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Twin-pregnancy increases susceptibility of ewes to hypoglycaemic stress and pregnancy toxaemia

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Abstract

Pregnancy toxaemia is a metabolic disorder with a high mortality rate and occurs in twin-bearing ewes in late gestation. Maternal hypoglycaemia is a characteristic symptom of the disease and has been attributed to an increase in glucose uptake by the twin-bearing uterus. The possibility that a reduced maternal glucose production rate might cause hypoglycaemia, has received little attention in the past. It was the aim of this study to investigate this explanation as a possible alternative. Six ewes were sequentially subjected to two types of hypoglycaemic stress, firstly by fasting for 14 h and secondly through induction of moderate hyperketonaemia. Glucose kinetics were assessed in each animal during the dry non-gestational period, during late gestation, and during early lactation. Application of these stress factors was associated with variation of plasma glucose concentration from 4.9 to 0.87 mmol L^{-1}.

The plasma glucose concentration was always significantly related to the glucose production rate. The greatest stress-induced reductions in glucose concentration and glucose production rate were seen during late gestation in twin-bearing ewes. The decline in the glucose production rate after an overnight fast and during induced hyperketonaemia was greater in twin-bearing ewes than in single-bearing ewes (59% and 43%, respectively, $p < 0.05$). The stress conditions resulted in the lowest levels of glucose concentration and glucose turnover rates in the stressed, hyperketonaemic, late gestation twin-bearing ewes. This illustrates that the glucose homoeostatic system of ewes bearing twins is significantly more susceptible to hypoglycaemic stress than that of ewes bearing single lambs. These findings also show that the primary cause of hypoglycaemia in late gestation twin-pregnant ewes is an increased susceptibility to a stress related reduction in glucose production rate. This metabolic condition leaves the twin-pregnant ewe predisposed for the development of pregnancy toxaemia.
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1. Introduction

Pregnancy toxaemia is a well-known metabolic disorder of glucose and fat metabolism in sheep and goats, which usually occurs spontaneously in the last three weeks before parturition. Ewes with multiple foetuses are mostly affected (Reid, 1968). The disease is associated with low plasma concentrations of glucose and markedly increased plasma concentrations of ketone bodies (Bickhardt et al., 1993; Scott et al., 1995; Sigurdsson, 1988; Van Saun, 2000). It is believed that the disorder is caused by an inability of the twin-pregnant ewe to meet the increased glucose demand of the uteroplacental unit (Reid, 1968; Rook, 2000). However, this theory does not explain why the disorder occurs during late gestation rather than during early lactation? Energy intake and glucose turnover are 40–100% greater during early lactation than during late gestation (Bergman et al., 1974; Perry et al., 1994; Wilson et al., 1983). This should make early lactating ewes more predis-

Abbreviations: BHB, β-hydroxybutyrate; D-BHB or DL-BHB, D- or DL-β-hydroxybutyrate.

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posed to ketosis than periparturient gestating ewes, as is the case with early-lactating and pregnant dairy cows. Similarly, the mortality rate from ovine pregnancy toxaemia is considerably higher than that of bovine ketosis and the efficacy of treatment is much lower even though the haematologic and metabolic features of the two conditions are similar (Henze et al., 1995; Sargison et al., 1994). Another noteworthy aspect of pregnancy toxaemia in sheep is that long-term underfeeding of twin-bearing ewes during late gestation is often tolerated and does not necessarily result in pregnancy toxaemia (Bergman et al., 1974; Leng, 1965). Under field conditions, the disease occurs sporadically and unpredictably even if good quality feed should ensure the availability of sufficient nutrients (Bickhardt et al., 1993; Reid, 1968). Still there is no doubt that a relative shortage of glucose availability accompanies pregnancy toxaemia. However, the general accepted view, that the ewe is over strained and develops pregnancy toxaemia due to an increasing supply of glucose to the growing uterus and conceptus appears to be a conclusion, drawn by analogy to ketotic dairy cows. From experimental data there is good evidence that foetal energy consumption and growth are related to the availability of nutrients, especially glucose (Clapp, 2006). It is also worth noting that the supply of glucose to the late-pregnant uterus is not increasing as dramatically as to the lactating mammary gland during early lactation. These observations lead to the question whether an impaired maternal glucose production contributes to the onset of hypoglycaemia and development of pregnancy toxaemia rather than an increased supply of glucose to the pregnant uterus. This assumption implies that the primary cause of pregnancy toxaemia is not due to an inadequately high foetal glucose consumption but rather a shortage of maternal glucose production possibly caused by an impaired maternal glucose homoeostatic system. In our study, isotope dilution techniques with labelled glucose were used to estimate glucose production and disposal in sheep pregnant with single or twin-lambs.

2. Materials and methods

2.1. Experimental animals

Six healthy, non-ketotic ewes (80 ± 8.0 kg) were purchased from a local breeder. The breeder was chosen, because of the superior general health status of his herd, as known from the Clinics for Pigs and Small Ruminants which is also located at the School of Veterinary Medicine in Hannover. The herd was farmed for mutton production for which a seasonal reproductive cycle was enforced. Selection criteria for the ewes were a good body condition score and an age of three years or older. The selection criterion "age" was chosen because we assumed that older animals were more susceptible to metabolic stress. Owing to the fact that the breeds "Texel" and "German Blackface" do not differ with respect to breeding performance and incidence rates of pregnancy toxaemia, breed of the animals was not a selection criterion. The mean number of lambs born per ewe for the German Blackface and Texel ewes were 1.68 and 1.69, respectively and their mean body weights at 105 days of age were 44.9 kg and 39.5 kg, respectively (Mendel and Zindath, 2004). There was also no difference in the incidence of pregnancy toxaemia between German Blackface and Texel ewes (9.5% and 8.6%, respectively; Bickhardt et al., 1998). Three of the selected animals were Texels and three were German Blackfaces. The gestational ages and numbers of foetuses were determined by ultrasonography before the beginning of the study. By chance, the three Texel ewes carried twins and the three German Blackface ewes carried singletons. The sheep were held in two group pens (3.5 m × 4 m, one pen for each breed) on straw bedding. Good quality hay containing 10.9% crude protein, 8.2% ash, and 8.7 megajoules metabolizable energy per kg dry matter was available *ad libitum*. Water was available at all times. During the last six weeks of pregnancy, the feed was supplemented twice daily with concentrate (Club-Schafkraft, Club Kraftfutterwerke Nord, 21107 Hamburg, Germany) containing 17% crude protein, 1.5% Ca, 0.5% P, and 10.2 megajoules metabolizable energy per kg of feed. Ewes bearing twins received 1000 g of concentrate per day, and ewes bearing singletons received 500 g of concentrate per day. The supplements were offered in two equal portions at 09:00 and 15:00. Food was withheld from about 17:00 on the day before the main experiments started until the end of the experiment (about 13:00 on the next day). The ewes had fasted for about 15 h before the experiments began (at 8:00).

Experimental animals, each with a companion animal, were transferred from their pens to the laboratory 1 h before the beginning of the experiments. Each experiment consisted of two phases in which glucose metabolism was measured, i.e., after an overnight fast and during induced hyperketonaemia. Hyperketonaemia depressed the plasma glucose concentration as shown by other investigators (Bergman et al., 1963; Kammula, 1976; Radcliffe et al., 1983). Six experiments of this kind were carried out with each animal; two during the dry non-gestating period (four weeks after their lambs were weaned at 100 d or more after parturition); two during late pregnancy (10 ± 8 d ante-partum; term = 147 ± 3 d); and two during early lactation (two to four weeks after lambing). This resulted in 36 experiments: 12 in the dry season, 12 during late gestation, and 12 in early lactation.

In the morning on the day before the experiments started, two indwelling polyethylene catheters (ID = 0.86 mm) were placed into both jugular veins. A venous blood sample was taken at about 17:00. The catheters were flushed, filled with saline (0.9% w/v NaCl) containing 750 U ml^{-1} heparin (U.S.P. XXI, Carl Roth, 76185 Karlsruhe, Germany), closed, and then secured to a bandage with adhesive tape that had been placed around the neck. One catheter was used for sampling and the other was used for infusion. The animal experiments were approved by the ethical committee on animal rights protection of the Hannover District

Government in accordance with German legislation on animal rights and welfare.

2.2. Experimental design

The objective of the study was to measure glucose concentration in plasma and glucose turnover after moderate hypoglycaemic stress consisting of an overnight (15–16 h) fast and subsequent induction of hyperketonaemia. Hyperketonaemia was induced by a continuous intravenous infusion of a DL-β-hydroxybutyrate (DL-BHB) solution for 3 h, which increased the D-BHB concentration to 3.7–5.7 mmol L^{-1}.

The rates of BHB infusion required to increase D-BHB levels varied between animals and between reproductive states. The required doses were determined from the results of a BHB tolerance test carried out on the day before the main experiment (from 10:00 to 13:00). For the tolerance test, a DL-BHB racemate solution containing 1.2 mol L^{-1} D-BHB was infused for 3 h, during which the infusion rate was increased eight times in a stepwise manner every 20 min. For the late gestation ewes, the initial D-BHB infusion rate was 8 μmol kg^{-1} min^{-1} and was incremented in steps of 2.5 μmol kg^{-1} min^{-1}. For the dry and lactating ewes, the initial D-BHB infusion rate was 11 μmol kg^{-1} min^{-1} and the increments were 3.0 μmol kg^{-1} min^{-1}. After each 20 min infusion period, a 4.0 ml blood sample was collected into heparinized test tubes for measurement of D-BHB concentration. Plots of plasma D-BHB concentrations against their corresponding D-BHB infusion rates were used to determine the D-BHB infusion rates required to elevate plasma D-BHB concentrations to the desired level for the main experiment.

