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Glucose Tolerance in Ewes and Susceptibility to Pregnancy Toxaemia

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Abstract

Intravenous glucose tolerance tests were undertaken on fed twin-pregnant ewes at about 120 days of gestation by injecting 0·4 g glucose per kilogram of live weight, then measuring glucose and insulin concentrations in plasma over the next 2 h. An insulin resistance index was calculated from the product of $T_{1/2}$ for glucose disappearance and the plasma insulin concentrations integrated over time. Approximately 10 days later, the ewes were starved to induce ovine pregnancy toxaemia. During this period, the course of the hypoglycaemia and ketonaemia were followed by measuring metabolite concentrations in jugular blood samples obtained every 2–3 days. The existence of dehydration, acid–base imbalance and renal failure was also determined from packed cell volumes, serum CO_2 content and serum concentrations of urea, creatinine and inorganic phosphate. Ewes that became recumbent and moribund with the disease were classified as susceptible whereas those asymptomatic after 10 days were classified as non-susceptible. Seven susceptible ewes had significantly higher insulin resistance indices (2043 ± 670 s.d.) than did six non-susceptible ewes (1261 ± 433 s.d.). It was concluded that poor control of glucose homeostasis may be an important predisposing factor in pathogenesis of the disease.

Introduction

Normoglycaemia in the healthy mammal is achieved through the operation of a number of feedback control mechanisms. Of these, the secretion of insulin upon ingestion of food or other hyperglycaemic stimuli and the secretion of glucagon following hypoglycaemia appear to be the most important (Bassett 1975; Brockman 1978, 1979).

The intravenous glucose tolerance test (IVGTT) is a simple technique for assessing an animal's ability to maintain glucose homeostasis through secretion of insulin. The test has been used in sheep to examine the effects of diet (Reid 1958), fasting (Boda 1964), and ketosis or ovine pregnancy toxaemia (Reid 1960) but it has not been used diagnostically as in non-ruminants to detect disorders of carbohydrate metabolism (Lundbaek 1962; Horton *et al.* 1968). The hypoglycaemia of ovine pregnancy toxaemia (Reid 1968), invariably seen as an early metabolic change preceding the development of clinical symptoms (McClymont and Setchell 1955, 1956) or fatal complications, indicates that glucose homeostasis is impaired in the underfed pregnant ewe. We hypothesized that the large variability observed between sheep in their susceptibility to ovine pregnancy toxaemia, following the withdrawal of food in late pregnancy, might be accompanied by a difference in their ability to maintain glucose homeostasis. In this report we describe the glucose tolerance and

insulin responses to an intravenous load of glucose for 16 fed, twin-pregnant sheep at about 120 days gestation. The sheep were later starved for 10 days to ascertain their susceptibility to ovine pregnancy toxaemia.

Materials and Methods

Animals and Experimental Protocol

IVGTTs were undertaken in duplicate (2 days apart) at about 120 days gestation in each pregnant ewe. The sheep, 4- and 5-year-old Perendale–Romney crossbred ewes, were mated on known dates and X-rayed at 80 days gestation. Sixteen ewes bearing twins were moved indoors from 90 days gestation and fed individually 40 g dry matter $kg^{-0.75}$ daily of a pelleted ration comprising 60% lucerne, 30% barley, 5% linseed meal and 5% molasses. The crude protein was not less than 17% of dry matter. The sheep received each day's ration in 24 hourly portions for 10 days before the IVGTT. One week before the IVGTT, a polyvinyl catheter (Deseret Pharmaceuticals, Utah, U.S.A.) was surgically implanted in a jugular vein and kept patent by flushing every 2 days with 0·9% saline (Travenol, Sydney, N.S.W.) containing 10000 units of heparin (Evans Medical Ltd, Liverpool, U.K.) and 100 mg of neomycin sulfate (Neobiotic, Upjohn Pty Ltd, N.Z.) per litre of saline.

