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Table 1. Changes with development in mean body and placental weights and in mean plasma hormone and glucose concentrations in fetal rabbits from nulliparous does sampled under anaesthesia

	Gestational age (days)					
	22	24	26	28	30	33 (suckled newborns, 24th postpartum)
Number of litters	3	3	2	3	7	2
Body weight (g)	6.35 ± 0.12 (29) (5.23 – 7.69)	11.72 ± 0.28 (22) (9.44 – 13.75)	20.15 ± 0.88 (18) (14.32 – 26.36)	26.21 ± 0.76 (28) (16.4 – 30.57)	40.69 ± 1.8[b] (56) (15.55 – 50.43)	56.77 ± 0.62 (16) (42.55 – 71.99)
Fetal placenta weight (g)	1.95 ± 0.07 (22) (1.21 – 2.4)	2.63 ± 0.115 (22) (1.55 – 3.60)	2.81 ± 0.19 (18) (1.50 – 4.10)	2.46 ± 0.10 (27) (1.51 – 3.51)	3.58 ± 0.12[b] (56) (1.05 – 5.3)	–
Plasma insulin (nmol/l)[a]	0.23 ± 0.007 (27) (0.09 – 0.45)	0.51 ± 0.005 (22) (0.17 – 1.10)	0.35 ± 0.008 (18) (0.16 – 0.79)	0.365 ± 0.01 (27) (0.11 – 0.70)	0.332 ± 0.017[b] (56) (0.03 – 0.60)	0.225 ± 0.007 (16) (0.09 – 0.47)
Plasma glucagon (nmol/l)[a]	–	–	0.103 ± 0.011 (16) (0.06 – 0.16)	0.226 ± 0.171 (27) (0.11 – 0.41)	0.163 ± 0.011 (40) (0.05 – 0.30)	0.206 ± 0.008 (16) (0.11 – 0.39)
Plasma glucocorticoids[a] (nmol/l)	–	70.7 (pooled sample)	71.3 ± 3.4 (4) (47.6 – 101)	33.8 ± 3.3 (19) (17.3 – 77.9)	29.7 ± 3.0 (30) (15.8 – 53.4)	–
Plasma glucose (mmol/l)	2.54 ± 0.37 (20) (1.22 – 6.0)	4.09 ± 0.40 (15) (0.83 – 6.5)	2.68 ± 0.13 (15) (1.72 – 3.44)	3.42 ± 0.08 (24) (2.1 – 5.0)	4.04 ± 0.19[b] (43) (0.9 – 7.1)	5.37 ± 0.40 (16) (3.0 – 7.5)
Pancreas weight (mg)	–	27 ± 1.3 (14) (18 – 32)	41 ± 4.7 (18) (10 – 68)	36 ± 2.4 (38) (14 – 63)	60 ± 3.5 (31) (20 – 99)	147 ± 10.4 (16) (79 – 241)
Pancreatic insulin (nmol/pancreas)	0.017 ± 0.002 (26) (0.005 – 0.035)	0.118 ± 0.012 (14) (0.067 – 0.23)	0.052 ± 0.005 (18) (0.023 – 0.085)	0.08 ± 0.018 (38) (0.017 – 0.11)	0.35 ± 0.05 (31) (0.058 – 1.15)	0.70 ± 0.47 (16) (0.3 – 1.12)

Results are expressed as mean ± SEM with number and the range below in parentheses; [a] Hormone values given are the antilogs of mean ± SEM values calculated using $\log_{10}$ transformed values. [b] Including one ectopic fetus weighing 15.55 g

Table 2. Correlations between body or placental weight and plasma hormone concentrations in individual fetal rabbits from nulliparous does sampled under anaesthesia