Each main experiment started with a 60 min adaptation period and lasted for 300 min (Fig. 1). All blood samples (5.0 ml) were collected into heparinized tubes (Na-Heparin Monovetten®, Fa. Sarstedt, 51588 Nürmbrecht, Germany). Two blood samples were drawn via the catheters at the beginning and end of the adaptation period ($t = 0$, $t = 60$ min) for measurement of plasma glucose concentrations. For measurement of glucose turnover, a bolus of 1.85 MBq D-2-[^{3}H]-glucose in 5 ml sterile 10% glucose solution was administered 60 min after the start of the experiment. Six serial blood samples (5.0 ml) were then collected at 70, 80, 90, 100, 110, and 120 min after the start of the experiment.

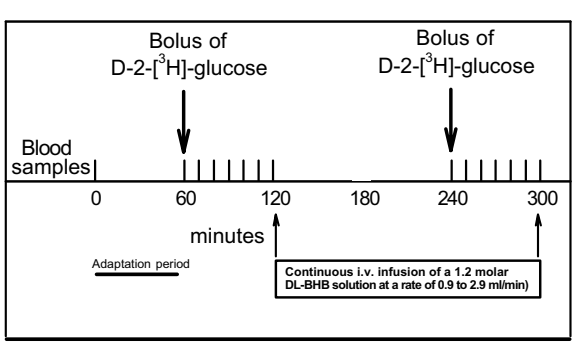

Fig. 1. Schematic representation of the experimental design.

At the end of this period ($t = 120$ min), hypoglycaemia was induced by a continuous 3 h ($t = 120$–300 min) intravenous infusion of 1.2 mol L^{-1} D-BHB using a peristaltic pump (Ismatic MV-CA4, 97877 Wertheim Monfeld, Germany). The previous day the solution had been autoclaved, filtered and the pH adjusted to 7.4. The solution was stored at 4 °C until used. The infusion rate was monitored by continuous weighing of the reservoir containing the infusate. Because of differences in BHB tolerances and body weights between animals, infusion volumes varied from 0.9 to 2.9 ml min^{-1}. After 2 h of DL-BHB infusion (at $t = 240$ min), glucose concentrations were decreased significantly. At this time, a second dose of 1.85 MBq of 2-[^{3}H]-glucose was administered to determine glucose turnover during the induced hypoglycaemia. In this second phase of the experiment glucose and D-BHB concentrations were determined in samples at 120, 240, 260, 280, and 300 min after the start of the experiment. Radioactivity of the glucose tracer was determined in plasma samples at 250, 260, 270, 280, 290, and 300 min after the start of the experiment. Blood samples were immediately placed on crushed ice and the plasma was separated at the end of the experiment by centrifugation at 3000*g* for 10 min at 4 °C. The plasma samples were divided into three sub-samples and stored at −20 °C until analysis.

2.3. Laboratory methods

The concentration of D-BHB was determined using the method of Williamson et al. (1962) after deproteinization with 1 M $HClO_4$, centrifugation, neutralization with 1 M K_3PO_4, and subsequent centrifugation. The intra-assay coefficient of variation of this method was 4.6 ± 3.9% (mean ± SD, $n = 160$) for duplicate measurements. The inter-assay coefficient of variation for the standard solution was 5.5 ± 4.6% (mean ± SD, $n = 53$). The glucose concentration in the plasma was determined with a reflection photometer (Reflolux®, Roche, 68305 Mannheim, Germany). Test experiments in our laboratory showed that glucose concentrations estimated using this method differed by <5% ($n = 40$) from those estimated using the conventional photometric Glucose-Perid® method (Roche Diagnostics, 68305 Mannheim, Germany). For measurement of D-2-[^{3}H]-glucose radioactivity, 1 ml plasma was deproteinized following the Somogyi (1952) procedure. This involved the addition of 0.25 M $Ba(OH)_2$, followed by 5.65% $ZnSO_4$ and centrifugation at 4 °C for 20 min at 3000*g*. The supernatants were decanted into scintillation vials, lyophilized to remove [^{3}H]-H_2O, dissolved in 2.0 ml deionized water, and mixed with 14 ml of a scintillation cocktail (Hydroluma™, Baker, 64521 Groß-Gerau, Germany). The emission of radioactivity from D-2-[^{3}H]-glucose in 10 min was measured using a liquid scintillation counter (Tri-Carb® 2500 TR, Canberra-Packard, 63303 Dreieich, Germany).

2.4. Chemicals

D-2-[^{3}H]-glucose (specific activity: 0.74–1.11 TBq mmol^{-1}) was purchased from New England Nuclear (NEN, 63303 Dreieich, Germany, now PerkinElmer). The sodium salt of the DL-BHB racemate was synthesized as described previously (Schlumbohm and Harmeyer, 1999). The proportion of D isomer in the DL-BHB racemate solution was 42% (quantified enzymatically according to Williamson et al., 1962). Concentrations of D-BHB in the infusate and in blood plasma were measured using NAD, D-BHB dehydrogenase, and D-β-hydroxybutyric acid as a standard (Schlumbohm and Harmeyer, 2003). These chemicals were obtained from Roche Diagnostics (68305 Mannheim, Germany). All other chemicals were purchased from VWR (VWR International GmbH, 64295 Darmstadt, Germany).

2.5. Calculation of glucose kinetics

Glucose turnover and the rate constant of glucose turnover were calculated from the decay curve of the 2-[^{3}H]-glucose concentration in the plasma vs time after tracer injection, the injected dose of tracer, and the mean concentration of glucose in the plasma for 60 min after injection of the tracer. The non-compartmental approach described by Shipley and Clark (1972), and modified by Schlumbohm and Harmeyer (1999), was used. The decay curve of the specific radioactivity of glucose in plasma $(SA)_t$ after the bolus injection of tracer was fitted (SigmaPlot^{TM}, Systat Software GmbH, 40688 Erkrath, Germany) to a two-exponential equation of the form:

$$(SA)_t[\text{Bq mmol}^{-1}] = E_1 \exp(-k_1 \cdot t) + E_2 \exp(-k_2 \cdot t)$$

where $(SA)_t$ is the specific radioactivity of glucose at time t after the bolus injection [Bq mmol^{-1} glucose], E_1, E_2 the coefficients [Bq mmol^{-1} glucose], k_1, k_2 the exponents [min^{-1}] and t the min after injection of tracer.

To prevent high $(SA)_t$ values from dominating the iteration process, the squares of the deviations of the measured data from the curve were weighted by dividing them by the squares of the measured data. The specific radioactivity of glucose at time t after the bolus injection $(SA)_t$ was calculated by dividing the glucose radioactivity per litre plasma by the glucose content per litre plasma:

$$(SA)_t[\text{Bq mmol}^{-1}] = \frac{(A)_t[\text{Bq L}^{-1}]}{(C)_t[\text{mmol L}^{-1}]}$$

where $(A)_t$ is the [^{3}H]-glucose radioactivity per litre plasma at time t relative to the time of the bolus injection ($t = 0$) [Bq L^{-1}] and $(C)_t$ the concentration of glucose in blood at time t relative to the time of the bolus injection ($t = 0$) [mmol L^{-1}].

Because plasma glucose concentrations of samples taken during the 50 min sampling periods varied by <5%, mean glucose concentrations were used in the calculation of $(SA)_t$. The sampling periods after the two tracer injections were relatively short compared with the duration of the decay curve, which theoretically extends from the time of tracer injection to infinity. For calculation of the two areas under the curves (AUC), the radioactivity vs time curves were extrapolated over a time period of 4 h, i.e., from the time of injection of tracer ($t = 0$ min) to 4 h after marker injection ($t = 240$ min) (cut-off). These times corresponded to $t = 60$ min and $t = 240$ min (starting points) and $t = 300$ min and $t = 480$ min (cut-offs) of the experimental program. The latter points were chosen as cut-offs of the curves since $(SA)_t$ was <1% of $(SA)_0$ at this time. The AUC for each decay curve was calculated using the measured data from 10 to 60 min after tracer injection and the areas under the extrapolated time segments for the time interval from tracer injection until 10 min and from 60 min to 240 min after each tracer injection. Glucose radioactivity in plasma $(A)_t$ after 2-[^{3}H]-glucose injection was extrapolated using the same procedure.

The turnover of glucose per litre of the glucose distribution volume ($(TO)_n$, normalized glucose turnover) was calculated by dividing $(A)_0$ by the AUC as follows:

$$(TO)_n[\text{mmol L}^{-1}\ \text{min}^{-1}] = \frac{(A)_0[\text{Bq L}^{-1}]}{\text{AUC}[\text{Bq mmol}^{-1}\ \text{min}]}$$

where $(TO)_n$ is the normalized glucose turnover, $(A)_0$ the concentration of [^{3}H]-glucose at the time of tracer injection [Bq L^{-1}]; corresponding to $t = 60$ min and $t = 240$ min of the experimental program and AUC the area under curve.

