Insulin action and glucose metabolism in sheep fed on dried-grass or ground, maize-based diets

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1. The effect of an exogenous supply of glucose, provided by the digestion of maize starch in the small intestine, on endogenous glucose metabolism and insulin action was studied in sheep using the euglycaemic insulin clamp procedure.

2. Insulin was infused intravenously at rates of 0·2, 0·5, 1·0 and 6·0 mU/min per kg live weight for four consecutive periods in each of four sheep fed on dried-grass and maize-based diets. Glucose was also infused intravenously at a variable rate, sufficient to maintain the plasma glucose concentration at basal levels. Whole-body rates of glucose metabolism were determined using a continuous infusion of [6-^{3}H]glucose.

3. From the resulting insulin dose-response curves, it was observed that, when the sheep were fed on the dried-grass diet, the responsiveness of glucose metabolism to insulin was less than that reported for non-ruminants.

4. When fed the maize-based diet, the glucose metabolic clearance rates (MCR) observed during insulin infusions were significantly greater ($P < 0·05$) than those observed for the dried-grass diet. However, after correcting for the non-insulin-mediated glucose disposal, differences between diets were not significant.

5. The sensitivity of glucose utilization to insulin was not affected by diet. The plasma insulin concentrations causing half-maximal insulin-mediated glucose MCR were 103 (SE 21) and 85 (SE 11) mU/l for the dried-grass and maize-based diets respectively.

6. The sensitivity of endogenous glucose production to insulin was also unaffected by diet. The plasma insulin concentrations resulting in the suppression of endogenous glucose production to half the basal level were 80 (SE 26) and 89 (SE 29) mU/l for the dried-grass and maize-based diets respectively.

7. It is concluded that the observed increase in glucose utilization on the maize-based diet was due partly to a slight change in responsiveness to insulin and also partly to a change in the rate of non-insulin-mediated glucose disposal.

The effect of insulin on glucose metabolism in ruminants differs from that in non-ruminants in that the former are less responsive to insulin with respect to hepatic production (Chandrasena *et al.* 1982; Brockman, 1983*a*; Weekes *et al.* 1983) and extrahepatic utilization of glucose (Prior & Christenson, 1978; Weekes *et al.* 1983). These differences probably result from differences in intermediary metabolism, particularly that of glucose (Etherton, 1982). The glucose requirement of the sheep is met largely by gluconeogenesis, since little glucose is normally absorbed from the digestive tract (Lindsay, 1970). However, diets containing non-heat-treated maize provide an exogenous supply of glucose through an increase in the flow of starch to the post-rumen tract (Tucker *et al.* 1966, 1968; Hogue *et al.* 1968; Ørskov *et al.* 1969; Beever *et al.* 1970). This starch is digested by the carbohydrases of the small intestine and absorbed as glucose (Huntington *et al.* 1980; Janes *et al.* 1985). The effects of an exogenous glucose supply on insulin action and endogenous glucose metabolism in ruminants is not known.

The objectives of the present study were to use the euglycaemic insulin clamp technique (De Fronzo *et al.* 1978*b*; Kolterman *et al.* 1980) to investigate the effects of an exogenous supply of glucose, provided by the digestion of maize starch in the small intestine, on insulin sensitivity and glucose metabolism in sheep. As previously employed in sheep (Weekes *et al.* 1983), use of the euglycaemic clamp technique to determine insulin dose-response curves

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requires several separate days of infusions. A modification of the original procedure used in humans involved sequential infusions of insulin, with little difference in the results obtained when compared with single infusion studies (Rizza *et al.* 1981). In the following experiment, sequential infusions of insulin were used and, in addition, a single infusion of insulin was also performed to enable the comparison of results obtained by the two methods.

A preliminary report of part of the present study has been published (Janes *et al.* 1984*b*).

METHODS

Animals and diets

Four cross-bred wethers, about 9 months of age and weighing approximately 40 kg at the beginning of the experiment were used. The experiment consisted of two dietary periods in which dried-grass or maize-based diets were fed. The composition of the diets and intakes were exactly the same as those used in a previous study to determine the amount of glucose absorbed from the small intestine (Janes *et al.* 1985). Each sheep received both diets in two experimental periods; the diets, which were randomized to periods independently for each sheep, were fed hourly by continuous-belt feeders for at least 14 d before any experiments were performed. Polyvinylchloride catheters (1·14 mm i.d. × 1·57 mm o.d.; Portex Ltd, Hythe, Kent) were inserted into both jugular veins on the day preceding an infusion.

Experimental procedure

The catheter in the right jugular vein was used for all infusions. Separate infusion lines between the pump and the catheter were used for the different infusates. Two peristaltic pumps (Watson-Marlow Ltd, Falmouth, Cornwall) were used, one set at a fixed rate (0·67 ml/min), the other calibrated for a variable infusion rate (0–3·0 ml/min). Blood samples were obtained via the catheter in the left jugular vein.