	Gestational age (days) (number of litters)				
	22 (3)	24 (3)	26 (2)	28 (3)	30[a] (7)
Bodyweight versus placenta weight	0.67 (27) $p < 0.01$	0.62 (22) $p < 0.01$	0.88 (19) $p < 0.001$	0.87 (28) $p < 0.001$	0.76 (55) $p < 0.001$
Log plasma insulin versus bodyweight	0.46 (27) $p < 0.02$	0.48 (22) $p < 0.05$	0.82 (18) $p < 0.001$	0.62 (27) $p < 0.001$	0.68 (55) $p < 0.001$
Log plasma insulin versus placenta weight	0.41 (27) $p < 0.05$	0.71 (22) $p < 0.001$	0.92 (18) $p < 0.001$	0.64 (25) $p < 0.01$	0.59 (55) $p < 0.001$
Log plasma glucagon versus bodyweight	–	–	−0.48 (16)	−0.43 (28)	NS

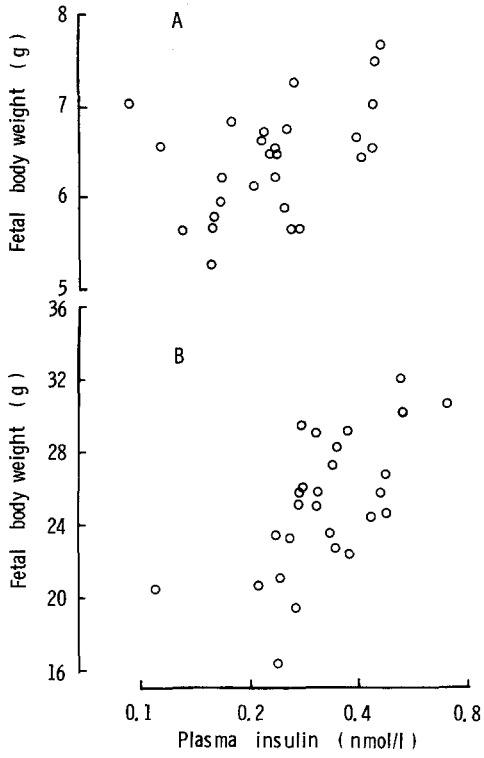

Fig. 1. The relationship of plasma insulin concentration and body-weight in individual fetal rabbits at *(A)* 22 days gestation and *(B)* 28 days gestation

related to maternal plasma glucose concentrations (6.89 ± 0.38 mmol/l, mean ± SEM), as these did not differ significantly with gestational age.

The mean plasma insulin concentration at 24 days gestation was significantly higher than at either 22 ($p < 0.001$) or 26 days ($p < 0.01$). Values later in gestation were similar to that at 26 days, but post-natally the value was lower ($p < 0.001$). Insufficient plasma was available from the younger fetuses to measure glucagon or glucocorticoids in individual samples. However glucocorticoid values at 28 and 30 days gestation appeared lower than those in younger fetuses (Table 1). Mean plasma glucagon concentrations did not show any clear trend with age (Table 1).

Pancreatic weight showed little change before 28 days of gestation, but by 30 days had increased significantly ($p < 0.001$) before the large increase occurring during the perinatal period (Table 1). In general, there were similar changes in the insulin content, although the concentration of pancreatic insulin was higher at 24 days than at 26 and 28 days.

Interrelations of Body and Placental Weights with Plasma Hormone Concentrations

As expected from earlier studies, fetal body and placental weights were highly correlated (Table 2). In addition, plasma insulin concentrations and body weights of individual fetuses were significantly correlated at each gestational age studied, but not post-natally (Table 2). Values for these parameters at 22 and 30 days gestation are illustrated in Figure 1. Similar significant correlations were also obtained when plasma insulin concentration was correlated with the weight of the fetal placenta (Table 2). Plasma glucose concentration was not correlated with either body weight or plasma insulin at any of the ages studied.

Plasma glucagon concentrations at 28 days of gestation were negatively correlated with body weight ($p < 0.05$; Table 2). At 26 days there was a similar negative correlation (p insignificant). At 30 days gestation there was no relation between plasma glucagon and fetal size.

Plasma glucocorticoids showed no significant relation to body weight. However, when the insulin: glucocorticoid ratio was related to fetal body weight at 30 days gestation, a significant correlation ($r = 0.622$, $n = 30$, $p < 0.001$).

