J Vet Intern Med 2013;27:359–366

Insulin Sensitivity during Late Gestation in Ewes Affected by Pregnancy Toxemia and in Ewes with High and Low Susceptibility to this Disorder

R. Duehlmeier, I. Fluegge, B. Schwert, and M. Ganter

Background: Insulin resistance during late gestation might act as 1 etiologic factor causing pregnancy toxemia in ewes.

Objective: Evaluation of pancreatic insulin secretion and peripheral insulin sensitivity in ewes with differing susceptibility to pregnancy toxemia and in ketotic ewes.

Animals: Pregnant ewes suffering from (PT, n = 5) and ewes with high (HR, n = 7) and low risk (LR, n = 5) of being affected by pregnancy toxemia.

Methods: In a case-control study, the pancreatic insulin release and the peripheral insulin sensitivity were assessed by means of the intravenous glucose tolerance test with subsequent measurement of the plasma concentrations of glucose, insulin, nonesterified fatty acids (NEFA), and β-hydroxybutyrate (β-HB). The ewes were tested during late pregnancy within 5 and 15 days antepartum.

Results: The insulin secretion after glucose administration was significantly lower in the HR and PT than in the LR ewes. The baseline rate of lipolysis was significantly increased in the HR ewes, but the NEFA clearance was similar in both risk groups, albeit delayed in the PT ewes. The baseline β-HB concentration was significantly higher in the PT than in the HR and LR ewes. In the HR and in the PT ewes, the plasma β-HB concentrations did not decrease after glucose administration.

Conclusion and Clinical Importance: There is reduced pancreatic first-phase insulin response and impaired insulin-dependent inhibition of the ketone body formation during late pregnancy in the HR and PT ewes. This insulin resistance might represent 1 causative factor in the pathogenesis of ovine pregnancy toxemia.

Key words: Insulin resistance; Pregnancy toxemia; Sheep.

Pregnancy toxemia (PT) in sheep is a disorder of the maternal energy metabolism during late pregnancy, characterized by plasma β-hydroxybutyrate (β-HB) concentrations usually higher than 3.0 mmol/L[1,2] and ketonuria. The disease predominantly affects older ewes carrying 2 or more fetuses during the last weeks of pregnancy.[3] Besides age and multiple pregnancy, an individual or breed-dependent susceptibility appears to be involved in the pathogenesis of PT.[4] The most common clinical signs are weakness, depression, mental dullness, disorientation, anorexia, blindness, and finally recumbency and death after 3–10 days.[5] Even if treated intensively, 40% of the affected ewes die. In 20% of cases, the offspring die before or immediately after parturition.[6]

The etiopathology of PT is still poorly understood. Several observations[4,5] indicate that insufficient energy utilization rather than deficient energy supply is most probably the primary cause of PT. One important regulator of nutrient partitioning is insulin. Insulin activates glucose uptake in maternal skeletal muscle and adipose tissue,[7] and glucose phosphorylation in skeletal myocytes.[8,9] Besides its effect on glucose homeostasis, insulin decreases the breakdown of triacylglycerols in adipocytes,[10] and depresses hepatic ketone body formation.[11] Thus, insulin resistance promotes impaired glucose supply to the maternal skeletal muscle and adipose tissue, increases lipolysis, and enhances ketone body synthesis. Because hypo- and hyperglycemia, lipemia, and hyperketonemia are common findings in PT, individual or breed-dependent insulin resistance might be 1 major predisposing factor of this disease.[4]

Abbreviations:

AUC	area under the curve
AUP	area under the peak
β-HB	β-hydroxybutyrate
HSL	hormone-sensitive lipase
IGR	insulin glucose ratio
IVGTT	intravenous glucose tolerance test
k-value	glucose elimination rate
LPL	lipoprotein lipase
mHS	mitochondrial 3-hydroxy-3-methylglutary CoA synthetase
NEFA	nonesterified fatty acids
SCOT	succinyl CoA: 3-ketoacetic CoA transferase

From the Clinic for Swine, Small Ruminants, Forensic Medicine and Ambulatory Service, University of Veterinary Medicine Hannover Foundation, Hannover, Germany. *This study was carried out at the Clinic for Swine, Small Ruminants, Forensic Medicine and Ambulatory Service, University of Veterinary Medicine Hannover, and the Institute of Farm Animal Genetics, Friedrich-Loeffler-Institute (FLI) Federal Research Institute for Animal Health, Neustadt, Germany. This study was supported by the German Research Organization (DFG) grant SA 160/17 - 2. Results of this trial were presented at the 7th International Sheep Veterinary Congress, Stavanger, Norway, June 12–16, 2009 and at the International Conference on Small Ruminant Health Management, Sellin, Germany, May 23–25, 2012..*

Corresponding author: R. Duehlmeier, Clinic for Swine, Small Ruminants, Forensic Medicine and Ambulatory Service, University of Veterinary Medicine Hannover, Foundation, Bischofsholer Damm 15, D-30173 Hannover, Germany; e-mail: reinhard.duehlmeier@tiho-hannover.de.