Single insulin infusion. This experiment was only performed on each of the four sheep when fed on the dried-grass diet. The procedure was similar to that used previously for sheep (Weekes *et al.* 1983). The priming dose and infusion rate of [6-^{3}H]glucose were 20 μCi and 0·2 μCi/min respectively. Insulin (Actrapid monocomponent porcine insulin; Novo Industri, Denmark) was administered as a primed continuous infusion at a rate of 6·0 mU/min per kg live weight, as described by Weekes *et al.* (1983). This rate of infusion has been shown to produce maximal responses in sheep (Weekes *et al.* 1983). The insulin infusate also contained 40 ml plasma/l to prevent adsorption of insulin to glassware and tubing, and also potassium chloride to supply 12·4 mg K^+/min to prevent hypokalaemia. Glucose distribution volume and the size of the mixable glucose pool were estimated as described previously (Weekes *et al.* 1983).

Sequential insulin infusions. The following procedure was performed on each of the four sheep when fed on the dried-grass and the maize-based diets. A primed continuous infusion of [6-^{3}H]glucose, similar to that used in the single insulin dose experiments, was commenced at −180 min. Blood samples were taken at 15-min intervals during the period −90 min to zero time for rapid determination of plasma glucose and subsequent determination of basal plasma insulin concentrations and glucose specific radioactivities. The time from zero to +8 h was divided into four 2 h periods with sequential insulin infusions at rates of 0·2, 0·5, 1·0 and 6·0 mU/min per kg live weight respectively. At the beginning of each period, a priming insulin dose, similar to that used in the single dose experiments was given. For the last three infusion rates the volume of the priming dose was reduced slightly to allow for the effect of the previous insulin infusion on the plasma insulin concentration. The concentration of the glucose infusate was, for the two lowest insulin infusion rates, 50 g/l and, for the two highest, 100 g/l. Blood samples were taken at 10-min intervals from zero

time to +8 h, the plasma glucose concentration rapidly determined and the glucose infusion rate adjusted to maintain euglycaemia.

Chemical analyses

Plasma glucose concentrations in heparinized blood samples were rapidly determined by centrifuging a small portion (0·2 ml) of the whole blood in a Beckman Microfuge (Beckman RIIC Ltd, High Wycombe, Bucks) for 45 s; 50 μl plasma were then injected into a YSI 23A Glucose Analyzer (Clandon Scientific Ltd, Aldershot, Hants). This procedure allowed the glucose infusion rate to be adjusted approximately 3 min after taking the blood sample. The remaining blood was centrifuged and the plasma stored at −20° for subsequent analysis within 3 months. A small portion of plasma was frozen separately for determination of immunoreactive insulin concentration (Fuller *et al.* 1977). The remaining plasma was deproteinized for determination of glucose specific radioactivity using the rapid ion-exchange method of Schmidt *et al.* (1975). A sample of the [6-^{3}H]glucose infusate was taken to check for any loss of activity.

Calculations and statistics

In the basal state, the glucose turnover rate (GTR) was determined from the expression:

$$\text{GTR (mg/min)} = \frac{\text{[6-}^3\text{H]glucose infusion rate (nCi/min)}}{\text{plateau specific activity (nCi/mg glucose)}}. \quad (1)$$

The basal plateau specific activity was taken as the mean of the specific activities obtained in the period −60 min to zero time. For the single insulin dose experiments only, the glucose distribution volume in steady-state was determined from the initial decay curve following the priming dose of [6-^{3}H]glucose (Steele *et al.* 1956). The 'mixable' glucose pool (Issekutz, 1981) may be obtained by applying a pool fraction term to the glucose distribution volume (Cowan & Hetenyi, 1971). A pool fraction of 0·65 has generally been assumed (Cowan & Hetenyi, 1971) although an estimated value of 0·7 has been used in sheep (Prior & Christenson, 1978). As an alternative, an approximation to the mixable glucose pool volume was obtained from the 'washout' curve of glucose specific activity after stopping the [6-^{3}H]glucose infusion as described by Weekes *et al.* (1983).

During the period +80 to +120 min of each insulin infusion, the rate of glucose infusion was relatively constant, in agreement with previous studies in sheep (Weekes *et al.* 1983) and humans (Rizza *et al.* 1981). All results reported for the insulin infusions are mean values over this plateau period. The mean glucose infusion rate over the plateau period, termed the steady-state glucose infusion rate (SSGIR), represents the sum of the insulin-induced suppression of gluconeogenesis and the insulin-induced increase in glucose utilization.

The rates of glucose appearance (RA) and utilization (RD) were calculated using non-steady-state kinetics (Cowan & Hetenyi, 1971). In calculating RA and RD, the 'mixable' glucose pool volumes obtained from the single insulin infusion experiments were used for the sequential insulin infusion experiments. No significant differences were observed in the 'mixable' glucose pool volumes obtained from the [6-^{3}H]glucose washout curves over a range of insulin infusion rates in a previous study in sheep (Weekes *et al.* 1983).

RA values during the plateau periods represent the sum of the endogenously produced glucose and the SSIGR and, thus, the endogenous glucose output can be determined by difference. When fed on the maize-based diet, the endogenously produced glucose also includes glucose absorbed from the small intestine. The value obtained previously (Janes *et al.* 1985) for glucose absorbed from the small intestine of slightly heavier sheep fed on the same maize-based diet, equivalent to 70 mg/min, was used to obtain the true endogenous *de novo* glucose production.