Effects of Litter Size Reduction

Curtailing litters to only two fetuses by surgery resulted in significant fetal hypertrophy by comparison with age matched controls from similar nulliparous does (Table 3) [13]. In such litter-reduced fetuses all the organs measured and the fetal portion of the placenta were significantly increased in size, whereas the maternal portion of the placenta was unaltered. Plasma concentrations of glucose, glucagon and glucocorticoids did not differ significantly from those of age-matched controls and the mean plasma insulin concentration was significantly increased (Table 3). Individual values, however, fell on the regression line relating insulin concentration to body weight in control fetal rabbits at 30 days gestation (Fig. 2). Pancreatic weight and insulin content were significantly greater than in control fetuses, but the concentration of insulin was unaltered (Table 3).

Plasma somatomedin activity determined in 10 litter-reduced fetuses was significantly higher than in 11 fetuses from two control litters at 30 days gestation (Table 2). The somatomedin activity in individual fetuses of litter-reduced and control litters was not significantly correlated with fetal body weight, plasma insulin or glucocorticoids, but was significantly correlated with the insulin : glucocorticoid ratio ($r = 0.617$, $p < 0.01$; Fig. 3).

Fetal rabbits occupying the ovarian end-most positions within the uterine horns are frequently amongst the largest of their litter [21]. The weight and plasma insulin concentrations of litter-reduced fetuses were therefore compared with the ovarian end-most fetuses of intact normal litters at 30 days of gestation. Mean fetal body weight and plasma insulin concentrations of ovarian end-most fetuses were 43.8 ± 1.7 g and 0.336 ± 0.04 nmol/l, respectively. These values were significantly less than the corresponding values for litter-reduced fetuses ($p < 0.001$).

Table 3. Body and placental weights and plasma hormone and glucose concentrations at 30 days gestation in fetuses from nulliparous does after litter-reduction and in fetuses from multiparous does compared with normal fetuses from nulliparous does at the same age

	Nulliparous does		Multiparous does	*p* Normal nulliparous fetuses versus	
	(7) Normal fetuses[a]	(6) Litter-reduced fetuses	(6) Normal fetuses	litter reduced	multiparous
Fetal body weight (g)	40.95 ± 1.21 (55)	57.95 ± 1.24 (12)	46.9 ± 1.89 (48)	< 0.001	< 0.05
Fetal placenta weight (g)	3.74 ± 0.08 (55)	5.40 ± 0.21 (12)	4.20 ± 0.12 (45)	< 0.001	< 0.01
Plasma insulin (nmol/l)[b]	0.345 ± 0.081 (55)	0.648 ± 0.085 (12)	0.423 ± 0.035 (48)	< 0.001	< 0.05
Plasma glucagon (nmol/l)[b]	0.163 ± 0.011 (40)	0.154 ± 0.017 (10)	0.117 ± 0.001 (47)	NS	< 0.05
Plasma glucocorticoids (nmol/l)[b]	29.7 ± 3.0 (30)	26.8 ± 3.26 (12)	25.4 ± 0.7 (47)	NS	< 0.05
Plasma somatomedin activity (U/ml)	0.596 ± 0.14 (11)	0.734 ± 0.14 (10)	–	< 0.02	–
Plasma glucose (nmol/l)	4.04 ± 0.19 (43)	4.68 ± 0.30 (10)	4.42 ± 0.20 (48)	NS	NS
Pancreatic insulin (nmol/g)	5.67 ± 0.08 (31)	7.60 ± 0.04 (10)	–	NS	–
Pancreas weight (mg)	60.3 ± 3.5 (31)	127.6 ± 3.4 (10)	–	< 0.001	–

Results are expressed as mean ± SEM with number of fetuses in parentheses; [a] excluding one ectopic fetus; [b] hormone values are antilogs of mean ± SEM values calculated using $\log_{10}$ transformed values; NS = not significant

