Effects of pancreatectomy on the growth and metabolite concentrations of the sheep fetus

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REVISED MANUSCRIPT RECEIVED 10 February 1986

ABSTRACT

The effects of fetal pancreatectomy on the growth and metabolism of the fetal sheep were investigated in chronically catheterized animals during the last third of gestation. Fetal pancreatectomy reduced body weight and crown–rump length at delivery near term (term 145 days). Body weight was affected more than body length so the ratio of weight to length was significantly less after pancreatectomy than in intact animals ($P < 0.05$). Pancreatectomized fetuses appeared to maintain a normal growth rate for 5–10 days after surgery but thereafter showed no further significant increase in body weight. When all the data from the intact and pancreatectomized fetuses were combined, there was a significant positive correlation between the plasma insulin concentration in utero and the body weight at delivery near term. The majority of organs studied were reduced in absolute weight after pancreatectomy but only the spleen and thymus were proportionally lighter when the weights were expressed as a percentage of body weight. Brain and placental weights were similar in intact and pancreatectomized fetuses. Over the range of values observed in utero, there were significant inverse correlations between the log plasma insulin level and the mean plasma concentrations of glucose, lactate, fructose and α-amino-nitrogen in individual intact and pancreatectomized fetuses. Insulin infusion into pancreatectomized fetuses restored the metabolite concentrations to their normal values within 48 h of infusion. The results demonstrate that insulin has a vital role in regulating fetal growth and metabolism in utero.

J. Endocr. (1986) 110, 225–231

INTRODUCTION

Many recent studies indicate that insulin is a major growth-promoting hormone in the fetus (Bassett & Fletcher, 1982; Gluckman & Liggins, 1984). Infants of diabetic mothers are large at birth while diabetic infants or infants with pancreatic agenesis have reduced birth weights (see Pedersen 1975; Hill, 1982). Experimental hyperinsulinaemia in utero produced by insulin infusion also increases birth weight in the rat, rabbit and monkey and alters the weights of specific organs in the fetal pig (Picon, 1967; Susa, McCormick, Widness et al. 1979; Fletcher, Falconer & Bassett, 1982; Garssen, Spencer, Colenbrander et al. 1983).

Much less is known about the effects of insulin deficiency on body size and organ weights of the fetus (see Hill, 1982). Fetal hypoinsulinaemia induced with diabetogenic drugs reduces body weight in fetal rabbits but has more variable effects on body and organ weights in fetal lambs and monkeys (Cheek & Hill, 1975; Harding, Young & Possmayer, 1975; Brinsmead & Thorburn, 1982). Insulin deficiency produced by ablation of the fetal pancreas has a more consistent effect on reducing body weight at delivery but no information is available on the organ weights or rate of growth in these fetuses, despite their abnormal metabolic environment (Felix & Jacquot, 1976; Fowden & Comline, 1984). In the present study the effects of fetal pancreatectomy on body size and specific organ weights have been investigated in fetal sheep during the last third of gestation. The extent of the metabolic disturbance caused by pancreatectomy and the effect of insulin in restoring the normal metabolic status of these fetuses were also examined.

MATERIALS AND METHODS

Animals

Forty-seven Welsh Mountain ewes of known gestational age were catheterized specifically for this
study. Fetal body weights were also obtained from a further 33 catheterized ewes used in other studies. All the ewes carried single fetuses. Thirty-eight ewes delivered spontaneously at a mean (±S.E.M.) gestational age of 140-9 ± 0-9 days (term 145 ± 2 days); the remainder of the ewes were delivered by Caesarian section between 112 and 141 days of gestation.

Operative procedures

Anaesthesia was induced with ethyl chloride and maintained with halothane after intubation. The pancreas was removed from 30 fetuses between 113 and 120 days of gestation using the surgical procedures described previously (Fowden & Comline, 1984). The mean weight of pancreatic tissue removed from these fetuses was 916 ± 41 mg, which accounts for about 90% of the fetal pancreas at this gestational age (Fowden & Comline, 1984). Sham-operations in which the pancreas was exposed but left intact were carried out in 17 fetuses between 115 and 123 days of gestation. The remaining 33 fetuses had no abdominal surgery. Catheters were inserted into the fetal dorsal aorta and caudal vena cava and into the maternal aorta of all the animals, using the surgical techniques described by Comline & Silver (1972). Antibiotic was given intravenously to the fetus at the end of surgery (100 mg ampicillin; Penbritin, Beecham Research Laboratories, Brentford, Middx) and intramuscularly to the mother on the day of surgery and for 3 days thereafter (10 ml Streptopen; Glaxo Ltd, Uxbridge, Middx). The ewes delivered by Caesarian section were anaesthetized with intravenous sodium pentobarbitone (6%, w/v, in physiological NaCl) between 5 and 38 days after the first operation.