Table 1. *Basal plasma insulin and glucose concentrations and glucose kinetics in sheep fed on either dried-grass or maize-based diets*

(Values are means with their standard errors for four sheep)

Diet...	Dried-grass		Maize-based		Statistical significance of between-diet difference
	Mean	SE	Mean	SE	
Body-wt (kg)	39·6	3·1	43·9	1·5	NS
Plasma glucose concentration (mg/l)	805	36	798	19	NS
Plasma insulin concentration (mU/l)	47	16	27	6	NS
GTR (mg/min per kg live wt)	1·75	0·21	3·11	0·61	NS
Glucose MCR (ml/min per kg live wt)	2·2	0·3	4·0	0·9	NS
Glucose pool size (g)	3·2	0·5	ND	—	—
Glucose distribution (V; l)	4·0	0·6	ND	—	—
V as a proportion of body-wt (ml/kg)	105	23	ND	—	—

ND, not determined; GTR, glucose turnover rate; MCR, metabolic clearance rate; NS, not significant.

The glucose and insulin metabolic clearance rates (MCR) were calculated as follows:

$$\text{Glucose MCR (ml/min)} = \frac{\text{RD (mg/min)}}{\text{plateau glucose concentration (mg/ml)}}, \quad (2)$$

$$\text{Insulin MCR (ml/min)} = \frac{\text{insulin infusion rate (mU/min)}}{\text{plateau} - \text{basal insulin concentration (mU/l)}}. \quad (3)$$

Insulin-independent glucose uptake was estimated by performing a linear regression analysis of plasma insulin concentration *v.* glucose MCR and extrapolating back to zero plasma insulin concentration (Gottesman *et al.* 1983). Mean values for all sheep in the basal state and the 0·2, 0·5 and 1·0 mU/min per kg live weight insulin infusions were used.

Slight differences occurred between individual animals in the insulin infusion rate and in the plateau plasma insulin concentration at any one dose. Statistical analysis, therefore, was by two-way analysis of covariance making comparisons between sheep and diets, with plasma insulin being used as the covariate. A comparison of the results from the single insulin infusion experiments, and those from the highest rate in the sequential insulin infusion experiments when the dried-grass diet was fed, was made by two-way analysis of variance, testing differences between sheep and infusion procedure.

RESULTS

Basal state

Mean values for the basal plasma insulin and glucose concentrations and glucose kinetics for the two dietary periods are shown in Table 1. In addition, values relating to the glucose pool size and distribution volume when the sheep were fed on the dried-grass diet are also shown. Plasma glucose concentrations in all experiments were constant during the basal

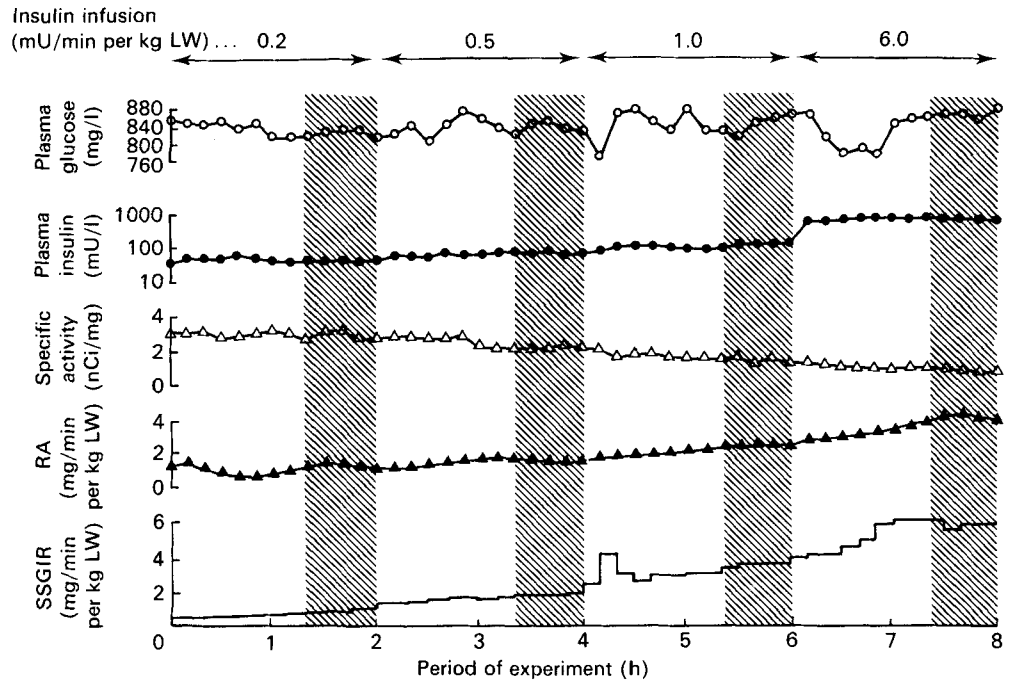

Fig. 1. A typical sequential insulin dose euglycaemic clamp experiment in one sheep fed on 1 kg dried-grass pellets/d, illustrating changes in plasma glucose (○) and insulin (●) concentrations, glucose specific radioactivity (△), rate of glucose appearance (RA) (▲) and steady-state glucose infusion rate (SSGIR) (——). [6-^{3}H]Glucose was infused (0·2 μCi/min) for 3 h before and throughout the 8 h sampling period. LW, live weight; ▨, plateau period.