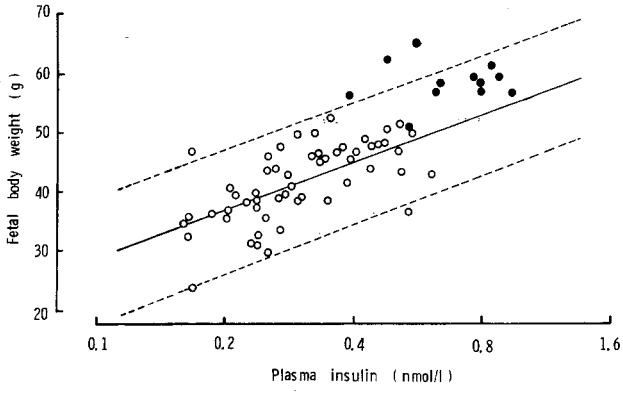

Fig. 2. The relationship of plasma insulin concentration and fetal body weight at 30 days gestation in litter-reduced fetal rabbits (●) after surgical reduction of litter size to two at 9 days gestation compared with that in normal 30 day fetal rabbits (○). The regression line shown is that derived for the normal fetuses. The dotted lines represent ± 2 SD

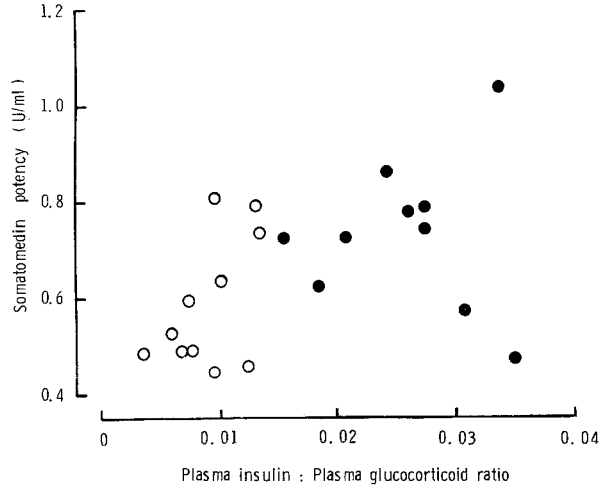

Fig. 3. The relationship of plasma somatomedin activity to the plasma insulin : glucocorticoid ratio in normal (○) and litter-reduced (●) fetal rabbits at 30 days gestation

Effects of Maternal Anaesthesia and Parity on Fetal Hormone Concentrations

Plasma hormone concentrations at 30 days gestation in fetuses from multiparous does were not significantly influenced by maternal treatment before their removal from the uterus. Mean values in fetuses from three litters sampled after maternal sacrifice were not significantly different from those in three litters where fetuses were removed from the uterus with the mother under pentobarbitone anaesthesia.

The mean weights of fetuses and their placentae from these multiparous does were significantly greater than those from the 30 days fetuses of nulliparous does used in the gestational age series (Table 3). The mean plasma insulin concentration was also greater but their

plasma glucagon and glucocorticoid concentrations were less than those in fetuses from nulliparous does. Fetal body size was significantly correlated with log plasma insulin in this group ($r = 0.523$, $p < 0.001$) and values appeared to lie on the same relation as those in the nulliparous group.

When values from fetuses of multiparous does at 30 days gestation were pooled with values from normal and litter-reduced fetuses from nulliparous does at 30 days gestation, fetal body weight and plasma insulin were highly correlated ($r = 0.693$, $p < 0.001$, $n = 116$).