Plasma glucose radioactivity after the second 2-[^{3}H]-glucose injection was confounded by carryover of radioactivity from the first injection of [^{3}H]-glucose. Carryover of radioactivity, which amounted to 5–11% of the AUC derived from the second tracer injection, was estimated from extrapolation of the SA vs time curve after the first tracer injection and subtracted from the second AUC. The turnover of glucose per kg body weight, TO [μmol kg^{-1} min^{-1}] was estimated by multiplying TO_n by the fractional distribution volume of glucose (V) [L kg^{-1}]. According to Bergman and Hogue (1967), the fractional distribution volume of glucose is relatively constant, ranging from 0.24 to 0.27. We estimated V by dividing the dose of tracer radioactivity [Bq] by the extrapolated concentration of tracer radioactivity at time zero $(A)_0$ [Bq L^{-1}] and the body weight of the ewe [kg]. The mean V of 0.24 was used for calculation of TO. The rate constant of glucose turnover, k, was calculated by dividing $(SA)_0$ by the AUC:

$$k[\text{min}^{-1}] = \frac{(SA)_0[\text{Bq mmol}^{-1}]}{\text{AUC}[\text{Bq mmol}^{-1}\ \text{min}]}$$

where $(SA)_0$ is the specific radioactivity of [^{3}H]-glucose at the time of bolus injection [Bq mmol^{-1}]; corresponding to $t = 60$ min and $t = 240$ min of the experimental program, Fig. 1.

2.6. Statistics

Data are presented as means ± SD. Mean glucose concentrations from the 70–120 min and 250–300 min sampling periods were used for subsequent statistical analysis. Plasma glucose concentrations, glucose turnover rates, and rate constants of glucose turnover were evaluated using three-way ANOVAs and SAS® software. The factors used in the three-way mixed model ANOVA were reproductive state, type of hypoglycaemic stress, and number of foetuses. Data related to reproductive state and type of hypoglycaemic stress were treated as repeated measures, and data related to the number of foetuses were treated as independent measures. The Wilcoxon test was not suitable for detecting the significance of differences between means derived from three observations. The three-way mixed model ANOVA enabled us to assess the significance of the effects of reproductive state (dry, pregnant, or lactating), type of hypoglycaemic stress (fasting or hyperketonaemia) and corresponding interactions using the *F*-test for repeated measures, and to assess the effects of the number of foetuses and corresponding interactions using the *F*-test for independent measures. Regression analysis testing of significance was carried out using the statistical program, SigmaStat® (Systat Software, 40688 Erkrath, Germany).

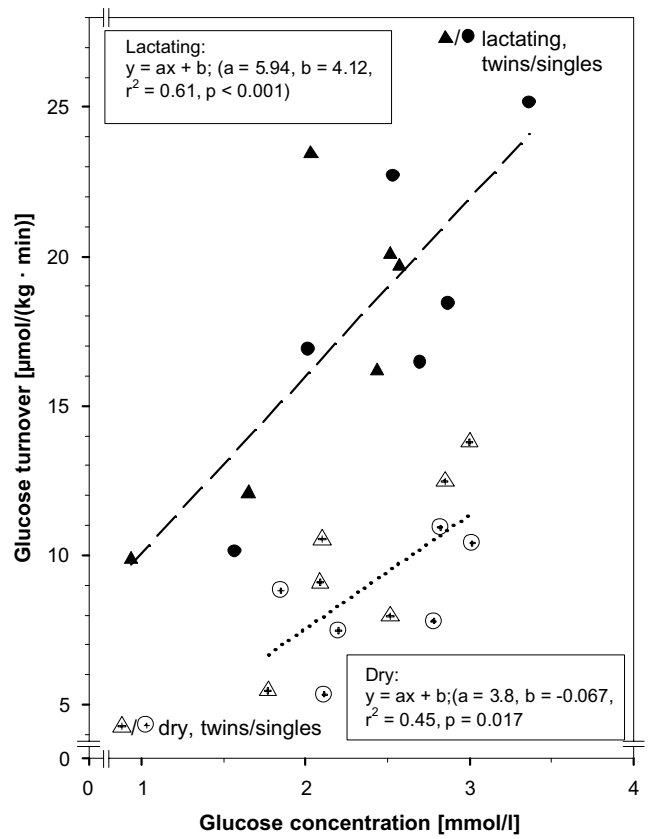

Fig. 2. Relationship between plasma glucose concentrations and glucose turnover rates in dry non-gestating and early-lactating ewes (12 experiments per group, each point represents one experiment).

3. Results

3.1. Effects of reproductive period, hypoglycaemic stress, and foetal number on kinetic parameters of glucose metabolism of ewes

Treatment means of effects of reproductive period, sequential hypoglycaemic stress and foetal number on kinetic parameters of glucose metabolism of ewes are summarized in Tables 1–4.

The mean *plasma glucose concentration* for all experiments was 2.7 ± 0.87 mmol L^{-1} (54 measurements) and

Table 1
Means ± SD of parameters of glucose metabolism of ewes with single and twins under different experimental conditions ($N = 3$)

			Glucose concentration (mmol/L)	Glucose turnover (µmol/kg min)	Rate constant of turnover (min^{-1})
Dry, non-mated	Postprandial	Single	3.11 ± 0.56	–	–
		Twins	3.38 ± 0.20	–	–
	Fasted	Single	2.87 ± 0.13	9.71 ± 1.67	0.014 ± 0.0018
		Twins	2.79 ± 0.25	11.4 ± 3.05	0.014 ± 0.0009
	Hyperketonaemic	Single	2.06 ± 0.18	7.21 ± 1.75	0.015 ± 0.0021
		Twins	1.99 ± 0.19	8.36 ± 2.61	0.013 ± 0.0016
Late gestating	Postprandial	Single	3.69 ± 0.35	–	–
		Twins	3.28 ± 0.32	–	–
	Fasted	Single	3.96 ± 0.90	18.4 ± 4.41	0.016 ± 0.0008
		Twins	2.33 ± 0.59	10.5 ± 3.50	0.019 ± 0.0021
	Hyperketonaemic	Single	2.76 ± 1.12	12.7 ± 3.51	0.018 ± 0.0024
		Twins	1.14 ± 0.38	5.93 ± 0.93	0.021 ± 0.0025
Lactating	Postprandial	Single	3.45 ± 0.17	–	–
		Twins	3.32 ± 1.02	–	–
	Fasted	Single	2.92 ± 0.42	22.1 ± 3.41	0.022 ± 0.0050
		Twins	2.37 ± 0.30	21.1 ± 2.06	0.022 ± 0.0016
	Hyperketonaemic	Single	2.09 ± 0.57	14.5 ± 3.78	0.020 ± 0.0024
		Twins	1.67 ± 0.75	12.7 ± 3.19	0.021 ± 0.0019

Table 2
Effect of reproductive state on kinetic parameters of glucose metabolism of ewes subjected to graded hypoglycaemic stress (means ± SD, () = number of experiments)

	Dry non-mated			Late gestating			Lactating		
	Post-prandial	Fasted	Hyperketonaemic	Post-prandial	Fasted	Hyperketonaemic	Post-prandial	Fasted	Hyperketonaemic
Glucose concentration mmol L^{-1} (6)	3.24 ± 0.40	2.83 ± 0.18	2.02 ± 0.17	3.49 ± 0.37	3.15 ± 1.12	1.94 ± 1.16	3.39 ± 0.66	2.65 ± 0.44	1.88 ± 0.64
Mean		2.70 ± 0.58 (18)			2.86 ± 1.13 (18)			2.64 ± 0.84 (18)	
Glucose turnover μmol (kg min)$^{-1}$ (6)	Not measured	10.6 ± 2.39	7.78 ± 2.09	Not measured	14.5 ± 5.63	9.33 ± 4.38	Not measured	21.6 ± 2.58	13.6 ± 3.28
Mean		9.17 ± 2.58[a] (12)			11.9 ± 5.49[a] (12)			17.6 ± 5.03[a] (12)	
Rate const. of glucose turnover, min^{-1} (6)	Not measured	0.014 ± 0.0013	0.014 ± 0.0019	Not measured	0.018 ± 0.0021	0.019 ± 0.0029	Not measured	0.022 ± 0.0034	0.021 ± 0.0020
Mean		0.014[b] ± 0.0015 (12)			0.019[b] ± 0.0026 (12)			0.021[b] ± 0.0027 (12)	

[a,b] Different with $p < 0.001$.

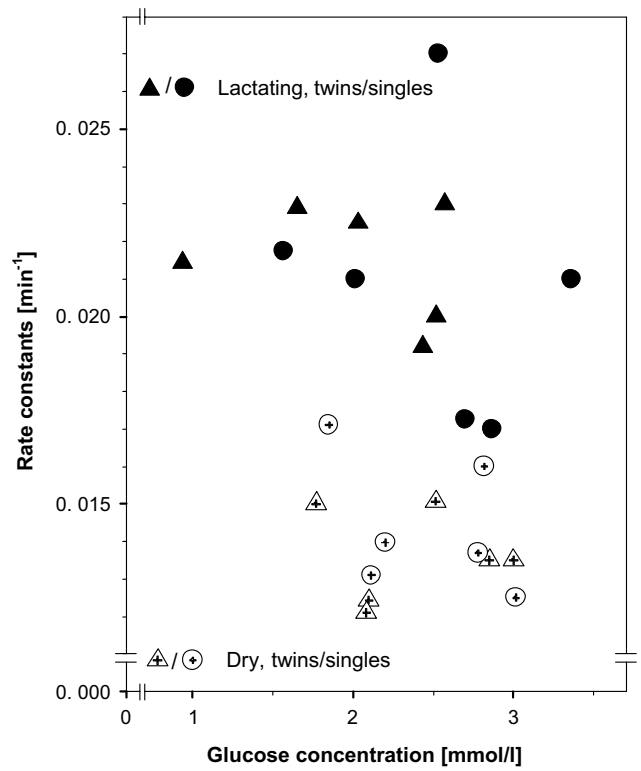

Fig. 3. Rate constants of glucose turnover plotted versus plasma glucose concentrations in dry non-gestating and lactating ewes (12 experiments per group, each point represents one experiment).

ranged from 4.9 to 0.87 mmol L^{-1} across all experimental conditions (Table 3). Postprandial plasma glucose concentration was not significantly different in the three reproductive states (Tables 2 and 5). Application of hypoglycaemic stress significantly lowered mean plasma glucose concentration in all reproductive states ($p < 0.001$) (Table 3). Mean reduction of plasma glucose concentration after fasting and during hyperketonaemia amounted to 15 and 34%, respectively. These effects were statistically not significantly different in different reproductive states ($p = 0.621$) (Tables 2 and 5). Mean glucose concentration in ewes with twins was significantly lower than in ewes with a single lamb ($p = 0.036$) (Tables 4 and 5). This effect was due to a significantly greater stress-induced reduction of plasma glucose in late gestating twin-bearing than single-bearing ewes ($p = 0.029$) (Tables 4 and 5 and Fig. 6). Fasting of late gestating single-pregnant ewes led to a slight (non-significant) 7% increase in glucose concentration (Fig. 6).