period, indicating satisfactory steady-state conditions with the animals fed hourly. Basal values obtained in the sheep fed on the dried-grass diet measured on separate days were similar. Basal plasma glucose and insulin concentrations did not differ with diet. GTR, although not significantly different, was higher when the maize-based diet was given. After correction for glucose absorbed from the small intestine, the basal rate of endogenous glucose synthesis when fed on the maize-based diet was 1·40 (SE 0·66) mg/min per kg live weight. This value is lower, although not significantly so, than that of 1·75 (SE 0·21) obtained for the dried-grass diet. The glucose MCR was greater, but not significantly so, when the maize-based diet was given.

Euglycaemic insulin clamp experiments

A typical sequential insulin dose experiment with the initial 3 h basal period omitted for clarity is illustrated in Fig. 1. Euglycaemic conditions were observed during the plateau period (+80 to +120 min of each infusion) when the exogenous glucose infusion rate was fairly constant. The mean coefficient of variation for plasma glucose concentration during the plateau period for all infusions was 4·1 (SE 0·5) %, and in only two out of the thirty-two infusions did it exceed 10%. There was a tendency for exogenous glucose infusion rates to rise steadily throughout any one insulin infusion. Thus the rate at +80 min was often, but not always, slightly lower than that at +120 min; such differences were never significant.

Plasma insulin concentrations during insulin infusions were more variable than the corresponding glucose concentrations. The mean coefficient of variation for plasma insulin concentration during the plateau period for all infusions was 15·4 (SE 1·5) %. No initial under-

Table 2. *Comparison of single and sequential euglycaemic insulin clamp procedures*

(Values are the means with their standard errors for four sheep obtained during euglycaemic insulin clamp experiments when insulin was infused at a rate of 6·0 mU/min per kg live weight, either as a single infusion or as part of a sequential infusion procedure. For the latter, the insulin infusion rate of 6·0 mU/min per kg live weight was preceded by three successive 2 h periods, during which insulin was infused at 0·2, 0·5 and 1·0 mU/min per kg live weight (for further details, see p. 460).

Measurement†	Infusion procedure				Statistical significance of difference between procedures
	Single		Sequential		
	Mean	SE	Mean	SE	
Insulin infusion rate (mU/min per kg live wt)	6·1	0·3	6·1	0·9	NS
Plasma glucose concentration (mg/l)	721	36	805	29	*
Plasma insulin concentration (mU/l)	633	90	634	122	NS
Steady-state glucose infusion rate (mg/min per kg live wt)	3·44	0·31	4·59	0·49	*
Rate of glucose utilization (mg/min per kg live wt)	3·52	0·71	3·91	0·26	NS
Rate of glucose appearance (mg/min per kg live wt)	3·52	0·71	3·91	0·26	NS
Endogenous glucose production (mg/min per kg live wt)	0·08	0·40	−0·68	0·32	NS
Glucose metabolic clearance rate (ml/min per kg live wt)	5·1	1·2	4·9	0·2	NS
Insulin metabolic clearance rate (ml/min per kg live wt)	10·0	1·0	12·4	3·7	NS

NS, not significant.
* $P < 0·05$.
† Calculated over the plateau period between 80 and 120 min of insulin infusion.

or overshoot was observed when the priming dose was given at the start of each insulin infusion.

The 'mixable' pool volume, calculated from the glucose specific activity washout curves in the four sheep fed on the dried-grass diet, was 2·49 (SE 0·26) litres.

Effect of antecedent insulin infusions. The influence of sequential insulin infusions on the maximal responses observed with an insulin infusion of 6·0 mU/min per kg live weight can be seen in Table 2. Plateau plasma insulin concentrations and insulin MCR in the two infusion procedures were not significantly different. The SSGIR required to maintain euglycaemia was significantly ($P < 0·05$) higher in the sequential, compared with the single infusion experiments. However, the plateau plasma glucose concentration was also significantly ($P < 0·05$) higher during the sequential infusions. For both infusion procedures, the difference between the basal and plateau period plasma glucose concentrations was not significant. However, in the sequential insulin dose experiments the mean plateau glucose concentration was slightly higher, and in the single insulin dose experiments it was slightly lower than basal. The small, non-significant, difference in basal plasma glucose concentration was, therefore, amplified during the plateau period.