Experimental procedures

Blood samples of 2 ml were taken daily from the fetal and maternal arteries for blood gas, pH, packed cell volume (PCV), metabolite and insulin determinations. Insulin was infused intravenously for at least 48 h into six pancreatectomized fetuses aged between 130 and 132 days of gestation at the beginning of the infusion (porcine insulin; Novo Actrapid MC Farillon Ltd, Romford, Essex; 0-5 units bolus then 1-0-2-0 units/24 h). Blood samples were taken at 24, 1 and 0 h before infusion and at 3, 6, 12, 24, 36 and 48 h after beginning the infusion. Values of metabolic clearance rate (MCR) for insulin were calculated from the rate of insulin infusion between 24 and 48 h and the mean plasma concentration of insulin during that period (Nathanielsz, 1976). All blood samples were centrifuged immediately at 4 °C and the plasma was stored at −20 °C until required for analysis.

Biochemical analyses

Blood gas tensions, pH and PCV were measured immediately on all the blood samples using standard radiometer equipment. The basal values observed were within the normal range and similar to those reported previously (Comline & Silver, 1972; Fowden & Comline, 1984). The plasma concentration of insulin was measured by a double-antibody radioimmunoassay (Hales & Randle, 1963) using crystalline ovine insulin as standards (Eli Lilly & Co., Indianapolis, IN, U.S.A.; Lot No.615-112B-108-I). The detailed procedures and specificity of the assay have been published previously (Fowden, 1980). The intra- and interassay coefficients of variation were 10-6 and 16-7% respectively and the minimum detectable quantity of insulin was 20 pmol/l. The methods used to measure the plasma concentration of glucose, lactate, fructose and α-amino-nitrogen have been described previously (Fowden & Comline, 1984).

Statistical analyses

Means and standards errors have been used throughout and statistical analyses were made according to the methods of Armitage (1971). The significance of differences between the intact and pancreatectomized groups of fetuses was evaluated using the conventional t-test. The correlation coefficients between two variables were calculated by simple linear regression and assessed for significance by Fisher’s t-test. The
effect of insulin infusion was assessed by paired t-test to the preinfusion concentrations (0 h sample). No data obtained within 36 h of surgery or spontaneous delivery have been included in the study.

RESULTS

Morphometric observations

Body size
The body weight of fetuses pancreatectomized between 113 and 120 days was similar to that of intact animals up to 125 days of gestation but thereafter the pancreatectomized fetuses weighed significantly \( (P<0.05) \) less than intact animals of a similar gestational age (Fig. 1). At delivery, after 137 days of gestation, the pancreatectomized fetuses weighed 28% less than the intact fetuses and had had significantly \( (P<0.05) \) lower plasma insulin concentrations for the last 15–30 days of gestation (Table 1). In the pancreatectomized fetuses, there was no significant increase in body weight between 120 and 140 days of gestation, while in the intact fetuses body weight increased by 50% during this period (Fig. 1, \( P<0.01 \)). Between 120 days and term there was a significant positive correlation between body weight and gestational age in the intact fetuses \( (r=0.74, \ n=40, \ P<0.01) \) but not in the pancreatectomized animals \( (r=0.27, \ n=30, \ P<0.05) \). For the fetuses delivered after 137 days of gestation, there was a significant positive correlation \( (r=0.71, \ n=35, \ P<0.01) \) between body weight and the mean plasma insulin concentration in utero in individual animals during the last third of gestation.

The crown-rump length of pancreatectomized fetuses delivered after 137 days was also significantly less than that of intact fetuses of a similar gestational age (Table 1), although the reduction in length was less than that in body weight. As a result the ratio of weight to length in the pancreatectomized fetuses was significantly less than that observed in the intact animals (Table 1). There was no significant difference

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<th>Table 1. Mean (± S.E.M.) gestational age, plasma insulin concentration, body length and placental and body weights of pancreatectomized and intact sham-operated fetal sheep delivered after 137 days of gestation. Numbers of animals are shown in parentheses</th>
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*\( P<0.05 \) compared with intact animals (t-test).

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<th>Table 2. Mean (± S.E.M.) body and organ weights of pancreatectomized and intact sham-operated fetal sheep after 137 days of gestation. Numbers of animals are shown in parentheses</th>
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in the placental weight between intact and pancreatectomized fetuses and hence the ratio of fetal to placental weight was significantly less in the pancreatectomized fetuses (Table 1).