The IVGTT procedure generally followed that of Reid (1958). The glucose load was made up as a 50% solution, sterilized by autoclaving and injected through the jugular catheter over a 1-min period. The glucose dose (0·4 g kg^{-1} live wt) was flushed in with saline and the catheter rinsed by repeated withdrawal and injection of blood.

Blood samples (5 ml) were collected 5 and 2 min before injection and at 3, 4, 5, 7, 10, 15, 20, 25, 30, 38, 45, 52, 60, 90 and 120 min after injection. They were stored on ice in tubes containing 25 mg sodium fluoride and 20 mg potassium oxalate, then centrifuged at 3000 rpm for 15 min at 4°C. Plasma was analysed immediately for glucose and a portion stored at −20°C for insulin assay.

Following the IVGTTs, at about 130 days of gestation, the ewes were starved in an open yard with free access to water to induce ovine pregnancy toxaemia. Jugular blood samples were obtained thrice weekly during the starvation period to monitor the metabolic changes of starvation. Whole blood was collected in a heparinized evacuated tube for measuring packed cell volumes, in a fluoride–oxalate evacuated tube for analysis of glucose and ketones in plasma, and in a siliconized evacuated tube for the analysis of urea, creatinine, inorganic phosphate and carbon dioxide content in serum. The plasma tubes were stored in ice pending analysis and the serum tubes were held at ambient temperature for 4 h to facilitate clotting, then stored overnight at 4°C to contract the clot.

Ewes that became recumbent with symptoms typical of ovine pregnancy toxaemia were designated susceptible (S) and those that failed to show symptoms after 10 days were regarded as non-susceptible (NS). Both groups were slaughtered for hepatocyte studies reported elsewhere (Wastney *et al.* 1978, unpublished data). Some NS ewes were found to be carrying dead foetuses *in utero* at the time of slaughter and ewe No. 330 was carrying three foetuses.

Analyses

Glucose

Glucose was measured on a Technicon Autoanalyser II by the method of Trinder (1969) with some modifications to increase the sensitivity. These included making up the enzyme solutions separately, excluding azide from the reagents (as it was found to be slightly inhibitory) and adding phenol to the reagent stream after the dialyser. Peroxidase and glucose oxidase (grade III) were from the Sigma Chemical Co. (St Louis, U.S.A.).

Packed cell volumes

Packed cell volumes were determined with a microhaematocrit centrifuge.

Ketones

Acetoacetate was measured by the Autoanalyser method of Salway (1969) with modifications (Dobbie, unpublished data) that improved specificity and sensitivity and provided stable flows in the manifold. The modifications were: (1) use of 0·05 M HCl in 1% Triton X-405 as the dialysis

recipient; (2) use of 0·20 M citrate (pH 4·8) in ethylene glycol (50% v/v), ethanol (20% v/v) and Triton X-405 (1% v/v) to buffer the reaction, ensure solubility of the chromaphore and normalize viscosity; and (3) use of pure 2,5-dichlorobenzene diazonium chloride (0·1% w/v) in 20% ethanol as colour reagent. The concentrations and flow rates of all reagents were optimized as recommended by Technicon (Anon. 1972). 3-Hydroxybutyrate was analysed by the method of Zivin and Snarr (1973) adapted for the Technicon Autoanalyser II.

Free fatty acids

Plasma free fatty acid determinations were by the semi-automated technique of Crane and Lane (1977).

Urea

Urea was measured in plasma by the standard Technicon method (method 01), based on the work of Marsh *et al.* (1965).

Creatinine

Serum creatinine was analysed by the Technicon AAII clinical method No. SE2-011FC4, which is based on the method of Chasson *et al.* (1961).

Inorganic phosphate

This was analysed by the Technicon AAII clinical method No. 4, which is based on the method of Kraml (1966).

Serum CO_2 content

Serum CO_2 content was analysed by the Technicon AAII clinical method No. 11–08, which is based on the method of Skeggs and Hochstrasser (1964).