RDs were greater, but not significantly so, in the sequential insulin dose experiments. The difference may have been due to the different plasma glucose concentrations. The glucose MCR provides a more valid index of insulin-induced glucose utilization when plasma glucose concentrations differ slightly. The glucose MCR were very similar for the two

Table 3. *Plasma insulin and glucose concentrations and insulin metabolic clearance rates during euglycaemic insulin clamp experiments on sheep fed on dried-grass and maize-based diets*

(Values as means with their standard errors for four sheep)

Measurement†	Insulin infusion rate (mU/min per kg live wt)	Diet: Dried-grass Mean	Dried-grass SE	Maize-based Mean	Maize-based SE	Statistical significance of difference between diets
Plasma insulin concentration (mU/l)	0·2	49	9	51	6	NS
	0·5	67	11	81	13	NS
	1·0	110	19	137	25	NS
	6·0	634	122	804	153	NS
Plasma glucose concentration (mg/l)	0·2	790	37	792	15	NS
	0·5	791	36	769	17	NS
	1·0	805	31	809	25	NS
	6·0	805	29	771	20	NS
Insulin metabolic clearance rate (ml/min per kg live wt)	0·2	45·1	29·2	9·2	1·6	NS
	0·5	15·4	7·3	10·9	2·2	NS
	1·0	11·0	6·1	11·3	2·7	NS
	6·0	12·4	3·7	8·7	1·8	NS

NS, not significant.
† Calculated over the plateau period between 80 and 120 min of insulin infusion.

Table 4. *Steady-state glucose infusion rates and glucose appearance rates during euglycaemic insulin clamp experiments on sheep fed on dried-grass or maize-based diets*

(Values are means with their standard errors for four sheep)

Measurement†	Insulin infusion rate (mU/min per kg live wt)	Diet: Dried-grass Mean	Dried-grass SE	Maize-based Mean	Maize-based SE	Statistical significance of difference between diets
Steady-state glucose infusion rate (mg/min per kg live wt)	0·2	0·75	0·06	0·75	0·15	NS
	0·5	1·45	0·25	1·63	0·40	NS
	1·0	2·37	0·54	2·95	0·21	NS
	6·0	4·59	0·49	5·12	0·24	NS
Rate of glucose appearance (mg/min per kg live wt)	0·2	1·82	0·22	3·49	0·77	NS
	0·5	2·18	0·22	4·05	0·42	*
	1·0	2·36	0·23	5·16	0·75	**
	6·0	3·91	0·26	6·04	0·54	*

NS, not significant.
* $P < 0·05$, ** $P < 0·01$.
† Calculated over the plateau period between 80 and 120 min of insulin infusion.

infusion procedures, indicating that the observed differences in RD were a result of the different plasma glucose concentrations.

During the infusion of insulin at 6·0 mU/min per kg live weight, the rate of exogenous glucose infused was often greater than the calculated RA. This clearly must represent experimental error, probably in the determination of RA, although errors involved in

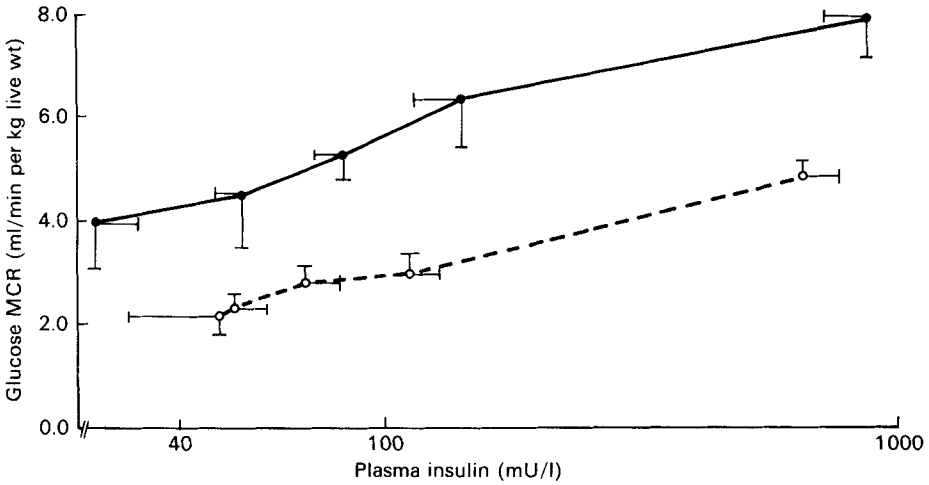

Fig. 2. The effect of diet on insulin dose-response curves for the glucose metabolic clearance rate (MCR). The points are mean values with their standard errors represented by vertical bars for four sheep fed on dried-grass (○- - -○) or maize-based (●—●) diets.