**Organ weights**
The weights of the liver, lungs, heart, rumen and abomasum, large intestine and spleen were significantly less in the pancreatectomized fetuses than in the intact animals delivered after 137 days of gestation (Table 2, \(P < 0.05\)). However, when the weights were expressed as a percentage of the body weight, only the spleen and the thymus were proportionally lighter after pancreatectomy (Table 2, \(P < 0.05\)). Brain weight was similar in the intact and pancreatectomized fetuses, so the brain was a significantly larger proportion of the body weight in the pancreatectomized animals (Table 2, \(P < 0.05\)). No significant differences in thyroid or adrenal weight were observed between the two groups of fetuses and only very small pancreatic remnants (<80 mg) were found in any of the pancreatectomized fetuses (Table 2).

**Metabolic observations**

**Basal metabolite concentrations**
The increases in the fetal plasma concentrations of glucose, fructose, lactate and \(\alpha\)-amino-nitrogen found after pancreatectomy were related to the degree of hypoinsulinaemia observed in utero. This relationship is shown in Fig. 2 for glucose and \(\alpha\)-amino-nitrogen. When all the data from the intact and pancreatectomized fetuses were combined, there were significant inverse correlations between the log plasma concentration of insulin and the mean plasma levels of glucose (Fig. 2a), fructose \((r = -0.60, n = 28, P < 0.01)\), lactate \((r = -0.71, n = 40, P < 0.01)\) and \(\alpha\)-amino-nitrogen (Fig. 2b) in individual animals during the last third of gestation. As fetal glucose concentrations vary with the maternal nutritional state (Bassett & Madill, 1974), the log plasma insulin level was better correlated with the ratio of fetal to maternal glucose concentrations \((r = -0.71, n = 40, P < 0.01)\) than with the fetal glucose level alone.

**Insulin infusion**
Infusion of insulin lowered the plasma concentrations of glucose, lactate, fructose and \(\alpha\)-amino-nitrogen in all six pancreatectomized fetuses studied (Fig. 3). The mean initial plasma concentrations of glucose, lactate, fructose and \(\alpha\)-amino-nitrogen were \(1.08 \pm 0.13 \text{ mmol/l}, 3.10 \pm 0.36 \text{ mmol/l}, 6.53 \pm 0.92 \text{ mmol/l} \) and \(9.77 \pm 1.02 \text{ mmol/l} \) respectively \((n = 6)\). These concentrations were reduced significantly by 3 h after beginning the infusion and reached values similar to those observed in intact fetuses by 24 h of infusion. All four metabolite concentrations returned to their preinfusion values within 24 h of ending the infusion (Fig. 3). The arterial \(pO_2\) and oxygen content also fell significantly during insulin infusion; the mean reductions at 48 h were \(0.24 \pm 0.05 \text{ kPa} \) and \(0.45 \pm 0.14 \text{ mmol/l} \) respectively \((n = 6, P < 0.05\) in both cases). The mean initial plasma concentration of insulin was \(29.3 \pm 10.0 \text{ pmol/l} \) and rose to a mean value of \(340.2 \pm 28.7 \text{ pmol/l} \) during the last 24 h of

![FIGURE 2. Relationship between the log plasma concentration of insulin and the plasma concentrations of (a) glucose \((y = 1.64 - 0.45 \log x, r = -0.57, n = 40, P < 0.01)\) and (b) \(\alpha\)-amino-nitrogen \((y = 19.19 - 6.63 \log x, r = -0.76, n = 40, P < 0.01)\) in pancreatectomized (○) and intact sham-operated (▲) sheep fetuses.](image)
Disruption

In the present study, ablation of the fetal pancreas produced intrauterine growth retardation. The close correlation observed between body weight and the plasma insulin concentration in individual fetuses after 137 days of gestation shows that the endogenous insulin concentration has an important role in regulating fetal growth in utero. Both body weight and body length were significantly reduced by fetal pancreatectomy but not equally so. The reduction in the fetal weight:length ratio observed after pancreatectomy is similar to that found in fetuses in which growth is retarded by placental insufficiency or maternal malnutrition (Alexander, 1974; Robinson, Kingston, Jones & Thorburn, 1979). This suggests that the growth of the soft tissues is affected more readily than that of bone when the supply of nutrients to the fetus is restricted in any way.

Pancreatectomized fetuses appeared to maintain a normal growth rate for about 5–10 days after surgery but showed no further significant increase in body weight thereafter. As insulin levels fall within 24 h of pancreatic ablation (Fowden & Comline, 1984), the present findings suggest that the cellular effects of insulin may last for several days after the circulating level of insulin has become undetectable. The mechanisms by which hypoinsulinaemia leads to a reduced growth rate have not been identified fully. Placental mass is determined well before the age at which the fetuses were pancreatectomized (Bancroft, 1946) and there was no significant difference in placental weight between the intact and pancreatectomized animals. However, the umbilical uptake of glucose is reduced in pancreatectomized fetuses and there are also changes in the fetal plasma concentration of the somatomedins in these animals (Fowden, Silver & Comline, 1984; Gluckman, Fowden, Butler & Comline, 1985).