Submitted March 22, 2012; Revised August 16, 2012; Accepted November 28, 2012.

Copyright © 2013 by the American College of Veterinary Internal Medicine

10.1111/jvim.12035

The aim of this trial was to evaluate the pancreatic insulin response and the peripheral insulin sensitivity during late pregnancy in ewes with probably different risks attributable to breed, age, and nutritional state of being affected by PT, and in ewes with this metabolic disorder.

Materials and Methods

In accordance with the German Animal Welfare Legislation, the animal tests included in this study were reported to the district government of Hannover, Lower Saxony, Germany (reference number: 33-42502–05/928).

Experimental Animals

The study was carried out on 5 ewes of different breeds suffering from PT, on 7 German Blackheaded Mutton (GBM) ewes, and on 5 Finnish Landrace (FL) ewes during late pregnancy (Table 1). GBM ewes are recognized as being highly susceptible to PT.[12] In the years 2007–2011, 47.1% of the ewes with PT admitted to the Clinic for Small Ruminants were GBM pure- or cross-bred ewes, while 31% of all sheep treated in the clinic during this period belonged to this breed. In contrast to the GBM breed, no cases of PT have been documented in pure-bred ewes of the highly reproductive[13] FL breed. All GBM and FL ewes were in good physical condition and healthy at the beginning of the investigation according to clinical examination and laboratory diagnostics (hematology, clinical chemistry). The diagnosis of "PT" depended on the clinical signs, weakness, depression, and anorexia during late pregnancy in combination with severe hyperketonemia. In this trial, the initial plasma β-HB concentration was on average 5.8 ± 1.0 mmol/L in the ketotic ewes. Only ewes suffering from primary PT, according to clinical and laboratory examination, were subjected to the study. All ketotic ewes survived until parturition, but 4 of the ewes died within 1–21 days after lambing. Concomitant laboratory findings in the ketotic ewes were increased activities of the creatine kinase in serum of 4 ewes and slight hypocalcaemia in 3 ewes. The activity of the liver enzyme glutamate dehydrogenase (GLDH) was enhanced slightly (21 IU/L) in one and severely (143 IU/L) in a second case compared with the reference value of 2–12 IU/L.[14] The latter ewe died 1 day postpartum because of liver rupture as a consequence of severe liver lipidosis. Necropsy of the other perished ewes revealed acute septicemia accompanied by slight lipidosis and slight leukocyte infiltration of the liver in 1 case. The cause of death in the remaining 2 ewes could not be identified and—because of histopathology—the livers of these ewes as well as the kidneys of all perished ketotic ewes were inconspicuous.

Irrespective of breed and body weight (BW), the GBM and FL ewes received a diet consisting of 500 g hay, 3 kg grass silage, and 300 g of mineralized concentrate daily. The resulting daily energy intake per sheep was approximately 21.1 MJ of metabolizable energy (ME). Because of this feeding regime, the FL ewes were moderately overnourished, whereas the GBM ewes were slightly underfed (Table 1). The ketotic ewes were fed on hay or grass silage, concentrate, and mineral premixes of different manufacturers at their origin farms. According to the owners' information, none of these ewes received an individual diet. Water was available ad libitum for all animals.

The GBM and FL ewes were kept in groups of four in a barn fitted with slatted flooring at the Friedrich-Loeffler-Institute, Neustadt, Germany. The ketotic ewes were housed on straw in single boxes at the Clinic for Small Ruminants, University of Veterinary Science, Hannover, Germany.

The experimental animals were divided into 3 groups; PT (= pregnancy toxemia), HR (= high risk), and LR (= low risk) ewes. According to the known predisposing factors responsible for susceptibility to PT (breed, age, dietary inadequacies), the HR group consisted of the older, slightly underfed GBM ewes. The young, moderately overnourished FL ewes represented the LR group.