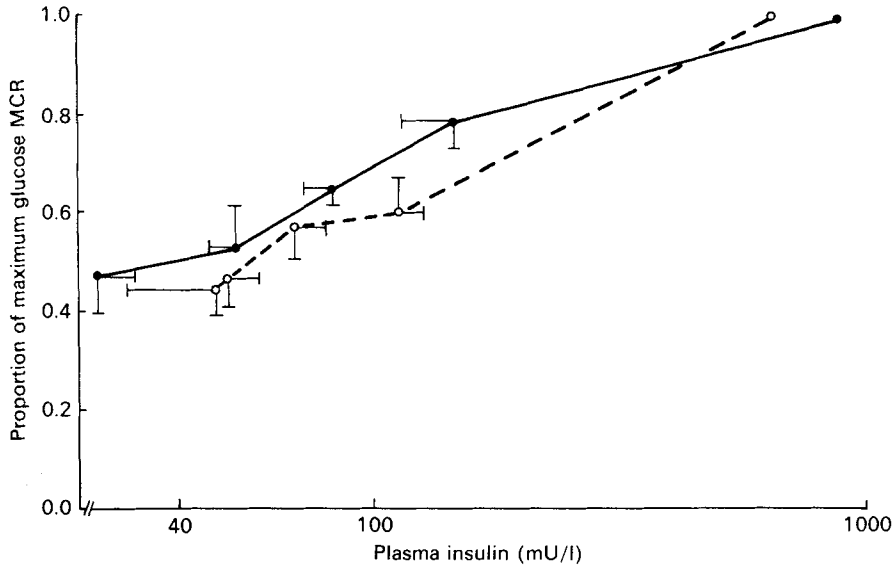

Fig. 3. The effect of diet on insulin dose-response curves for the glucose metabolic clearance rate (MCR) expressed as a percentage of the observed maximum. The points are mean values with their standard errors represented by vertical bars for four sheep fed on dried-grass (○- - -○) or maize-based (●—●) diets.

measuring the SSGIR may also contribute. The resulting negative endogenous glucose production rates, obtained from the difference between the SSGIR and RA, occurred in both the single and sequential insulin infusions. There was no significant difference between the infusion procedures for the rate of endogenous glucose production. In calculating endogenous glucose produced as a proportion of the basal rate when insulin was infused, the negative values were taken as zero. In most cases, complete suppression of basal endogenous output occurred, and there was no significant difference between infusion

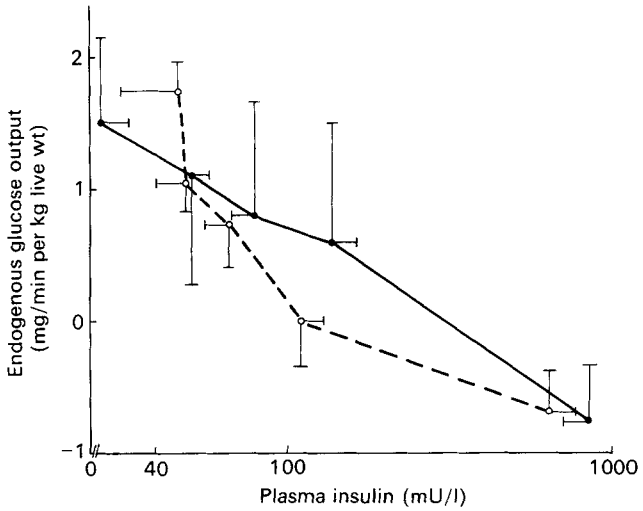

Fig. 4. The effect of diet on insulin dose-response curves for the rate of endogenous glucose output, calculated from the difference between rate of glucose appearance (RA) and the steady-state glucose infusion rate; when sheep were fed on the maize-based diet, a value of 70 mg/min was also subtracted from the RA to allow for glucose absorption from the small intestine (for further details, see p. 461). The points are mean values with their standard errors represented by vertical bars for four sheep fed on dried-grass (○- - -○) or maize-based (●—●) diets.

procedures for the amount of endogenous glucose produced as a proportion of the basal rate.

Effect of diet. Although there were slight differences in the rate of insulin infusion, the plasma insulin concentrations at all insulin infusion rates did not differ significantly between diets (Table 3). The insulin MCR was not significantly affected by either insulin infusion rate or diet. The effects of diet and the insulin infusion rate on the SSGIR and glucose RA are shown in Table 4. Assuming that maximal responses were observed at an insulin infusion rate of 6·0 mU/min per kg live weight, there was no significant between-diet difference in the responsiveness to insulin with respect to the SSGIR. Also, diet had no significant effect on the plasma insulin concentration resulting in half-maximal SSGIR; such values, obtained from individual curves for SSGIR *v.* log insulin concentration, were 100 (SE 24) and 111 (SE 10) mU/l for the dried-grass and maize-based diets respectively. Glucose RA was significantly higher when the sheep were fed on the maize-based diet for insulin infusion rates of 0·5 ($P < 0·05$), 1·0 ($P < 0·01$) and 6·0 ($P < 0·05$) mU/min per kg live weight. A large contribution to this effect must represent glucose absorption from the small intestine.

The effect of diet on glucose utilization, independent of slight, non-significant, differences in plasma glucose concentration, is shown by plotting glucose MCR *v.* plasma insulin concentration (Fig. 2). Clear differences, significant at insulin infusion rates of 0·5 ($P < 0·05$), 1·0 ($P < 0·05$) and 6·0 ($P < 0·01$) mU/min per kg live weight, can be seen between the two diets. The shape of the dose-response curve for glucose MCR, independent of the maximal glucose MCR, is provided by expressing glucose MCR as a proportion of the observed maximum (Fig. 3). At all insulin infusion rates, except 6·0 mU/min per kg live weight, the proportion of the maximal glucose MCR was slightly higher, although not significantly so, when the sheep were fed on the maize-based diet. The half-maximally-effective plasma insulin concentration was obtained from individual curves for the proportion of the maximal glucose MCR *v.* log insulin concentration; mean values were 55 (SE 19) and 32 (SE 9) mU/l for the dried-grass and maize-based diets respectively. These values assume that