Insulin

Plasma insulin was measured by the charcoal separation radioimmunoassay procedure of Albano and Ekins (1970) on only one of the IVGTTs undertaken on each animal. Ovine insulin was used for standards, and porcine ^{125}I-labelled insulin as tracer. Labelled insulin was prepared by the method of Hunter and Greenwood (1962), and the antisera were obtained by raising antibodies in guinea pigs to porcine insulin. Bell *et al.* (1970) showed that such guinea pig antisera reacted with ovine insulin in a manner that was almost identical to the reaction with antisera raised against ovine insulin. The coefficients of variation between six assays were 15% at 20 μU ml^{-1} and 9% at 44 μU ml^{-1}. The within-assay coefficients of variation between triplicate samples were 4·6, 21 and 21% at 4, 50 and 100 μU ml^{-1}, respectively.

Calculations

Glucose tolerance

Glucose tolerance, as the half-life ($T_{1/2}$) of the injected glucose, was calculated from linear regression of log of change of glucose from basal over the 10–60-min period.

Extravascular insulin

Sherwin *et al.* (1974) published details of a three-compartment model for insulin kinetics in man. They found that glucose utilization was not directly related to plasma insulin but was proportional to the insulin concentration in a large, slowly turning-over, third compartment, known as the extravascular compartment. Extravascular insulin levels were calculated from plasma values by solving the Sherwin model:

$$dC_3(t)/dt = -L_{13}\,C_3(t) + L_{31}\,(V_1/V_3)\,C_1(t),$$

such that,

$$C_3(t) = C_3(0)\exp(-L_{13}\,t) + L_{13}\exp(-L_{13}\,t)\int_0^t \exp(L_{13}\,\tau)\,d\tau,$$

where C_1 is plasma insulin concentration (μU ml^{-1}), C_3 is extravascular insulin concentration (μU ml^{-1}), t is time (min), L_{13} is the rate constant for the movement of insulin from the extravascular compartment (3) to the plasma compartment (1) (min^{-1}), L_{31} is the rate constant for the movement of insulin from compartment 1 to compartment 3 (min^{-1}) and V_1 and V_3 are volumes of compartments 1 and 3 (ml).

Insulin resistance index

An insulin resistance index (R) was calculated from the product of $T_{1/2}$ and extravascular insulin concentration (I'), when plasma glucose was 5·56 mM (100 mg dl^{-1}) above basal. This glucose level was on the linear part of the decay curve of glucose for all sheep. As only small quantities of glucose are excreted in the urine at this concentration (Judson and Leng 1973), the disappearance of glucose can be attributed to (1) the effect of insulin and (2) the effect of hyperglycaemia *per se* (Cherrington *et al.* 1978). The magnitude of this second effect has not been quantitated in sheep and for our purposes was assumed invariant between animals. The R value was thus considered an index of the dependence of glucose utilization on insulin concentration.

Statistical Analysis

The group means were generally compared using Students t-test. The Wilcoxon two-sample rank test was used if an F-test of the variances was significant.

Results

Classification of Ewes

Seven of the ewes were classified as susceptible (S) to ovine pregnancy toxaemia as they became recumbent and appeared moribund during the period of starvation. All these ewes carried live foetuses at the time of autopsy. The results presented in the lower part of Table 1 (S–L) provide information on the metabolic status of these sheep. All but two (Nos 309 and 344) had elevated concentrations of urea and creatinine, indicating the development of renal failure. The hyperphosphataemia in Nos 229 and 310 would also appear to be due to renal failure. Two (Nos 294 and 299) were hyperglycaemic and three (Nos 294, 299 and 356) were markedly dehydrated as indicated by the high packed cell volumes. All but two (Nos 309 and 344) had reduced serum CO_2 contents indicative of a metabolic acidosis. This pattern of clinical manifestations is typical for sheep that succumb to pregnancy toxaemia (Wolff, unpublished data).