Experimental Procedure

Intravenous Glucose Tolerance Tests (IVGTTs). The pancreatic insulin release and the peripheral insulin sensitivity were determined by the IVGTT. Retrospectively, the IVGTTs took place 4.6 ± 1.5 days antepartum (a.p.) in the PT ewes, 10.6 ± 2.3 days a.p. in the HR ewes, and 15.3 ± 4.5 days a.p. in the LR ewes. Because of this experimental design, it was concluded that the hormones of gestation and parturition which contribute to insulin resistance (ie, progesterone, cortisol, estrogens, prolactin[15]) were on a similar level in all 3 groups.[16] After an overnight fast, an indwelling cannula (Vygonüle T G 16[a]) was inserted into the jugular vein at 08:30 h. After a 30-min rest, 1 mmol glucose/kg BW was administered to the ewes as a sterile 40% solution[b] within 1 min. The glucose dosage of 1 mmol/kg BW was used because the assumed maximum plasma glucose concentrations during the IVGTT should not exceed the renal glucose threshold of 10 mmol/L.[17] Blood samples were collected 10 and 1 min before the glucose injection with heparinized tubes (Li-Heparin Monovette Sarstedt[c]). These samples were pooled

Table 1. Characteristics and reproductive performance of the experimental ewes.

	Experimental Group		
	PT "Pregnancy Toxemia"	HR "High Risk"	LR "Low Risk"
Breed	German Blackheaded Mutton (n = 3) Merino Landrace (n=1) Hornless Heath Sheep (n = 1)	German Blackheaded Mutton (n = 7)	Finnish Landrace (n = 5)
Age	3–7 years[ab]	4.5–6.5 years[a]	2.5 years[b]
Body weight	74.5 (45–102.5) kg[ab]	100.3 ± 4.3 kg[a]	65.6 ± 2.4 kg[b]
Nutritional status [14]**	unknown	undernourished by 4%	overnourished by 13%
Lambs born to each ewe*	2.2 ± 0.2[a]	2.0 ± 0.3[a]	2.6 ± 0.6[a]
Absolute offspring weight	9.9 ± 0.7 kg[a]	10.0 ± 0.8 kg[a]	7.1 ± 1.3 kg[a]
Relative offspring weight	10.9 ± 0.7%[a]	11.8 ± 1.0%[a]	12.7 ± 2.5%[a]

**The energy supply to the HR and LR ewes was calculated according to Kamphues et al.[47]
*In the HR and LR ewes, all lambs were born alive. This was only the case in 2 of the PT ewes.
Values without common superscript letters are significantly different ($P < .05$).

for determining the baseline value. Further blood samples were taken 5, 10, 15, 20, 30, 40, 50, 60, 80, 100, 120, 150, 180, 210, and 240 min after glucose administration. Samples were immediately chilled on ice and centrifuged for 10 min at 2000 × *g* and 4°C within 1 hour. Plasma was separated and stored at −20°C until further analyses.

Biochemical Analyses

Plasma concentrations of glucose were assayed by means of the hexokinase method by the commercially available Gluco-quant Glucose/HK test kit.[d] The intra-assay coefficient of variance (CV) of the test was 4.5% while the interassay CV was 6.0%.

Plasma insulin concentrations were determined by the Coat-A-Count Insulin RIA kit with human insulin as standard and antibody-coated tubes.[e] The intra-assay CV of the insulin RIA was 1.4%, and the interassay CV 5.1%.

Concentrations of nonesterified fatty acids (NEFA) in the ewes plasma were analyzed by the enzymatic color test kit NEFA C ACS-ACOD,[f] which has recently been demonstrated to represent an appropriate method for determining NEFA levels in ovine plasma.[18] The intra-assay CV and the interassay CV were 1.8% and 6.8%, respectively. The recovery in diluted pool plasma ranged between 90.9 ± 0.4% (1 : 8 dilution) and 98.1 ± 2.1% (1 : 2 dilution).

Plasma concentrations of D-β-HB were measured by the D-β-HB-dehydrogenase method described by Williamson and Mellanby.[19] The intra-assay CV and the interassay CV of this procedure were 12.6 and 15.6%, respectively.

In preliminary assays in the HR and LR ewes, the plasma NEFA and β-HB started to decrease 30 minutes after glucose administration. Therefore, in the LR and HR ewes, both parameters were measured only before and 5, 10, 30, 50, 60, 80, and 240 minutes after the glucose challenge.

Calculations

To compare the glucose tolerance among the PT, HR, and LR ewes, the areas under the glucose curves ($AUC_{glucose}$) were calculated as described previously.[20] Furthermore, the maximum percentage increase in the plasma glucose concentration attributable to glucose administration and the glucose elimination rates (k-value) was evaluated as previously described.[21]

To determine the insulin secretion during the IVGTT, the maximum plasma insulin values were normalized for baseline levels and the areas under the curves ($AUC_{insulin}$) and peaks ($AUP_{insulin}$) were calculated. The AUP was defined as the difference between the AUC and the area under the baseline (baseline insulin concentration*240 min).