The mean *glucose turnover rate* in all experiments ($N = 36$) was 12.9 ± 5.67 μmol kg^{-1} min^{-1} and ranged from 23.5 to 5.2 μmol kg^{-1} min^{-1} across all experimental conditions (Table 3). It was highest in fasted lactating ewes nursing singleton lambs (22.1 ± 3.4) and lowest in late-gestating hyperketonaemic twin-pregnant ewes (5.9 ± 0.93) (Fig. 7, upper section). In contrast to plasma glucose concentration, reproductive state had a significant ($p < 0.015$) effect on glucose turnover, being 30% and 92% higher in late gestation and during lactation, respectively, compared

Table 3
Effect of sequential standardized hypoglycaemic stress on parameters of glucose metabolism in ewes (means ± SD, () = number of experiments)

	All experiments	Postprandial	Fasted	Hyperketonaemic
Plasma glucose concentration (mmol/l)	2.7 ± 0.87 Min: 0.87 Max: 4.9 (54)	3.4 ± 0.48 (18)	2.9 ± 0.70[a] (18)	1.9 ± 0.73[a] (18)
Glucose turnover (μmol/(kg min))	12.9 ± 5.67 Min: 5.23 Max: 23.5 (36)	Not measured	15.5 ± 5.91[b] (18)	10.2 ± 4.08[b] (18)
Rate constant of glucose turnover (min^{-1})	0.0179 ± 0.00360 Min: 0.0121 Max: 0.0270 (36)	Not measured	0.0178 ± 0.00340 (18)	0.0180 ± 0.00368 (18)

[a] $p < 0.001$.
[b] $p = 0.002$.

to the dry non-pregnant period (Table 2). Elevation of plasma D-BHB concentration effectively depressed mean glucose turnover ($p = 0.002$) by 34% (Table 3). It was important to note that glucose turnover in late gestation ewes bearing twins was significantly ($p = 0.046$) 47% lower

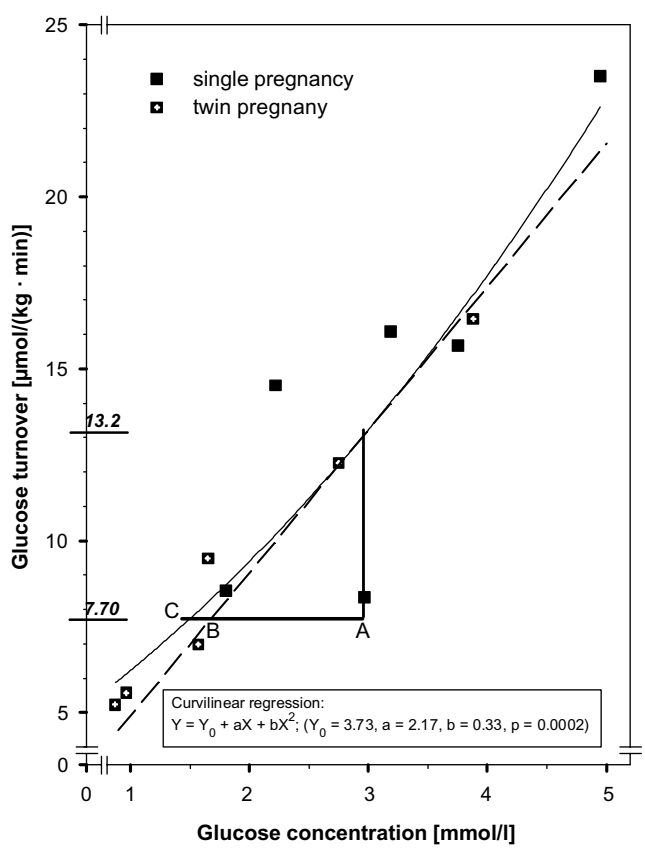

Fig. 4. Regression line showing the relationship between plasma glucose concentration and glucose turnover in late-gestation ewes with one or two foetuses. Each point represents one of 12 experiments. The regression line is described by a quadratic polynomial equation. The straight dashed line is the tangent at 3.0 mmol L^{-1}. It describes the change in glucose concentration, if – starting at this point – the plasma glucose concentration would only be affected by the glucose production rate.

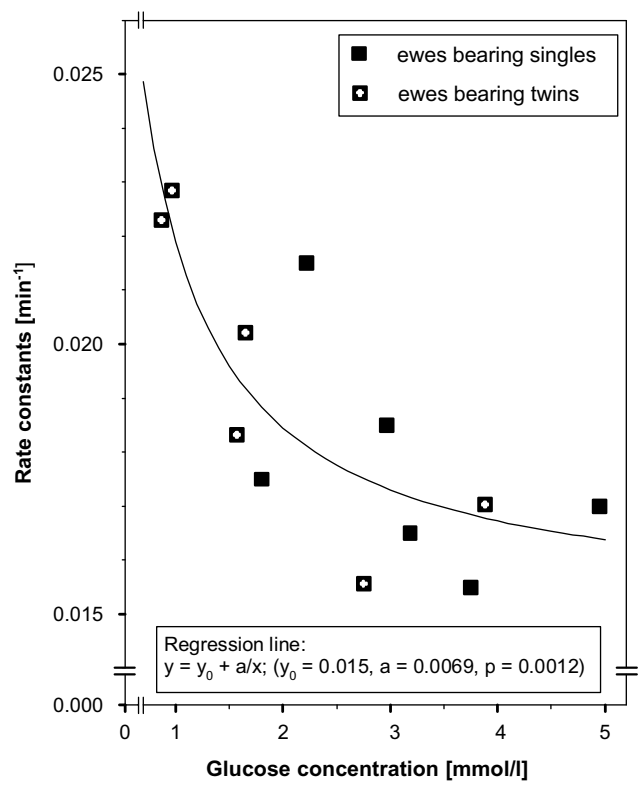

Fig. 5. Relationship between plasma glucose concentrations and rate constants of glucose turnover in late-gestation ewes bearing one or two foetuses (each point represents one of 12 experiments).

(8.2 vs 15.6 μmol kg^{-1} min^{-1}) than in ewes bearing a single lamb (Table 4).

The mean *rate constant of glucose turnover* in ewes from all experiments was 0.0179 ± 0.0036 min^{-1} (36 experiments) and ranged from 0.0121 to 0.020 min^{-1} across all experimental conditions (Table 3). The reproductive period was the only cause of significant variation for this parameter (Tables 2 and 5). In late gestation and during early lactation the rate constant was significantly increased ($p < 0.0012$) by 36% and 50%, respectively compared to the dry non-gestating period (Table 2). Both, foetal number

Table 4
Kinetic parameters of glucose metabolism of ewes related to single or twin pregnancies in three reproductive periods (Means ± SD_x() = number of experiments)

	All experiments		Dry non-mated		Late gestating		Lactating	
	Single	Twin	Single	Twin	Single	Twin	Single	Twin
Glucose concentration, mmol L^{-1}	3.0 ± 0.79 (27)	2.5 ± 0.82 (27)	2.7 ± 0.57 (9)	2.7 ± 0.63 (9)	3.5 ± 0.92[a] (9)	2.3 ± 1.01[a] (9)	2.8 ± 0.70 (9)	2.5 ± 0.97 (9)
Glucose turnover, μmol (kg min)$^{-1}$ (6)	14.1 ± 5.84 (27)	11.6 ± 5.38 (27)	8.46 ± 2.05 (9)	9.89 ± 3.04 (9)	15.6 ± 4.73[a] (9)	8.20 ± 3.38[a] (9)	18.3 ± 5.26 (9)	16.9 ± 5.17 (9)
Rate const.of glucose turnover, min^{-1} (6)	0.017 ± 0.0036 (27)	0.018 ± 0.0039 (27)	0.014 ±0.0018 (9)	0.014 ± 0.0013 (9)	0.017 ± 0.0017 (9)	0.020 ± 0.0023 (9)	0.021 ± 0.0037 (9)	0.022 ± 0.0016 (9)

[a] Different with $p < 0.05$.

and hypoglycaemic stress exerted no significant effect upon the rate constant of glucose turnover (Tables 3 and 4).

The levels of significance, probabilities of single and combined effects of the three factors: reproductive period, level of hypoglycaemic stress, and foetal number, are summarized in Table 5.