In the non-suceptible group (NS), three of the ewes (NS-D) were found to be carrying dead foetuses. The high plasma glucose and generally lower ketone concentrations were thus expected from the decreased uteroplacental glucose uptake. There was also evidence in these three ewes of renal failure, dehydration and acid–base imbalance, which are often irreversible, even on refeeding.

Heterogeneity was also observed in the six NS ewes with live foetuses at autopsy (NS-L). Three (Nos 330, 366 and 390) had elevated urea concentrations indicative of some impaired renal function, but in none of these did creatinine approach concentrations generally found in severe renal failure (Osbaldiston 1970). Based on our previous studies (Wolff, unpublished data), all but one of these NS-L ewes could be expected to recover when turned out to fresh pasture after the 10 days of starvation. The exception (No. 390) had a low serum CO_2 content that has often been associated with non-recovery from starvation.

Table 2 shows the plasma glucose concentrations for all sheep, before and for days 3, 5 and 7 of the starvation period. Using two-way analysis of variance, no

statistically significant differences were found between any of the groups. Overall, there was a general tendency for variability between sheep to increase during the period of starvation. Although within-animal variability was comparable in the NS-D and NS-L groups ($s^2 = 0{\cdot}15$ and $0{\cdot}12$), it was higher in the SL group ($s^2 = 0{\cdot}94$). This was due mainly to the terminal hyperglycaemic values for sheep Nos 294 and 299 and eliminating these data reduced s^2 to $0{\cdot}17$. These results show that the degree of hypoglycaemia failed to indicate whether or not the ewes would succumb to toxaemia.

Table 1. Circulating metabolite concentrations and packed cell volumes of ewes before slaughter

Ewes were susceptible (S) or non-susceptible (NS) to pregnancy toxaemia with live (L) or dead (D) lambs *in utero* at slaughter. Ketones, acetoacetate plus 3-hydroxybutyrate; F.F.A., free fatty acids; P.C.V., packed cell volume; Creat., creatinine; P_i, inorganic phosphate; CO_2, serum CO_2 content. Means with shared superscripts do not differ ($P<0{\cdot}05$) based on Wilcoxon's two-sample rank test

Group and animal No.	No. of days of starvation	Glucose (mM)	Ketones (mM)	F.F.A. (mM)	P.C.V. (%)	Urea (mM)	Creat. (μM)	P_i (mM)	CO_2 (mM)
NS-L									
114	10	0·77	5·7	1·88	34	8·3	77	3·20	22·0
313	10	1·12	10·7	1·62	40	11·5	99	4·87	23·7
330	10	2·24	3·3	1·51	39	18·8	106	2·45	21·8
357	10	1·75	3·7	1·38	39	12·8	111	5·36	16·0
366	10	1·07	12·3	1·04	38	14·4	179	4·38	14·1
390	10	2·20	7·7	0·46	41	19·2	190	4·94	10·6
Mean		1·53[a]	7·2[ab]	1·32	39	14·2	127[a]	4·20[a]	18·0
s.d.		0·63	3·7	0·50	2	4·3	46	1·14	5·2
NS-D									
4	11	3·85	1·3	0·50	40	14·0	93	8·22	15·0
302	11	4·63	1·2	0·70	38	46·3	390	6·14	10·4
368	11	5·17	5·0	0·84	44	42·3	540	9·77	11·5
Mean		4·55[b]	2·5[a]	0·68	41	34·2	341[ab]	8·04[b]	12·3
s.d.		0·66	2·2	0·17	3	17·6	228	1·82	2·4
S-L									
294	5	4·29	7·7	0·87	46	18·9	247	3·27	14·8
299	8	3·84[A]	7·0[A]	0·60	45	63·0	642	10·55	7·4
309	7	1·29	10·7	1·20	32	12·7	125	5·97	18·2
310	10	1·44	2·3	0·90	30	53·0	422	10·89	15·9
344	7	2·18	7·4	1·22	31	13·6	137	5·39	21·4
356	7	1·12	9·7	0·77	46	18·7	200	5·84	12·3
363	7	1·13	17·6	1·14	33	13·5	204	5·52	10·5
Mean		2·18[ab]	8·9[b]	0·96	38	27·6	282[b]	6·78[b]	14·4
s.d.		1·48	4·7	0·24	8	21·1	187	2·84	4·7

[A] Values for day 7 of starvation are presented.