To evaluate the potency of glucose as insulin secretagogue, the insulin-glucose ratio (IGR) was calculated. The IGR reveals the amount of insulin secreted by the stimulus of 1 mmol plasma glucose. The total IGR was defined as the area under the IGR curve (AUC_{IGR}).

To estimate the effect of insulin on NEFA and β-HB metabolism, the percentage decreases of both parameters after the glucose challenge were estimated in relation to the baseline (ie, the plasma NEFA and β-HB levels before glucose administration) values.

Statistics

Results are expressed as means and standard errors (SEM). Statistical analyses were carried out by SigmaStat 3.0.[g] Data were checked for normal distribution by means of the Kolmogoroff-Smirnoff test. Insulin and β-HB data had to be log-transformed to achieve normal distribution. Baseline concentrations were compared with postglucose application levels by one-way analysis of variance with repeated measures (ANOVA) in each experimental group to evaluate changes of the plasma metabolite and insulin values caused by the glucose challenge. To compare the PT, HR, and LR ewes, the calculated describing parameters (minimum, maximum, final concentrations, AUC, AUP, k-value, percentage increase and decrease) were compared by means of the one-way ANOVA and—if the ANOVA yielded a significant difference among the 3 groups—a subsequent Tukey test. The significance level was set at $P < .05$ in all statistical tests.

Results

Reproductive Performance

Ewes in both risk groups exhibited a similar reproductive performance with regard to the average number of lambs born alive, and the absolute and relative offspring weight. In the PT group, only the lambs of 2 ewes were born alive. Nevertheless, the number of lambs born to each PT ewe as well as the corresponding absolute and relative offspring weights did not differ significantly from those in the HR and LR groups (Table 1).

Plasma Glucose Concentrations

The baseline plasma glucose concentrations of 3.3 ± 0.8 mmol/L (PT ewes), 2.0 ± 0.2 mmol/L (HR ewes), and 2.5 ± 0.2 mmol/L (LR ewes) did not differ significantly among the 3 groups. The intravenous glucose administration increased the plasma glucose concentrations 3 to 3.5 fold in both risk groups and in the PT ewes in a similar manner (Fig 1). The glucose utilization—according to comparable $AUCs_{glucose}$ and similar glucose elimination rates (PT ewes: 10.7 ± 1.1, HR ewes: 13.2 ± 1.6, LR ewes: 15.3 ± 3.7)—also did not differ among the 3 groups (Fig 1).

Plasma Insulin Concentrations

For the ewes in all groups, the insulin secretion profiles were characterized by large interindividual variation. Thus, the baseline plasma insulin concentrations did not differ significantly between the experimental groups. After glucose administration, the maximum log-transformed plasma insulin concentration was significantly higher in the LR compared with the HR ewes (Fig 2A). When the plasma insulin concentrations were normalized to baseline values, the maximum log-transformed plasma insulin concentration was significantly higher as well as the $AUP_{insulin}$ being larger ($P < .05$) in the LR than in the PT and HR ewes (Fig 2B).

Insulin-Glucose Ratio (IGR)

The IGR data (Fig 3) paralleled the plasma insulin concentrations. During the IVGTT, the log-transformed maximum IGR was significantly higher in the

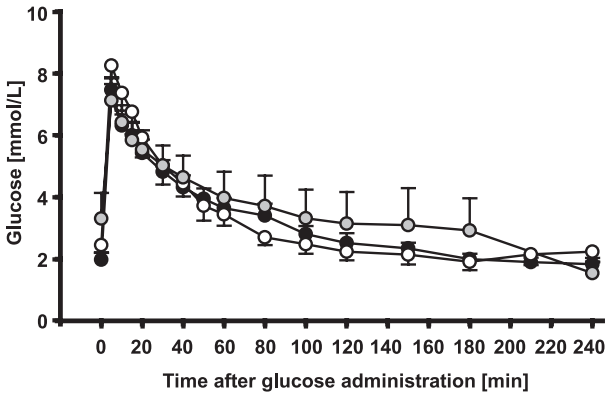

Fig 1. Mean (± SEM) plasma glucose concentrations during late pregnancy in ewes suffering from pregnancy toxemia (PT: ●; n = 5), and in ewes with high (HR: ●, n = 7) and low risk (LR: ○, n = 5) of being affected by pregnancy toxemia after a bolus glucose injection (1 mmol/kg BW).