3.2. Regressions between kinetic parameters of glucose metabolism

In order to further elucidate whether a stress-related decline in plasma glucose concentration was associated primarily with changes in glucose production rate or glucose disposal, regressions were calculated between plasma glucose and glucose turnover and in a second step between glucose concentration and rate constants of glucose turnover. All data from fasted and hyperketonaemic experiments were included in the calculation, separated for reproductive status and singleton or twin pregnancies.

Regression analysis showed that during the dry non-gestating period and in early lactation, stress-induced decline of plasma glucose concentration was linearly related to reduction in glucose turnover (Fig. 2) but was unrelated to changes in the rate constant of glucose turnover (Fig. 3). This demonstrated, that in dry and lactating sheep the lowering in plasma glucose concentration was only caused by a reduction in glucose turnover and not by an increase in the fractional rate of glucose disposal. This conclusion follows from the notion, that the rate constant of turnover denotes the fraction of the glucose pool which turns over per unit of time. The curves displayed in Figs. 2 and 3 also show, that the shape of the regression line was the same for ewes that were associated with single or twin pregnancies.

The parallel shift of the regression line of glucose turnover from lactating ewes compared to the dry ewes along the y-axis (Fig. 3) results from the fact that the mean rate constant of turnover during early lactation was throughout 50% greater than during the dry period (Table 2).

In contrast to dry and lactating ewes, plasma glucose concentration in late gestation ewes was significantly related to both, glucose turnover and rate constant of turnover (Figs. 4 and 5). The shape of the regression curves was curvilinear. This indicated that in late gestation changes in plasma glucose are associated with both, changes in glucose production rate and changes in the fractional rate of glucose disposal. The curves in Figs. 4 and 5 show that the rate of glucose turnover decreases and the rate constant increases when plasma glucose concentration decreases. These relationships demonstrate that in late gestation ewes a decrease in blood glucose concentration is associated with a decrease in the rate of glucose production and an increase in the fractional rate of glucose disposal. Again, values from single- and twin-bearing ewes behaved essentially the same.

Table 5
Probabilities of main effects and interactions of kinetic parameters of glucose metabolism

	Main effects of:			Interactions between:			
	Reproductive status	Hypoglycaemic stress	Foetal number	Repro./stress	Repro./foetal number	Stress/foetal number	Repro./stress/foetal number
Plasma glucose concentration mmol L^{-1}	0.720	**<0.0001**	**0.036**	0.621	0.125	**0.029**	0.633
Glucose turnover μmol/(kg min)	**0.0013**	**0.0022**	0.065	0.113	**0.046**	0.973	0.890
Rate constant of glucose turnover, min^{-1}	**0.0012**	0.873	0.242	0.127	0.313	0.972	0.539

Effects < 0.05 are printed in boldface.

The two curves of Figs. 4 and 5 were complemented by a third curvilinear relationship between glucose turnover (x) and the rate constant of glucose turnover (y) (figure not shown). As a consequence of the two relationships of Figs. 4 and 5, it also points to the fact that in the late gestation ewe a decrease in the glucose production rate is associated with an increase in the fractional rate of glucose disposal. This third relationship was best described by an inverse first-order equation of the form, $y = y_0 + a/x$ ($y_0 = 0.015$ min^{-1}; $a = 0.037$). The physiological implications of these findings are discussed in Section 4.1.

3.3. Different hypoglycaemic responses in late gestation ewes with one or two lambs

In order to examine the question, why late gestation ewes bearing twins preferably develop pregnancy toxaemia, we further examined the alterations of glucose metabolism separately in these two groups of ewes while they were subjected to hypoglycaemic stress. Responses of plasma glucose and of glucose kinetics in different reproductive states and classed according to the number of foetuses are illustrated in Table 1 and in Figs. 6 and 7.

Fig. 6 illustrates that plasma glucose concentrations after overnight fasting were significantly ($p = 0.036$) lower (41%) in twin- than in single-bearing ewes (2.3 vs 4.0 mmol L^{-1}). Hyperketonaemia depressed plasma glucose concentration further in both groups of ewes. This stress factor resulted in a 59% lower mean plasma glucose concentration in twin- than in singleton-bearing ewes (1.1 vs 2.8 mmol L^{-1}; $p = 0.04$). In summary, sequential application of short-term starvation and hyperketonaemic stress had led to the lowest plasma glucose concentrations in the study in twin-bearing ewes. The effect was accompanied by equivalent reductions in glucose turnover rates (Fig. 7, upper section). The glucose turnover rate in ewes bearing twins during hyperketonaemic stress was 53% lower than in ewes bearing singleton lambs (5.7 vs 12.7 μmol kg^{-1} min^{-1}, $p = 0.04$; Fig. 7, upper section). This stress condition also produced the lowest glucose turnover rate in the study.

The hypoglycaemic stress imposed by an overnight fast had the same effect on the rate constants of glucose turnover in single- and twin-bearing ewes (Fig. 7, lower section). The hypoglycaemic stress imposed by D-BHB infusion resulted, however, in 20% higher rate constants of glucose turnover in late gestation ewes with twins than in ewes with singleton lambs (0.0212 vs 0.0176 min^{-1}).

3.4. BHB status

Mean BHB concentrations are presented in Table 6.

Blood D-BHB concentrations after the overnight fast were 2.7 times higher (not significant) in twin-bearing ewes than in single-bearing ewes. The 3 h intravenous infusion of a DL-BHB solution elevated the D-BHB concentration in blood to 3.7–5.7 mmol L^{-1}. The degree of hyperketonaemia in the late gestation ewes with twins was maintained slightly below that of the other groups in order to avoid abortion.

4. Discussion

In ewes, glucose is the principal carbon source for placental and foetal oxidative metabolism and tissue synthesis (Bell, 1993; Hay, 1991). Thirty percent to 50% of maternal glucose production in late gestation is taken up by uterine and foetal tissues (Bell et al., 1999; Bell, 1993; Hay et al., 1983; Lindsay, 1971), and 50–70% of this amount is used by the uteroplacenta (Hay, 1991; Owens et al., 1989).

The results of this study do not support the widely held belief (Baird, 1981) that an increased uptake of glucose by the conceptus is the primary cause of hypoglycaemia in the twin-bearing ewe in late gestation. Our findings support the opinion that a reduced maternal glucose production causes hypoglycaemia in the twin-bearing late gestation ewe. The experiments presented here were carried out under near steady-state conditions in which the concentration of glucose in the plasma was almost constant. Therefore, the rate of glucose turnover was assumed to be equivalent to the rate of glucose entry and the rate of glucose disposal. Responses of ewes in various reproductive states to two hypoglycaemic stress conditions were evaluated using glucose kinetics. Results were interpreted according to the following concepts. If a stress-induced decrease in plasma glucose concentration is accompanied by a decrease in glucose turnover rate and no change or a regulatory increase in the rate constant of glucose turnover, it must have

resulted from a reduction in the glucose production rate. However, if a stress-induced decrease in plasma glucose concentration is accompanied by an increase in the rate constant of glucose turnover and no change or a regulatory increase in glucose turnover rate, it must have resulted from an increase in the glucose disposal rate.

4.1. Hypoglycaemia in the late gestation twin-bearing ewe results primarily from a reduction in the glucose production rate

The linear relationships between plasma glucose concentrations and glucose turnover rates during the dry and lactating periods (Fig. 2) indicate that in these two reproductive states, the glucose production rate was the only determinant of plasma glucose concentration. The observation that the rate constants of glucose turnover were not related to changes in plasma glucose concentration under these conditions (Fig. 3) supports this conclusion.

In contrast to the dry and lactating periods, during late gestation a curvilinear relationship was evident between plasma glucose concentration and glucose turnover. This indicated that the response of glucose metabolism to hypoglycaemic stress in late gestation ewes differs from that in dry and lactating ewes. The form of the regression line (Fig. 4) shows that changes in plasma glucose concentration were not determined by changes in glucose production rate alone. The decrease in the slope of the curve with decreasing plasma glucose concentration demonstrates that the reduction in glucose turnover rate per unit decrease in plasma glucose concentration was decremental, and was thus too small to account for the entire decrease in plasma glucose concentrations. This indicated that an additional factor was involved in mediating the decrease in plasma glucose concentration. The only factor that could be responsible for this effect is an increase in glucose disposal rate. That this did occur is evident from the curvilinear relationship illustrated in Fig. 5, which shows that the rate constant of glucose turnover increased with decreasing plasma glucose concentrations.

It is of interest to estimate the relative changes in glucose production and disposal rates associated with the decrease in plasma glucose concentration after hypoglycaemic stress. An example of such an estimate is illustrated in Fig. 4. It was assumed that plasma glucose concentration decreased from 3.0 to 1.5 mmol L^{-1}. This decrease in concentration (line segment AC in Fig. 4) was accompanied by a reduction in glucose production rate from 12.2 to 7.7 μmol kg^{-1} min^{-1} (indicated on the *y*-axis). The effect of this reduction in glucose production rate on the overall decrease in glucose concentration is shown by line segment AB, where B is the point of intersection between line segment AC and the tangent to the curve at 3.0 mmol L^{-1} (dashed line). The effect of an increased disposal rate on the overall decrease in glucose concentration is shown by line segment BC, which accounts for only 12% of the decrease in plasma glucose concentration. This example demonstrates that the reduction in glucose production rate accounted for the greatest proportion (88%) of the stress-induced decrease in plasma glucose concentration from 3.0 to 1.5 mmol L^{-1}. Furthermore, the increase in glucose disposal rate after hypoglycaemic stress seems to be a minor secondary event, which probably maintains a minimal supply of glucose to the uterine tissues.