Combined foetal weight at the time of autopsy (*c.* 140 days gestational age) is also shown in Table 2. There was no indication that ewes carrying heavier foetuses were more susceptible to toxaemia.

Repeatability of the IVGTT in sheep was assessed by plotting the first estimate of $T_{1/2}$ against the second estimate on the same animal. Fig. 1 illustrates these data

for the 16 pregnant ewes plus two non-pregnant ewes, which constituted a pilot study. The 95% confidence interval about the line is ±6·7 min.

Table 2. Plasma glucose concentrations before and during starvation, and foetal weights

Group and animal No.	Plasma glucose concentration (μM)[A] −2 days	0 days	+3 days	+5 days	+7 days	Combined foetal wt (kg)
NS-L						
114	3·39	2·95	1·29	1·27	1·02	4·6
313	3·49	3·50	1·20	1·53	2·23	—
330	3·20	2·51	1·18	1·14	1·33	6·2
357	3·30	3·41	1·33	0·99	1·39	6·0
366	3·44	3·42	1·04	1·39	1·16	6·1
390	3·28	3·09	1·32	1·50	1·66	5·3
Mean	3·35	3·14	1·23	1·30	1·47	5·6
s.d.	0·11	0·38	0·11	0·21	0·44	0·7
NS-D						
4	3·31	3·33	1·79	2·23	1·71	6·4
302	2·04	2·24	1·40	1·05	1·25	6·7
368	3·61	3·52	1·70	1·44	1·33	5·1
Mean	2·98	3·03	1·63	1·57	1·43	6·1
s.d.	0·83	0·69	0·21	0·60	0·25	0·9
S-L						
294	2·94	2·33	0·98	4·29	—	6·1
299	3·47	3·33	1·35	1·40	3·84	7·8
309	2·75	2·30	—	1·02	1·29	6·3
310	3·13	3·56	1·32	0·99	1·07	5·2
344	3·03	2·91	1·45	1·17	2·18	5·0
356	2·96	3·28	1·23	1·58	1·12	4·8
363	3·15	3·31	1·37	2·39	1·13	6·7
Mean	3·06	3·00	1·28	1·83	1·77	6·0
s.d.	0·22	0·51	0·17	1·18	1·10	1·1

[A] Days are relative to start of starvation.

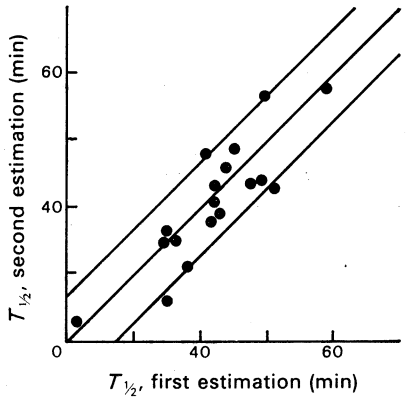

Fig. 1. Glucose tolerance tests with 95% confidence interval of ±6·7 min around the mean of two estimates of the half-life ($T_{1/2}$) of glucose.

The responses of three sheep to the IVGTT are shown in Fig. 2. The first, an S-L ewe (Fig. 2*a*), had a high insulin production but slow glucose disappearance, which resulted in a high resistance value and implied that the resistance was in the periphery.

Low resistance occurred in an NS-L ewe (Fig. 2*b*) when a high production was associated with fast glucose disappearance and in an NS-D ewe (Fig. 2*c*) when a low insulin production rate was accompanied by a very slow disappearance rate of glucose.

