-cells of CD rats appeared to be profoundly affected by starvation, and in the second phase insulin release evoked by glucose was significantly reduced.

The total secretion of insulin caused by 16.7 mM glucose during 20-min stimulation and the insulin content of the perfused pancreas under various conditions are summarized in Table 2. The total secretion of insulin from CD rats was only 28% of that from control rats in the nonpregnant state. The insulin secretion ratio was significantly lower in CD rats than in control rats. Therefore, the reduced insulin secretion observed in the CD nonpregnant rats...
Table 2. Effects of pregnancy and starvation on insulin secretion, content and secretion ratio of the perfused pancreas in control and CD rats.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Insulin Secretion (Is, mU)</th>
<th>Insulin Content (Ic, mU)</th>
<th>Secretion Ratio Is/Ic+Ic (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonpregnant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>6</td>
<td>1.8±0.2</td>
<td>312.6±12.4</td>
<td>0.57±0.07</td>
</tr>
<tr>
<td>CD</td>
<td>5</td>
<td>0.4±0.1</td>
<td>253.0±21.0</td>
<td>0.15±0.03</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>n.s.</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Pregnant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>7</td>
<td>5.0±0.3</td>
<td>675.8±66.2</td>
<td>0.73±0.08</td>
</tr>
<tr>
<td>CD</td>
<td>6</td>
<td>3.4±0.3</td>
<td>452.3±18.2</td>
<td>0.75±0.06</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.005</td>
<td>&lt;0.05</td>
<td>n.s.</td>
</tr>
<tr>
<td>Starved</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnant</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>6</td>
<td>1.2±0.2</td>
<td>352.6±5.3</td>
<td>0.33±0.05</td>
</tr>
<tr>
<td>CD</td>
<td>5</td>
<td>0.7±0.1</td>
<td>366.4±17.6</td>
<td>0.18±0.03</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM. n.s.=not significant

was due not only to decreased insulin content but also to impaired insulin stimulus-secretion coupling.

Pregnancy markedly enhanced insulin secretion and insulin secretion ratio evoked by glucose. Although the amounts of insulin released were significantly lower in CD rats than in control rats, pregnancy caused a 8.5-fold increase in insulin release from CD rats. It was also apparent that pregnancy increased insulin content in the two groups. However, the magnitude of the increase in insulin content induced by pregnancy was greater in control rats than in CD rats (218% vs. 182% of their non-pregnant levels), indicating the limitation of β-cell replication in CD rats. Of special interest was the fact that the insulin secretion ratio was similar in the two groups.

Forty-eight-hour fasting during day 19–21 of gestation was associated with markedly diminished insulin secretion, insulin content and insulin secretion ratio, while these parameters were not significantly different in the two groups (Table 2). Although the pancreases of the CD rats secreted less insulin than the normal rat pancreases, the insulin content after starvation was not significantly different in the two groups.

Discussion

In vitro studies illustrated in Figs. 2 and 3 reveal that pregnancy ameliorates insulin secretory activity in diabetic β-cells during late gestation, since insulin secretion was strongly abolished in CD virgin rats (Fig. 1). Bone and Taylor (1976) and Green et al. (1981) demonstrated that pregnancy augments insulin secretory activity and biosynthesis. However, dynamic insulin release from the perfused pancreas in late gestation had never been demonstrated until our previous observation (Tanigawa et al., 1989). The present study for the first time revealed dynamic insulin secretory activity by using the isolated perfused pancreas of CD rats during late gestation.

The release of insulin caused either by arginine or by glucose was significantly inhibited in diabetic β-cells. Insulin response to nonglucose stimuli in diabetic patients may appear to be similar to that of non-
diabetic subjects (Pfeifer et al., 1981). However, the magnitude of insulin release in response to arginine in CD pregnant rats was consistently lower than in normal pregnant rats. A prominent feature of the diabetic β-cells is the delay in the rise of the first phase of insulin release. These observations suggest that pancreatic β-cells of diabetic pregnant women cannot regain the ability to respond promptly to glycemic stimulation. The insulin content of the perfused pancreas in CD nonpregnant rats was lower than that of control nonpregnant rats, as shown in Table 2. In addition, the magnitude of the increase in replication caused by pregnancy was much greater in control rats than in CD rats, indicating a limitation of the replication of diabetic β-cells (Van Assche et al., 1979). Ziegler et al. (1985) demonstrated that rats which received an amount of STZ similar to that used in the present study showed a decrease in plasma glucose and an increase in plasma and pancreatic insulin concentrations during gestation. The present study provides additional evidence that the reduced insulin content of the diabetic pancreas cannot be raised to a level similar to that in the normal pancreas during pregnancy. It is also quite possible that augmented β-cell replication in pregnant rats occurs from the 19th to the 21st day of gestation. The reason for this is that the insulin content of control and diabetic rats was not different after 2-day starvation.

We previously studied the effect of fasting on secretory activity and fuel metabolism in late pregnancy in normal female rats (Tanigawa et al., 1989). Even in the fed state, plasma ketone body concentrations were higher in pregnant rats than in nonpregnant rats. Fasting resulted in about a 10-fold increase in plasma ketone bodies in the nonpregnant rats and a 20-fold increase in the pregnant rats, indicating enhanced lipolysis of adipose tissue and an accelerated rate of hepatic ketogenesis in late pregnancy.

As shown in Table 1, the prominent metabolic feature of the diabetic pregnancy is hyperketonemia. This observation was strongly confirmed when pregnant CD rats were starved for 48 hr. Ketone bodies were strikingly increased in diabetic pregnancy, whereas the other parameters in the two groups were not significantly different. Since we did not determine free fatty acid or glycerol levels in the rats, it is impossible to conclude whether the higher ketone body concentrations in CD rats resulted from accelerated hepatic ketogenesis, enhanced lipolysis or both. Magee et al. (1990) recently demonstrated that calorie restriction in pregnant women with gestational diabetes results in an increase in β-hydroxybutyrate, while free fatty acid and glycerol concentrations are not markedly altered. Thus, the hyperketonemia observed in CD rats in late gestation may be more closely linked to the rate of hepatic ketogenesis than to lipolysis. This assumption may be convincingly shown in studies with the isolated, perfused rat liver. Ketone bodies influence growth and malformation of the offspring (Freinkel, 1988; Sadler et al., 1989). Therefore it is very important to regulate ketone body production during gestation.

The increased plasma insulin and augmented insulin release in late pregnancy, as shown in the present study, have been accompanied by progressive insulin resistance (Edmond et al., 1985; Hauguel et al., 1987; Puavilai et al., 1982; Schmitz et al., 1985). Leturque et al. (1984) clearly demonstrated that insulin resistance during late pregnancy in the rat is characterized by a decreased sensitivity of liver and peripheral tissues to insulin. The anti-insulin hormones such as glucagon and catecholamine have been shown to stimulate lipolysis and ketogenesis both in vitro (McGarry et al., 1975; McGarry and Foster, 1980) and in vivo (Bahnsen et al., 1984; Shade and Eaton,
Since those hormones are increased in patient with diabetes mellitus, the hyperketonemia observed in CD pregnant rats may be due to excess secretion of ketogenic hormones, in addition to insulinopenia.

Acknowledgements

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References