These conclusions are in accordance with observations of other investigators who showed that induction of foetal hypoglycaemia by foetal insulin infusion resulted in a 40–50% increase in umbilical glucose uptake, but an insignificant 4–10% decrease in maternal glucose concentration (DiGiacomo and Hay, 1990; Bloch et al., 1986).

The higher mean rate constants of glucose turnover in late gestation and early lactation compared with the dry period (Table 2) do not contradict the previous statement because they are not involved in an acute stress-induced decrease in plasma glucose concentration. A greater rate constant of glucose turnover during gestation and lactation than during the dry period was observed by Bergman (1964), Bergman (1973), Bergman et al. (1974), Oddy et al. (1985), Van Der Walt et al. (1983), and Chaiyabutr et al. (1982). This reflects the greater ease of glucose uptake by the entire peripheral body tissues in late gestation and early lactation compared with the dry period.

It may be argued that the hypoglycaemic conditions applied in our study do not mimic naturally occurring conditions. This assumption is probably unfounded. The types of hypoglycaemic stresses applied in this study appear to occur naturally in herds with multifoetate ewes that are housed indoors and fed once or twice daily. Animals held under such conditions may also encounter other forms of stress such as management-related stress, psychological stress, and stress induced by environmental factors. These stress factors are probably equally effective in reducing the glucose production rate and causing hypoglycaemia. The different hypoglycaemic responses to the graded stress conditions in late gestation ewes bearing singletons or twins were not caused by individual differences between animals because the hypoglycaemic responses were the same in these animals during the dry period (Figs. 6 and 7).

It is worth noting that marked hypoglycaemic responses of late gestation twin-bearing ewes to various stimuli have been observed by many other investigators. These authors also mentioned that the plasma glucose concentration was mainly modified by changes in the glucose production rate. These studies include those in which measurements were derived from ewes with single- or twin-pregnancies (Van Der Walt et al., 1980; Petterson et al., 1993), ewes fed different levels of nutrition (Chandler et al., 1985; Oddy et al., 1985; Judson and Leng, 1968), ewes starved for 1–3 d (Bassett and Madill, 1974; Boyd et al., 1973), ewes with normoketonaemia or hyperketonaemia (Bergman, 1973; Bergman et al., 1974), and ewes with pregnancy toxaemia (Ford, 1965). Direct support for our finding is provided by the results of Hay et al. (1984) and Leury et al.

(1990a,b). These authors measured maternal and foetal glucose utilization simultaneously and demonstrated that the foetal uptake of glucose is always linearly related to the maternal plasma glucose concentration under normal feeding conditions, during starvation and induced hypoglycaemia. From a historical point of view, it appears of

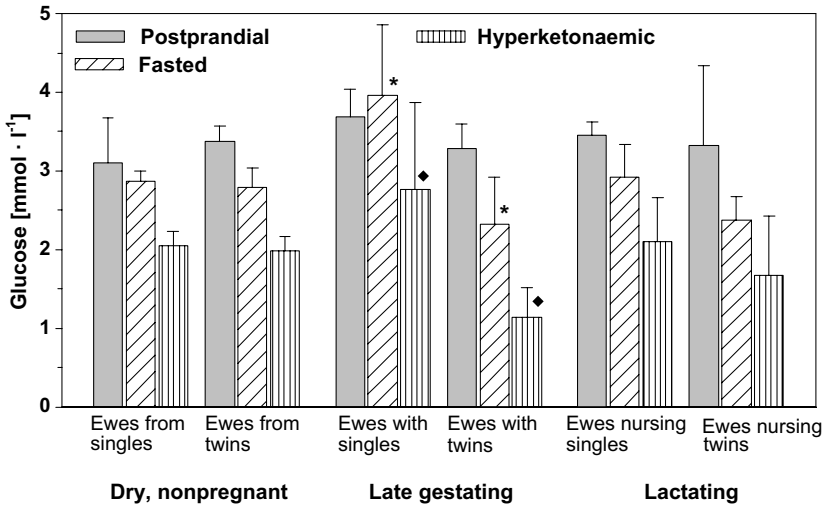

Fig. 6. Effects of standardized hypoglycaemic stress on plasma glucose concentrations in ewes in three reproductive periods (∗, ♦: $p < 0.05$).

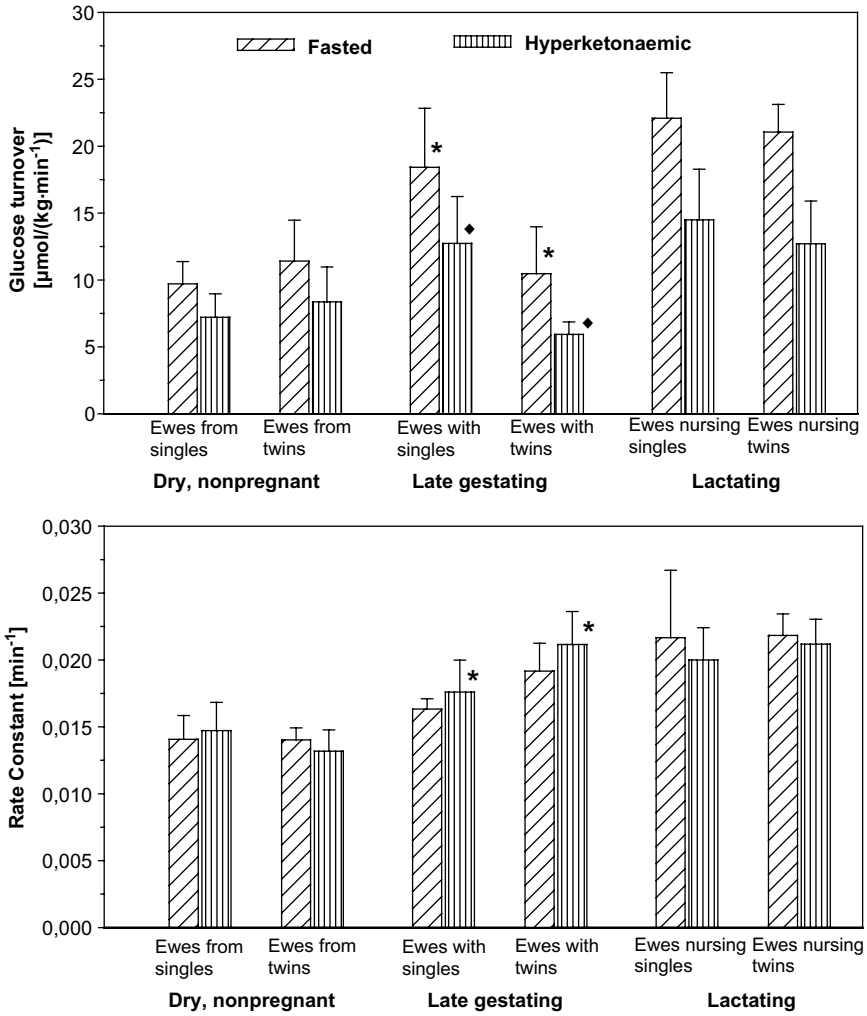

Fig. 7. Effects of standardized hypoglycaemic stress on glucose turnover (upper section) and rate constants of glucose turnover (lower section) in dry non-gestating, pregnant, and lactating ewes (∗, ♦: $p < 0.05$).

Table 6
D-BHB concentrations in blood of ewes after fasting and during D-BHB infusion rates ($N = 3$)

BHB metabolism	Dry, non-mated		Late gestation		Lactation	
	Single	Twin	Single	Twin	Single	Twin
Pre-infusional D-BHB concentration (mmol L^{-1})	0.42 ± 0.14	0.29 ± 0.07[a]	0.59 ± 0.30	1.61 ± 0.95[a]	0.48 ± 0.18	0.86 ± 0.35
Infusion rate (mmol/(kg min)$^{-1}$)	22 ± 0	22 ± 0	18 ± 0	12.7 ± 4.6	24.7 ± 4.1	24.7 ± 4.1
Elevated D-BHB (mmol L^{-1})	5.66 ± 0.62	5.14 ± 1.14	4.28 ± 1.74	3.73 ± 0.66	5.76 ± 1.05	6.49 ± 1.68

[a] Different with $p < 0.05$.

interest to note that Ford suggested in 1965 that hypoglycaemia in ketotic sheep probably resulted from an inadequate glucose supply rather than from an increased rate of utilization of glucose.

4.2. The effects of hypoglycaemic stress are greatest in late gestation twin-bearing ewes

The decrease in plasma glucose concentration and the concomitant reduction in glucose turnover after hypoglycaemic stress were greatest in late gestation ewes bearing twins (Figs. 6 and 7). This observation demonstrates that the late gestation ewe bearing twins is particularly at risk of responding to hypoglycaemic stress factors. This increased sensitivity to hypoglycaemic stress probably renders the late gestation twin-bearing ewe susceptible to pregnancy toxaemia, and may explain why pregnancy toxaemia occurs almost exclusively in multiparous late gestation ewes, why the mortality rate of the disease is high, and why the efficacy of treatment is low. Significantly greater hypoglycaemic responses in twin-pregnancies than in singleton pregnancies have been reported to occur in sheep (Reid and Hogan, 1959; Reid and Hinks, 1962) but also in humans (Casele et al., 1996).