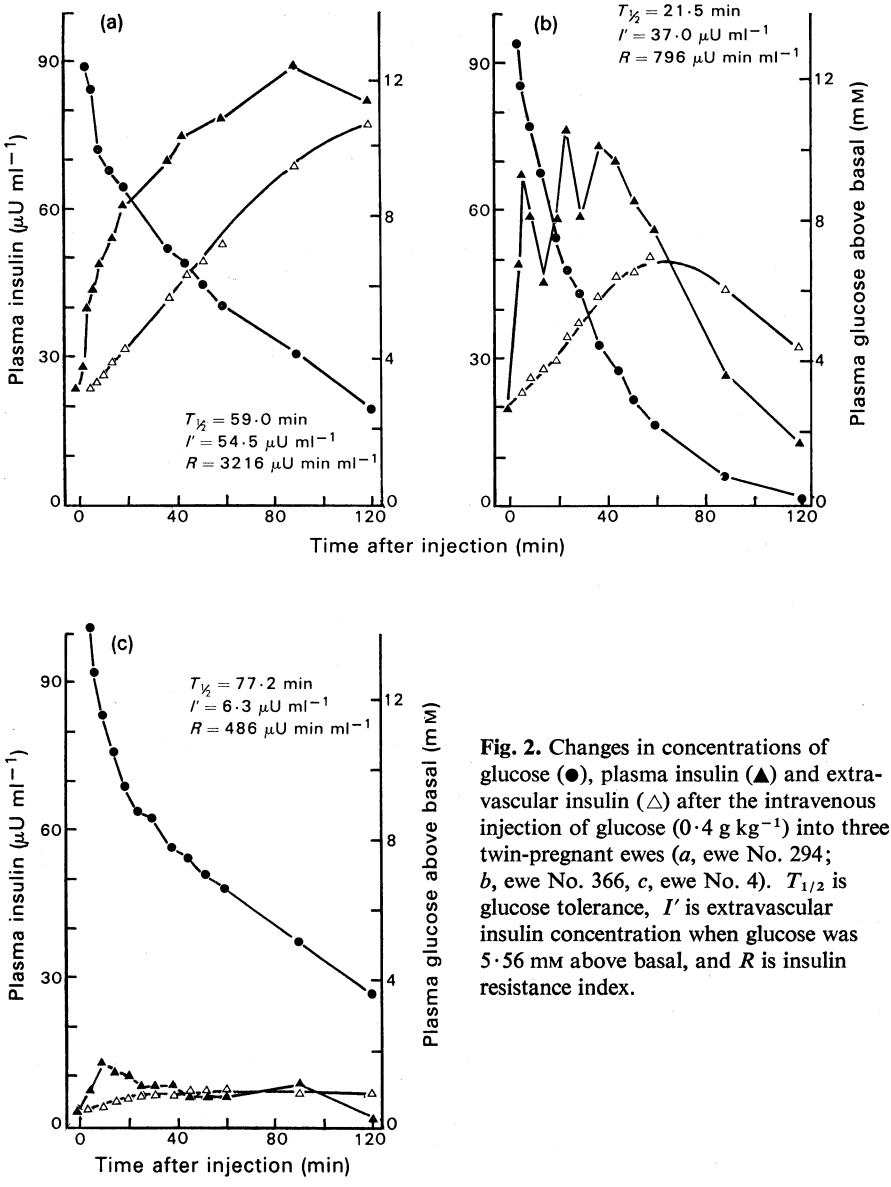

Fig. 2. Changes in concentrations of glucose (●), plasma insulin (▲) and extravascular insulin (△) after the intravenous injection of glucose (0·4 g kg^{-1}) into three twin-pregnant ewes (*a*, ewe No. 294; *b*, ewe No. 366, *c*, ewe No. 4). $T_{1/2}$ is glucose tolerance, I' is extravascular insulin concentration when glucose was 5·56 mM above basal, and R is insulin resistance index.