the basal glucose MCR is entirely insulin-mediated. Linear regression of plasma insulin concentration *v.* glucose MRC for the dried-grass (r 0·973, $P < 0·05$) and maized-based (r 0·998, $P < 0·01$) diets indicated that at zero plasma insulin concentration, RDs were 1·44 and 2.56 mg/min per kg live weight respectively (insulin-independent glucose utilization rates). When expressed as a proportion of basal glucose utilization, values of 0·82 and 0·85 were obtained. Thus, assuming that insulin-independent glucose utilization values are 0·82 and 0·85 of the total basal rate, insulin-dependent glucose MCR was calculated by difference. The plasma insulin concentrations causing half-maximal stimulation of insulin-dependent glucose MRC were thus 135 (SE 34) and 111 (SE 7) mU/l for the dried-grass and maize-based diets respectively; the difference between diets was not significant. After making allowance for the non-insulin-mediated glucose disposal, the maximal glucose MCR was still greater when the maize-based diet was given but the difference between diets was not significant.

Although there were no significant effects of diet, endogenous glucose production rates (Fig. 4) tended to fall more rapidly with increasing plasma insulin concentration when the dried-grass diet was fed. The plasma insulin concentrations which resulted in the suppression of endogenous glucose production to half basal levels were 80 (SE 26) and 89 (SE 29) mU/l for the dried-grass and maize-based diets respectively.

DISCUSSION

Effect of sequential insulin infusions

The use in sheep of sequential insulin infusions in euglycaemic insulin clamp experiments has not previously been validated. In the present study, differences in SSGIR obtained in single or sequential infusion experiments could be attributed to slight differences in plasma glucose concentration. When glucose concentrations differ slightly, the glucose MCR, rather than the SSGIR, can be used to compare the results obtained from in vivo insulin clamp studies (Doberne *et al.* 1982). In man, the use of sequential insulin infusions has been questioned, since the rate of exogenous glucose infusion required to maintain euglycaemia and glucose MCR continue to increase during a prolonged infusion (Doberne *et al.* 1981). Although a similar tendency was apparent between +80 and +120 min of insulin infusion in the present study, glucose MCR in the single and sequential infusions were very similar. Thus, the use of sequential insulin infusions in sheep appears valid.

Effect of diet on glucose production and utilization

The mean basal GTR determined in the sheep when fed on the dried-grass and maize-based diets are similar to those obtained in a previous study using the same techniques (1·76 and 2·42 mg/min per kg live weight respectively; Janes *et al.* 1985). A greater GTR in sheep fed on maize-based, compared with roughage-based diets, has been reported previously (Evans & Buchanan-Smith, 1975; Huntington *et al.* 1980; Janes *et al.* 1985). These differences are due to the absorption of large amounts of glucose from the small intestine when non-heat-treated maize is fed to sheep (Janes *et al.* 1985).

The glucose distribution volume as a function of body-weight determined in the sheep fed on the dried-grass diet is lower than published values of 180–240 ml/kg (White *et al.* 1969; Weekes *et al.* 1983). The rapidly mixing glucose pool volume was also less than that reported by Weekes *et al.* (1983), and was equal to the volume obtained by applying a pool fraction term of 0·65 to the total glucose distribution volume. This fraction is lower than the range of 0·81–0·84 determined by Weekes *et al.* (1983) using similar techniques, but is similar to the value of 0·7 assumed by Prior & Christenson (1978).

The shape of the insulin dose-response curve, with changes in either the maximal response

(change in responsiveness) or the rate at which the maximal response is reached (change in sensitivity), has been used to characterize insulin action (Kahn, 1980). In the present study, maximal SSGIR observed in the sheep when fed on the dried-grass diet are similar to those obtained in sheep fed on lucerne (*Medicago sativum*) and kept in a warm environment (Weekes *et al.* 1983). They are however, lower than comparable values obtained in humans (Kolterman *et al.* 1980; Rizza *et al.* 1981), pigs (Chandrasena *et al.* 1982) and in sheep maintained in a cold environment (Weekes *et al.* 1983). No change in responsiveness was observed when the sheep were fed on the maize-based diet. These results confirm that ruminants may be regarded as being less responsive to insulin in terms of glucose metabolism than non-ruminants. Weekes *et al.* (1983) suggested that the sensitivity to insulin was similar in humans and sheep, with half-maximal SSGIR achieved at insulin concentrations of approximately 50 mU/l. In the present study, values obtained for both diets are approximately twice this value, suggesting that, in addition to being less responsive, sheep are also less sensitive to insulin compared with non-ruminants. Such differences enable the ruminant animal to conserve glucose by minimizing insulin-stimulated catabolism.