It is well-known that maintenance of glycaemia is particularly important for the twin-bearing ewe in late gestation. Hypoglycaemia induces the release of stress hormones (Mellor et al., 1987), mobilization of body fat (Reid and Hinks, 1962), and the production of ketone bodies (Varnam et al., 1978) to a greater extent in twin-bearing ewes than in ewes bearing single lambs (Pethick and Lindsay, 1982). Hyperketonaemia depresses plasma glucose concentration further, an effect that occurs in sheep (Kammula, 1976; Radcliffe et al., 1983) and other mammalian species (Balasse and Fery, 1989; Beylot et al., 1986; Pi-Sunyer et al., 1970).

Late gestation ewes with single lambs did not exhibit hypoglycaemic responses to overnight fasting; the close association between plasma glucose concentration and glucose turnover rate led us to conclude that this was a real phenomenon. Similar observations have been made by other investigators. Bassett et al. (1990) also observed the absence of a decrease in plasma glucose concentration after 12 h of starvation of gestating ewes with single lambs at 125–130 d of gestation. At 115–141 d of gestation, an increase in feed intake to 55% above maintenance by ewes pregnant with single lambs resulted in a 20% increase in plasma glucose concentration (Mühlhäusler et al., 2002).

4.3. Concluding comments

The stress-induced decrease in plasma glucose concentration and the concomitant reduction in glucose turnover were only significant in late gestation twin-bearing ewes (Figs. 6 and 7). Two factors seem to be responsible for this phenomenon: pregnancy *per se* and the number of live foetuses. Wastney and co-workers (1983) showed that *in vivo* turnover rates of glucose were 263% greater in twin-bearing ewes in which the foetuses were dead than in twin-bearing ewes in which the foetuses were alive. Pregnancy results in profound metabolic alterations (Carpenter, 1993). The presence of multiple live foetuses seems to increase responsiveness to hypoglycaemic stress more than the presence of one foetus. In twin-bearing ewes near term, many hormonal responses are attenuated (Gardner et al., 2004; Keller-Wood, 1994; Rossi et al., 1993). The regulatory capacities of pregnant ewes, particularly those that have multiple foetuses and are close to term, seem to be under severe strain and in many cases are not able to cope with environmental and management-related challenges (Bell, 1993).

Support for this assumption is also provided by the studies of Everts and Kuiper (1983), who reported that the incidence of subclinical and clinical ketosis in prolific Isle de France × Finnish Landrace ewes was influenced by litter size, but not by energy intake. It is of interest that increased responsiveness to hypoglycaemic stress in late gestation is not confined to multifoetate ewes. Severe hypoglycaemia after short-term starvation also occurs in dogs (Connolly et al., 2000), guinea pigs (Gilbert et al., 1985), rats (Holness and Sugden, 1997; Lopez-Soldado et al., 2002; Rossi et al., 1993), and humans (Casele et al., 1996; Diamond et al., 1992; Metzger and Freinkel, 1987). This metabolic condition is called accelerated starvation. Studies have shown that the hypoglycaemic effect cannot be explained by short-term withholding of dietary energy alone. The late gestation multifoetate ewe is unique because its homoeorhetic capacity for maintenance of glucose homoeostasis is more limited than that of the aforementioned monogastric species. The susceptibility of twin-bearing ewes to pregnancy toxaemia at about 120 d of gestation was assessed by Wastney and co-workers (1982) with glucose tolerance tests. The authors reported

that animals that exhibited very poor control of glucose homoeostasis were predisposed to pregnancy toxaemia irrespective of their nutritional status. Therefore, it might be useful to assess in detail the hormonal, metabolic, and homoeorhetic features of individual sheep that are predisposed to hypoglycaemia or pregnancy toxaemia in forthcoming etiological studies on pregnancy toxaemia of the ovine.

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References

Baird, G.D., 1981. Lactation, pregnancy and metabolic disorder in the ruminant. Proc. Nutr. Soc. 40, 115–120.

Balasse, E.O., Fery, F., 1989. Ketone body production and disposal: effects of fasting, diabetes, and exercise. Diabetes Metab. Rev. 5, 247–270.

Bassett, J.M., Madill, D., 1974. The influence of maternal nutrition on plasma hormone and metabolite concentrations of foetal lambs. J. Endocrinol. 61, 465–477.

Bassett, N.S., Oliver, M.H., Breier, B.H., Gluckman, P.D., 1990. The effect of maternal starvation on plasma insulin-like growth factor I concentrations in the late gestation ovine fetus. Pediat. Res. 27, 401–404.

Bell, A.W., 1993. Pregnancy and fetal metabolism. In: Forbes, J.M., France, J. (Eds.), Quantitative Aspects of Ruminant Digestion and Metabolism, first ed. C.A.B. Int, UK, pp. 405–432.

Bell, A.W., Hay Jr., W.W., Ehrhardt, R.A., 1999. Placental transport of nutrients and its implications for fetal growth. J. Reprod. Fertil. Suppl. 54, 401–410.

Bergman, E.N., 1964. Glucose turnover rates in pregnant and non-pregnant sheep. Nature 202, 1333.

Bergman, E.N., 1973. Glucose metabolism in ruminants as related to hypoglycemia and ketosis. Am. J. Physiol. 215, 865–873.

Bergman, E.N., Hogue, D.E., 1967. Glucose turnover and oxidation rates in lactating sheep. Am. J. Physiol. 213, 1378–1384.

Bergman, E.N., Konk, K., Katz, M.L., 1963. Quantitative measurements of acetoacetate metabolism and oxidation in sheep. Am. J. Physiol. 205, 658–662.

Bergman, E.N., Brokman, R.P., Kaufman, C.F., 1974. Glucose metabolism in ruminants: comparison of whole-body turnover with production by gut, liver and kidneys. Fed. Proc. 33, 1849–1854.

Beylot, M., Khalfallah, Y., Riou, J.P., Cohen, R., Normand, S., Mornex, R., 1986. Effects of ketone bodies on basal and insulin-stimulated glucose utilization in man. J. Clin. Endocrinol. Metab. 63, 9–15.

Bickhardt, K., Henze, P., Sallmann, H.P.,1993. Glucose-Stoffwechselstörungen bei erwachsenen Schafen und ihre Behandlungen. DVG Tagung "Krankheiten Der Kleinen Wiederkäuer, Giessen, pp. 92–100.

Bickhardt, K., Henze, P., Ganter, M., 1998. Klinische Befunde und Differentialdiagnose bei Ketose und Hypocalcämie der Schafe. Dtsche. Klin. Wschr. 105, 413–419.

Bloch, C.A., Banach, W., Landt, K., Devaskar, S., Sperling, M.A., 1986. Effects of fetal insulin infusion on glucose kinetics in pregnant sheep: a compartmental analysis. Am. J. Physiol. 251, E448–E456.

Boyd, R.D., Morriss Jr., F.H., Meschia, G., Makowski, E.L., Battaglia, F.C., 1973. Growth of glucose and oxygen uptakes by fetuses of fed and starved ewes. Am. J. Physiol. 225, 897–902.

Carpenter, M.W., 1993. Metabolic changes in gestational diabetes. Clin. Perinatol. 20, 583–591.

Casele, H.L., Dooley, S.H., Metzger, B.E., 1996. Metabolic response to meal eating and extended overnight fast in twin gestation. Am. J. Obstet. Gynecol. 175, 917–921.

Chaiyabutr, N., Faulkner, A., Peaker, M., 1982. Glucose metabolism in vivo in fed and 48 h starved goats during pregnancy and lactation. Brit. J. Nutr. 47, 87–94.

Chandler, K.D., Leury, B.J., Bird, A.R., Bell, A.W., 1985. Effects of undernutrition and exercise during late pregnancy on uterine, fetal and uteroplacental metabolism in the ewe. Brit. J. Nutr. 53, 625–635.

Clapp, J.F., 2006. Influence of endurance exercise and diet on human placental development and fetal growth. Placenta 27, 527–534.

Connolly, C.C., Holste, L.C., Aglione, L.N., Neal, D.W., Lacy, D.B., Smith, M.S., Diamond, M.P., Cherrington, A.D., Chiasson, J.L., 2000. Alterations in basal glucose metabolism during late pregnancy in the conscious dog. Am. J. Physiol. 279, E1166–E1177.

Diamond, M.P., Reece, E.A., Caprio, S., Jones, T.W., Amiel, S., Degennaro, N., Laudano, A., Addabbo, M., Sherwin, R.S., Tamborlane, W.V., 1992. Impairment of counterregulatory hormone responses to hypoglycemia in pregnant women with insulin-dependent diabetes mellitus. Am. J. Obstet. Gynecol. 166, 70–77.

DiGiacomo, J.E., Hay Jr., W.W., 1990. Fetal glucose metabolism and oxygen consumption during sustained hypoglycemia. Metabolism 39, 193–202.

Everts, H., Kuiper, H., 1983. Energy intake and pregnancy toxaemia in prolific ewes. In: Proc. 5th Int. Conf. Prod. Disease in Farm Anim., Uppsala, Sweden, pp. 133–136.

Ford, E.J.H., 1965. Glucose utilization in spontaneous pregnancy toxaemia of sheep. Brit. Vet. J 121, 139–144.

Gardner, D.S., Jamall, E., Fletcher, A.J.W., Fowden, A.L., Giussani, D.A., 2004. Adrenocortical responsiveness is blunted in twin relative to singleton ovine fetuses. J. Physiol. 557, 1021–1032.