Pre-injection plasma glucose concentrations (Table 3) were similar in all groups as were the mean plasma insulin concentrations. The average $T_{1/2}$ value for glucose disappearance in ewes later shown to be susceptible was significantly slower (50 min) than that of NS ewes with live lambs (38 min). Thus, NS-L ewes were able to use the injected glucose more rapidly. In the NS-D group, glucose tolerance values were

scattered. One animal (No. 4) had a glucose tolerance of 77 min (equivalent to a rate constant of less than 1% per minute) and could be considered a gestational diabetic according to criteria used by Kalhan and Adam (1980) for human subjects.

The mean extravascular insulin concentrations, when plasma glucose was 5·56 mM above basal, were similar in each group, but there was large variability between animals. The insulin resistance values of the susceptible group, however, were significantly higher (2043 μU min ml^{-1}) than the NS-L group (1261 μU min ml^{-1}).

Table 3. Live weights, basal glucose and insulin concentrations, glucose tolerance and insulin resistance data of pregnant ewes

Means with shared superscripts do not differ ($P<0·05$) based on the *t*-test

Group and animal No.	Live weight (kg)	Basal concentration		IVGTT parameter[A]		
		Plasma glucose (mM)	Plasma insulin (μU ml^{-1})	$T_{1/2}$ (min)	I' (μU ml^{-1})	R (μU min ml^{-1})
NS-L						
114	50	3·70	19·0	41·8	30·5	1275
313	53	3·44	29·0	32·7	55·5	1814
330	50	2·63	17·0	43·6	37·0	1613
357	55	2·98	5·5	51·0	26·3	1341
366	54	3·43	20·0	21·5	37·0	796
390	53	3·23	7·0	34·6	21·0	727
Mean	53	3·24	16·3	37·5[a]	34·6	1261[a]
s.d.	2	0·38	8·8	10·3	12·0	433
NS-D						
4	61	2·85	3·3	77·2	6·3	486
302	53	2·28	11·5	41·8	20·5	857
368	59	3·55	19·5	35·4	51·5	1823
Mean	58	2·89	11·4	51·5[ab]	26·1	1055[ab]
s.d.	4	0·64	8·1	22·5	23·1	690
S-L						
294	58	2·81	24·0	59·0	54·5	3216
299	58	3·02	25·3	60·2	43·3	2607
309	57	3·75	10·4	44·9	27·3	1226
310	52	3·09	19·0	42·4	48·0	2035
344	51	2·96	15·5	49·3	34·5	1701
356	48	2·94	17·5	36·3	44·0	1597
363	57	3·80	17·0	59·0	32·5	1918
Mean	54	3·34	18·4	50·2[b]	40·6	2043[b]
s.d.	4	0·42	5·1	9·5	9·5	670

[A] Intravenous glucose tolerance test: $T_{1/2}$, half-life of injected glucose; I', extravascular insulin concentration; R, insulin resistance index.

Discussion

In recent years, several protocols have been devised for quantifying glucose homeostatic feedback mechanisms. Of these, the oral glucose tolerance test is inappropriate for ruminants unless the dose can be delivered to the abomasum, and the glucose clamp procedure (DeFronzo *et al.* 1979) requires equipment for rapid estimation of plasma glucose concentrations. By comparison, the intravenous glucose tolerance test is simple to conduct and is also amenable to direct interpretation.

It was, therefore, used to examine the homeostatic control mechanism in pregnant sheep.

There was considerable variability between sheep in all the parameters calculated from the IVGTT. Glucose tolerance differed between NS-L and S-L groups with the NS-D being most variable. The mean $T_{1/2}$ value of the NS-L group (38 ± 10 min) was comparable to values published by Reid (1958) for non-pregnant sheep fed lucerne roughage. Thus, pregnancy *per se* would not seem to cause glucose intolerance in sheep. Many of the S-L and NS-D ewes, however, did have impaired glucose tolerance. This could be due to a decreased rate of utilization or continued endogenous production of glucose. The former is the more likely explanation as Judson and Leng (1973) showed that an intravenous infusion of glucose suppressed gluconeogenesis.