The rate of endogenous glucose production, calculated from the previously determined rate of glucose absorption from the small intestine (Janes *et al.* 1985), was lower on the maize-based diet compared with the dried-grass diet. The findings of Huntington *et al.* (1980) suggest an increased rate of endogenous glucose production in lambs fed on a maize-based concentrate, compared with lucerne hay. Differences in the techniques used to measure glucose absorption from the small intestine in these studies may be important (Janes *et al.* 1984*a*). The process by which endogenous glucose production is inhibited in ruminants with an exogenous supply of glucose available is not clear. In humans, hepatic glucose production is effectively controlled by the plasma insulin concentration (Rizza *et al.* 1981). Hepatic sensitivity to insulin in ruminants compared with non-ruminants is generally low (Brockman *et al.* 1975; Weekes *et al.* 1983), although Brockman (1983*a*) suggested that the sheep liver was only slightly less sensitive to insulin than the human liver. In the present study, the plasma insulin concentrations resulting in the suppression of endogenous glucose production to half basal levels (80–89 mU/l) are greater than those reported for humans (approximately 22–25 mU/l; Rizza *et al.* 1981; Kolterman *et al.* 1980) and are probably not within the physiological range for sheep. Also, basal plasma insulin concentrations when the maize-based diet was given were slightly lower than when the dried-grass diet was given. These results support the suggestion that plasma insulin concentration does not play a major role in controlling endogenous production of glucose in sheep. Glucose production is also inhibited by hyperglycaemia (Prior & Christenson, 1978, Brockman, 1983*b*) although, in the present study, peripheral plasma glucose concentrations were not affected by diet. The glucose concentration in the blood perfusing the liver may be more important and this is likely to have been slightly greater when the maize-based diet was given.

The difference between diets in the maximal glucose MCR suggests an increased responsiveness to insulin in terms of glucose utilization when the maize-based diet was given. However, part of the observed differences between diet in glucose MCR appeared to be due to a change in the rate of non-insulin-mediated glucose disposal, which was greater when the maize-based diet was given. There was, therefore, only a slight, non-significant increase in the responsiveness of insulin-mediated glucose MCR to insulin when the maize-based diet was given.

The proportion of basal glucose utilization calculated to be non-insulin-mediated is similar to that derived from the values of Brockman (1983*a*) for sheep fasted for 24 h (0·89) and to the range of 0·75–0·85 reported in humans fasted overnight (Gottesman *et al.* 1983). The processes responsible for insulin-independent glucose utilization are not established for

ruminants and the reason for an increased rate of utilization when given the maize-based diet is also uncertain. In non-ruminants, glucose uptake by the brain is independent of insulin (Hom *et al.* 1984) and has been suggested as accounting for approximately 74% of the rate of non-insulin-mediated glucose disposal in humans (Gottesman *et al.* 1983). In sheep, the maximal glucose uptake by the entire central nervous system is probably only 0·2 mg/min per kg live weight (Lindsay, 1979) which in the present experiment would account for only 8–14% of the rate of insulin-independent glucose utilization. This suggests that, unlike in humans, tissues other than the central nervous system account for a large proportion of the rate of non-insulin mediated glucose disposal in sheep. It is unlikely that glucose uptake by the central nervous system was increased when the maize-based diet was given.

Diet is more likely to influence glucose metabolism by the splanchnic bed. In humans, splanchnic glucose uptake is greater after oral than after intravenous glucose administration (De Fronzo *et al.* 1978*a*). Under euglycaemic conditions, splanchnic glucose uptake is not increased by hyperinsulinaemia (De Fronzo *et al.* 1983). This would be interpreted as insulin-independent glucose uptake using the procedure of Gottesman *et al.* (1983), although insulin is required for hyperglycaemia to stimulate splanchnic glucose uptake (De Fronzo *et al.* 1983). The combination of a substantial amount of absorbed glucose and a moderately increased glucose concentration in the blood perfusing the liver may, therefore, have been responsible for an apparent increase in insulin-independent glucose uptake when the maize-based diet was given. A limitation to the calculation of insulin-dependent glucose utilization is the assumption that there is a linear relation between insulin concentration and glucose utilization, which may not be valid at very low plasma insulin concentrations. However, in vitro studies with muscle and adipose tissue from non-ruminant species suggest that glucose uptake is linearly related to insulin concentration over the range 0–25 mU/l (Ciaraldi *et al.* 1979; Le Marchand-Brustel & Freychet, 1979).

The sensitivity of glucose utilization to insulin was not affected by diet. The values obtained for the plasma insulin concentration causing half-maximal insulin-mediated glucose MCR are greater than those reported previously for sheep, when basal glucose MCR was assumed to be totally insulin-independent (42 mU/l; Weekes *et al.* 1983). The values obtained in the present study are probably not within the physiological range for sheep, indicating a limited role of insulin in glucose disposal.

We conclude that the increased utilization of glucose observed when sheep were fed on a maize-based diet was not mediated by a change in the sensitivity to insulin and was only partly due to a slight increase in the responsiveness to insulin. It has been suggested that the absence of glucose absorption from the small intestine of sheep fed on roughage diets may cause a lower tissue sensitivity to insulin in comparison with non-ruminants (Etherton, 1982). An increase in the amount of glucose absorbed from the small intestine when the sheep were fed on the maize-based diet was unable to increase the effectiveness of the actions of insulin on glucose metabolism to that observed in non-ruminants.

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