Discussion

While changes with development in the plasma insulin concentration of fetal rabbits and in the regulation of insulin release from pancreatic tissue in vitro have been studied extensively [1, 9–11, 22, 23], only Metzger and Brachet [11] have reported any association between plasma insulin concentrations and fetal body size. By contrast, the present study demonstrates unequivocally a clear positive association, independent of age, between individual fetal body weight and plasma insulin concentrations at all gestational ages studied between 22 days and term. A similar relationship was observed in fetal rats at term [24]. This consistent close correlation may reflect an involvement of insulin in regulation of fetal growth rather than simply an age-dependent associative relation. The findings that fetuses from multiparous does are not only larger than those from nulliparous does, but also have higher plasma insulin concentrations and that surgical reduction of litter size in nulliparous does leads to fetal hyperinsulinaemia as well as to somatic hypertrophy, also argue strongly in favour of a causal relationship. The experiments of Picon [3], recently confirmed by Angervall et al. [25], suggested that chronic hyperinsulinaemia in the rat fetus during late gestation could result in increased body size. Insulin administration to fetal monkeys via implantable osmotic mini-pumps [4] also caused significant fetal and placental hypertrophy. However, the degree of hyperinsulinaemia in these studies was extreme and there is still no indication whether alterations of insulin concentration within the physiological range result in alterations in fetal or placental growth rate.

Marked placental and somatic hypertrophy follow reduction in litter size if surgery is performed early in gestation [12]. Hormonal mechanisms involving ovarian-placental interaction or general systemic effects related to less uterine crowding and improved maternal blood supply to individual placentae, have been invoked to explain this phenomenon [26]. Whatever the primary cause of the variation in fetal and placental size, it is clearly associated with fetal hyperinsulinaemia. It is an attractive hypothesis that growth of the fetal placenta, like that of the fetal body, is also mediated by increased concentrations of insulin within the fetal circulation. The observations on insulin-treated monkeys [4] and the relationship of placental insulin receptor populations to fetal size [27] give strong support to this hypothesis.

Whatever the role of insulin, the present results suggest its possible effects cannot be considered in isolation from those of other hormones. Mean plasma concentrations of glucagon and glucocorticoids were comparable to those observed in earlier investigations [17, 28, 29]. There was evidence from the 26 and 28 day age group that concentrations of glucagon were negatively correlated with fetal size, a finding consistent with observations on normal and growth-retarded guinea pigs [30]. Glucocorticoid concentration was not associated with fetal size, although it was lower in the larger fetuses of multiparous does. Somatomedin activity was increased in the larger fetuses following litter reduction (Table 3). The work of Hill and Milner [31] suggests that the magnitude of this difference may have been greater if fetal rabbit, rather than porcine cartilage, had been utilised in the bioassay system. However, the role of somatomedins in control of growth and the factors regulating their activity either pre- or post-natally are not well understood [32, 33]. Post-natally insulin has been indicated as an important stimulus to somatomedin release [33] but contradictory findings have emerged regarding this role of insulin in utero. Hill and Milner [34] reported increased somatomedin activity following insulin injection into rabbit fetuses, but Brinsmead and Liggins [35] found a decreased somatomedin activity following insulin infusion into fetal lambs. By contrast, exogenous glucocorticoids depress somatomedin activity and are potent inhibitors of both pre- and post-natal growth [35–37]. With these findings in mind, the correlation observed between somatomedin activity and the plasma insulin : glucocorticoid ratio is of considerable interest.

Variation in glycaemia between fetuses within a litter, does not appear to be associated with variation in insulin concentration. The sampling procedure probably precludes any meaningful correlation between fetal glucose and insulin concentrations. It is evident, however, from the significant relation between plasma insulin and body weight at 22 days of gestation that physiologically important variation in plasma insulin, and presumably insulin secretion, precedes the reported development of glucoregulatory mechanisms in the fetal rabbit pancreas [22, 23].

In conclusion, the results of the present study demonstrate that the size of normal rabbit fetuses and their plasma insulin concentrations are highly correlated throughout the last third of gestation. Large fetuses produced by surgical litter reduction are shown to be relatively hyperinsulinaemic compared to appropriate controls.

Fetal hypertrophy and hyperinsulinaemia, comparable to that seen in infants of human diabetic mothers, has been difficult to achieve in small laboratory animals

following induction of maternal diabetes with chemical agents [38–41]. The hypertrophic, hyperinsulinaemic fetus of the litter-reduced rabbit may be of value for further study of the developmental consequences of hyperinsulinaemia.

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