The diagnostic value of the IVGTT and its value for predicting susceptibility to ovine pregnancy toxaemia were enhanced when the insulin response was also determined. In most sheep, plasma insulin concentrations peaked at 10 min and returned to normal after 2 h. In this respect, they were similar to the responses observed in wethers by Boda (1964) but as Fig. 2 shows, there were also substantial differences between animals in their pancreatic response to the constant glucose challenge. These differences should be quantified using a model that incorporates information on the kinetics of insulin metabolism in sheep.

Since embarking on the investigation, two publications have appeared (Cunningham and Heath 1978; Bergman *et al.* 1979) advocating that the data be used to calculate parameters of a glucose–insulin model. Our procedure is a simplification of their calculations. A key element of all methods is the use of model parameters for insulin kinetics determined by Sherwin *et al.* (1974) in human subjects. Although one can expect that procedures that use all the observed data are likely to yield better parameter estimates than those relying on portions of it, there are two considerations that vindicate our adoption of the extravascular insulin concentration when plasma glucose is 5·56 mM (100 mg dl^{-1}) above basal as the point at which to determine insulin resistance. Firstly, the extravascular insulin concentrations at this time (see Figs 2*a*, 2*b*, 2*c*) reflected the magnitude of the plasma insulin response curve. Secondly, it occurred on the portion of the plasma glucose curve that was well described by a single exponential term. For these reasons, we anticipate that the more detailed analyses will introduce only small refinements in the calculated parameters.

A real limitation of our procedure concerns the application of parameters estimated in non-pregnant humans to the pregnant sheep, but we know of no published data for a model of insulin kinetics in pregnant sheep. Insulin kinetics might also vary between animals, but in one study with pregnant human subjects (Bellmann and Hartmann 1975), no such variability was apparent between normal, gestational diabetic or normal twin-pregnant subjects.

Several causes of increased insulin resistance are well established. Obesity is frequently associated with insulin resistance in humans (Olefsky *et al.* 1974) and has been often observed as a predisposing factor in ovine pregnancy toxaemia (Reid 1968) but it would not appear to be a factor in this study as there was no significant liveweight difference between the groups and no individual ewe was excessively fat.

The antagonistic effects of placental lactogen on the action of insulin could also contribute to the resistance. In humans with gestational diabetes, Kalkhoff *et al.* (1969)

showed that the 12-h infusion of a placental lactogen preparation impaired glucose tolerance, even though plasma insulin concentrations increased. In normal pregnant women, however, the placental lactogen infusion had no effect and this suggests that placental lactogen is not alone responsible for the diabetic state. To support this, Spellacy and Buhi (1980) found no difference in glucose tolerances between twin-pregnant and single-pregnant women, despite an increased placental mass and higher circulating concentrations of placental lactogen in the twin-pregnant subjects. Thus, the twin-pregnant ewe with insulin resistance and glucose intolerance may serve as an experimental model for studying the pathogenesis of gestational diabetes.

High concentrations of adrenal corticoids might also contribute to the insulin resistance. Reid and Hogan (1959) suggested that the state of hypoglycaemic ketosis in acutely underfed sheep was analogous to an adrenal steroid diabetes, with the ewe being hypoglycaemic rather than hyperglycaemic because of the high foetal demand for glucose. This theory did not gain support when Saba *et al.* (1966) were unable to measure increased plasma cortisol in pregnant sheep with a toxaemia induced by starvation and stress.

Apart from overfatness and large foetuses (Reid 1968), there are no predictive indices of susceptibility to ovine pregnancy toxaemia. Like us, Kronfeld and Raggi (1966) observed an increased variance for plasma glucose concentrations in their toxaemic animals but as this usually arises from a terminal hyperglycaemia in some animals, its predictive value is limited. Thus, our result of an abnormal response to an IVGTT and an increased insulin resistance in susceptible ewes is a positive indication that impaired glucose homeostasis (preceding starvation) contributes to the pathogenesis of the disease. Further studies are indicated to understand the cause of the resistance.

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