The growth retardation observed after pancreatectomy did not affect the weight of the fetal brain. A similar sparing effect on the brain has been found in sheep fetuses in which growth is retarded by thyroidectomy, placental insufficiency and maternal malnutrition (Emmanouilides, Townsend & Bauer, 1968; Creasy, Barrett, de Swiet et al. 1972; Hopkins & Thorburn, 1972; Alexander, 1974; Robinson et al. 1979). These observations indicate that the requirements for cerebral growth are met preferentially whatever the prevailing fetal endocrine or metabolic environment. Of the other fetal organs, only the spleen and thymus were significantly reduced as a proportion of body weight after pancreatectomy. Relatively small thymuses and spleens are also common in other types of fetal growth retardation and depletion of the thymic tissue has been observed in a growth-retarded human infant with pancreatic agenesis (Alexander, 1974; Dodge & Laurensen, 1977; Gluckman & Liggins, 1984). The explanation for the reduced growth rate of these specific organs remains obscure.

The present study confirms the initial findings of high fetal metabolite concentrations after pancreatectomy (Fowden & Comline, 1984) and extends those observations to show that the extent of the metabolic disturbance is related to the degree of hypoinsulinaemia produced by pancreatic ablation. The close correlations observed between the log plasma insulin

**FIGURE 3.** Mean (± S.E.M.) changes in the plasma concentrations of insulin, glucose, lactate, fructose, and α-amino-nitrogen before, during and after infusion of insulin (stippled bars) into six pancreatectomized sheep fetuses. Details of the dose of insulin are given in the text. *P < 0.05 compared with 0 h value (paired t-test).

**Infusion.** The mean MCR for insulin calculated from these infusion experiments was 23.7 ± 2.4 ml/min or 13.3 ± 2.2 ml/min per kg fetal body weight (n = 6).

**DISCUSSION**

In the present study, ablation of the fetal pancreas produced intrauterine growth retardation. The close correlation observed between body weight and the plasma insulin concentration in individual fetuses after 137 days of gestation shows that the endogenous insulin concentration has an important role in regulating fetal growth in utero. Both body weight and body length were significantly reduced by fetal pancreatectomy but not equally so. The reduction in the fetal weight:length ratio observed after pancreatectomy is similar to that found in fetuses in which growth is retarded by placental insufficiency or maternal malnutrition (Alexander, 1974; Robinson, Kingston, Jones & Thorburn, 1979). This suggests that the growth of the soft tissues is affected more readily than that of bone when the supply of nutrients to the fetus is restricted in any way.

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The present study confirms the initial findings of high fetal metabolite concentrations after pancreatectomy (Fowden & Comline, 1984) and extends those observations to show that the extent of the metabolic disturbance is related to the degree of hypoinsulinaemia produced by pancreatic ablation. The close correlations observed between the log plasma insulin
level and the various plasma metabolite concentrations show that insulin has a vital role in the control of the metabolic balance in the fetus. Normal metabolite concentrations can be restored in the pancreatectomized fetuses by infusion of exogenous insulin. However, the plasma concentration of insulin required to achieve this was twice the normal value. As the MCR for insulin in the pancreatectomized fetus was similar to that in intact animals when expressed on a weight basis (Creswell, Susa, Cowett et al. 1983), these results suggest that the tissue sensitivity to insulin may be reduced after pancreatectomy. There may be changes in receptor number or affinity after pancreatectomy, or in the concentration of hormones such as growth hormone which antagonizes the action of insulin in utero (Gluckman & Liggins 1984; Parkes & Bassett, 1985). Certainly, the concentration of the somatomedin that binds to insulin receptors in the human fetus is raised in the sheep fetus after pancreatectomy (Sara, Hall, Misaki et al. 1983; Gluckman et al. 1985). Whatever the precise mechanisms involved, these observations clearly demonstrate that the fetal pancreas is essential for normal development in utero.

ACKNOWLEDGEMENTS

We should like to thank Miss T. Grimes and Mr P. Hughes for their help during surgery and sampling, Mr D. Clarke and Mr I. Cooper for their care of the animals and Mrs J. Knox and Mr K. Richardson for their assistance with the biochemical analyses. This work was supported by grants from the Wellcome Trust and British Diabetic Association. Mr Xin Zhi Mao was an Academic Exchange Visitor from the Chinese Academy of Sciences, who came under the auspices of the Royal Society.

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