Gilbert, M., Sparks, J.W., Girard, J., Battaglia, F.C., 1985. Effects of fasting on glucose turnover rate and metabolite levels in conscious pregnant guinea pigs. Biol. Neonate 48, 90–99.

Hay Jr., W.W., 1991. The role of placental–fetal interaction in fetal nutrition. Semin. Perinatol. 15, 424–433.

Hay Jr., W.W., Sparks, J.W., Wilkening, R.B., Battaglia, F.C., Meschia, G., 1983. Partition of maternal glucose production between conceptus and maternal tissues in sheep. Am. J. Physiol. 245, E347–E350.

Hay Jr., W.W., Sparks, J.W., Wilkening, R.B., Battaglia, F.C., Meschia, G., 1984. Am. J. Physiol. 246, E237–E242.

Henze, P., Bickhardt, K., Fuhrmann, H., Sallmann, H.P., 1995. Versuche zur Behandlung der Gestationsketose bei kleinen Wiederkäuern mit Insulin. Fachtg. Dsch.Vet.med.Ges., Über Haltungsprobleme und Krankheiten der kleinen Wiederkäuer, Gießen.

Holness, M.J., Sugden, M.C., 1997. Glucoregulation during progressive starvation in late pregnancy in the rat. Am. J. Physiol. 72, E556–E561.

Judson, G.J., Leng, R.A., 1968. Effect of diet on glucose synthesis in sheep. Proc. Austral. Soc. Anim. Prod. 7, 354–358.

Kammula, R.G., 1976. Effect of experimentally induced hyperketonemia on glucose metabolism of ovine brain in vivo. Am. J. Vet. Res. 37, 935–938.

Keller-Wood, M., 1994. Corticotropin responses to hypoglycemia and hypotension during ovine pregnancy. Am. J. Physiol. 266, R180–R187.

Leng, R.A., 1965. Ketone body metabolism in normal and underfed pregnant sheep and in pregnancy toxaemia. Res. Vet. Sci. 6, 433–441.

Leury, B.J., Bird, A.R., Chandler, K.D., Bell, A.W., 1990a. Glucose partitioning in the pregnant ewe: effects of undernutrition and exercise. Brit. J. Nutr. 64, 449–462.

Leury, B.J., Chandler, K.G., Bird, A.R., Bell, A.W., 1990b. Effects of maternal undernutrition and exercise on glucose kinetics in fetal sheep. Brit. J. Nutr. 64, 463–472.

Lindsay, D.B., 1971. Changes in the pattern of glucose metabolism in growth, pregnancy and lactation in ruminants. Proc. Nutr. Soc. 30, 272–277.

Lopez-Soldado, L., Betancor-Fernandez, A., Herrera, E., 2002. Differential metabolic response to 48 h food deprivation at different periods of pregnancy in the rat. J. Physiol. Biochem. 58, 75–85.

Mellor, D.J., Flint, D.J., Vernon, R.G., Forsyth, I.A., 1987. Relationships between plasma hormone concentrations, udder development and the production of early mammary secretions in twin-bearing ewes on different planes of nutrition. Q. J. Exp. Physiol. 72, 345–356.

Mendel, C., Zindath, W., 2004. Zuchtbericht der Bayrischen Herdbuchgesellschaft für Schafzucht. Der bayrische Schafhalter 3, 7–11.

Metzger, B.E., Freinkel, N., 1987. Accelerated starvation in pregnancy: implications for dietary treatment of obesity and gestational diabetes mellitus. Biol. Neonate 51, 78–85.

Mühlhäusler, B.S., Roberts, C.T., McFarlane, J.R., Kauter, K.G., McMillen, I.C., 2002. Fetal leptin is a signal of fat mass independent of maternal nutrition in ewes fed at or above maintenance energy requirements. Biol. Reprod. 67, 493–499.

Oddy, V.H., Gooden, J.M., Hough, G.M., Teleni, E., Annison, E.F., 1985. Partitioning of nutrients in merino ewes. II. Glucose utilization by skeletal muscle, the pregnant uterus and the lactating mammary gland in relation to whole body glucose utilization. Aust. J. Biol. Sci. 38, 95–108.

Owens, J.A., Falconer, J., Robinson, J.S., 1989. Glucose metabolism in pregnant sheep when placental growth is restricted. Am. J. Physiol. 257, R350–R357.

Perry, K.W., Janes, A.N., Weekes, T.E.C., Parker, D.S., Armstrong, D.G., 1994. Glucose and L-lactate metabolism in pregnant and in lactating ewes fed barley- or ground maize-based diets. Exp. Physiol. 79, 35–46.

Pethick, D.W., Lindsay, D.B., 1982. Metabolism of ketone bodies in pregnant sheep. Brit. J. Nutr. 48, 549–563.

Petterson, J.A., Dunshea, F.R., Ehrhard, R.A., Bell, A.W., 1993. Pregnancy and undernutrition alter glucose metabolic responses to insulin in sheep. J. Nutr. 123, 1286–1295.

Pi-Sunyer, F., Campbell, R.G., Hashim, S.A., 1970. Experimentally induced hyperketonemia and insulin secretion in the dog. Metabolism 19, 263–270.

Radcliffe, A.G., Wolfe, R.R., Colpoys, M.F., Mühlbacher, F., Wilmore, D.W., 1983. Ketone- glucose interaction in fed, fasted, and fasted-infected sheep. Am. J. Physiol. 244, R667–R675.

Reid, R.L., 1968. The physiopathology of undernourishment in pregnant sheep, with particular reference to pregnancy toxaemia. Adv. Vet. Sci. 12, 163–238.

Reid, R.L., Hinks, N.T., 1962. Studies on the carbohydrate metabolism in sheep XVIII. The metabolism of glucose, free fatty acids, ketones, and amino acids in late pregnancy an lactation. Aust. J. Agric. Res. 13, 1112–1123.

Reid, R.L., Hogan, J.P., 1959. Studies on the carbohydrate metabolism of sheep VIII. Hypoglycaemia and hyperketonaemia in undernourished and fasted pregnant ewes. Austral. J. Agric. Res. 10, 81–96.

Rook, J.S., 2000. Pregnancy toxaemia of ewes, does, and beef cows. Vet. Clin. North Am. Food Anim. Pract. 16, 293–317.

Rossi, G., Lapaczewski, P., Diamond, M.P., Jacob, R.J., Shulman, G.I., Sherwin, R.S., 1993. Inhibitory effect of pregnancy on counter regulatory hormone responses to hypoglycemia in awake rat. Diabetes 42, 1440–1445.

Sargison, N.D., Scott, P.R., Penny, C.D., Pirie, R.S., Kelly, J.M., 1994. Plasma enzymes and metabolites as potential prognostic indices of ovine pregnancy toxaemia – a preliminary study. Brit. Vet. J. 150, 271–277.

Schlumbohm, C., Harmeyer, J., 1999. Effect of hypocalcaemia on glucose metabolism in hyperketonaemic piglets. Exp. Physiol. 84, 707–723.

Schlumbohm, C., Harmeyer, J., 2003. Hypocalcemia reduces endogenous glucose production in hyperketonemic sheep. J. Dairy Sci. 86, 1953–1962.

Scott, P.R., Sargison, N.D., Penny, C.D., Pirie, R.S., Kelly, J.M., 1995. Cerebrospinal fluid and plasma glucose concentrations of ovine pregnancy toxaemia cases, inappetant ewes and normal ewes during late gestation. Brit. Vet. J. 151, 39–44.

Shipley, R.A., Clark, R.E., 1972. Tracer Methods for In Vivo Kinetics, Theory, and Applications. Academic Press, New York and London.

Sigurdsson, H., 1988. The effects of pregnancy and feeding on the insulin and glucose concentrations in blood of ewes in late pregnancy. Acta Vet. Scand. 29, 401–405.

Somogyi, M., 1952. Determination of blood sugar. J. Biol. Chem. 195, 19–23.

Van Der Walt, J.G., Procos, J., Labuschagne, F.J., 1980. Glucose turnover, tolerance and insulin response in wethers, ewes and pregnant ewes in the fed and fasted state. Onderstepoort J. Vet. Res. 47, 173–178.

Van Der Walt, J.G., Baird, G.D., Bergman, E.N., 1983. Tissue glucose and lactate metabolism and interconversions in pregnant and lactating sheep. Brit. J. Nutr. 50, 267–280.

Van Saun, R.J., 2000. Pregnancy toxemia in a flock of sheep. J. Am. Vet. Med. Assoc. 217, 1536–1539.

Varnam, G.C., Jeacock, M.K., Shepherd, D.A., 1978. Hepatic ketone-body metabolism in developing sheep and pregnant ewes. Brit. J. Nutr. 40, 359–367.

Wastney, M.E., Arcus, A.C., Bickerstaffe, R., Wolff, J.E., 1982. Glucose tolerance in ewes and susceptibility to pregnancy toxaemia. Aust. J. Biol. Sci. 35, 381–392.

Wastney, M.E., Wolff, J.E., Bickerstaffe, R., 1983. Glucose turnover and hepatocyte glucose production of starved and toxaemic pregnant sheep. Aust. J. Biol. Sci. 36, 271–284.

Williamson, D.H., Mallanby, J., Krebs, H.A., 1962. Enzymatic determination of D(−)-β-hydroxybutyric acid and acetoacetic acid in blood. Biochem. J. 82, 90–96.

Wilson, S., MacRae, J.C., Buttery, P.J., 1983. Glucose production and utilization in non-pregnant, pregnant and lactating ewes. Brit. J. Nutr. 50, 303–316.