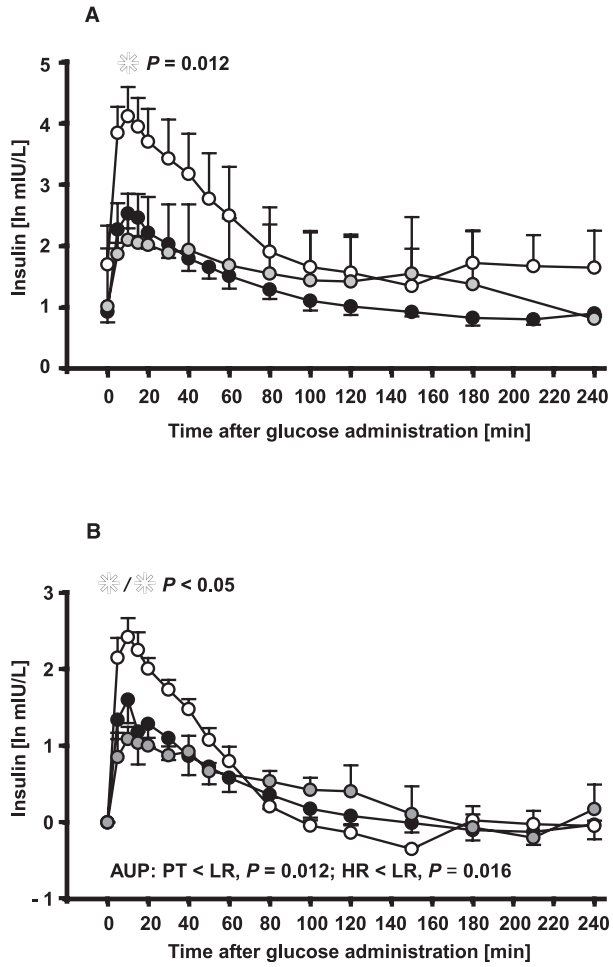

Fig 2. Mean (± SEM) log-transformed total plasma insulin concentrations (Fig 2A) and insulin levels normalized for baseline concentrations (Fig 2B) during late pregnancy in ewes suffering from pregnancy toxemia (PT: ●; n = 5), and in ewes with high (HR: ●, n = 7) and low risk (LR: ○, n = 5) of being affected by pregnancy toxemia after a bolus glucose injection (1 mmol/kg BW). AUP: area under the peak. ❁ = significantly higher log-transformed plasma insulin concentration in the LR than in the HR. ❁/❁ = significantly higher log-transformed plasma insulin concentration in the LR than in the HR and PT ewes.

LR than in the HR ewes. Comparing the LR and PT ewes, the maximum IGR was not higher in the LR than in the PT ewes (P = .089) (Fig 3).

Plasma NEFA Concentrations

In the ewes of all groups, plasma NEFA values decreased significantly after glucose administration. The percentage decrease in the plasma NEFA concentrations after the glucose challenge was comparable between the HR and LR ewes, although the HR ewes exhibited significantly higher baseline NEFA values than the LR ewes (Fig 4A). In the PT ewes, the percentage plasma NEFA values 30 minutes after the glucose application were significantly higher compared with the HR and LR ewes, indicating a delayed NEFA clearance (Fig 4B).

Plasma β-HB Concentrations

Significantly higher baseline β-HB concentrations were recorded in the PT ewes than in the ewes of the other groups (Fig 5A). During the IVGTT, the β-HB concentrations did not decrease significantly in the time period 30–80 minutes after the glucose challenge in the HR and PT ewes. As a consequence, the β-HB clearance during this gestational stage, ie, the percentage decrease in the β-HB concentrations 30–60 minutes after glucose administration, was significantly lower in the HR and in the PT ewes than in the LR sheep (Fig 5B).

Discussion

The objective of the present trial was to evaluate pancreatic insulin release and peripheral insulin sensitivity in ewes affected by PT (PT group) and in ewes with high risk of being affected by this disease (HR group). The main results were impaired pancreatic first-phase insulin response and abrogated β-HB elimination after intravenous glucose administration in the PT and HR ewes. The implication of these results on insulin resistance and its role in the pathogenesis of PT has to be discussed.

Insulin-Dependent Glucose Utilization

Early stages of noninsulin-dependent diabetes mellitus (NIDDM) and impaired glucose tolerance in humans are characterized by reduced first-phase insulin secretion after a glucose challenge.[22] In the present experiment, the PT and the HR ewes exhibited a lower first-phase insulin response to glucose and—in the HR ewes—an impaired IGR in contrast to the LR sheep. Thus, the responsiveness of the endocrine pancreas to glucose was impaired in the HR and in the PT ewes. However, neither the HR nor the hyperketonemic PT ewes exhibited impaired glucose tolerance. This finding in the PT ewes corresponds well with findings in

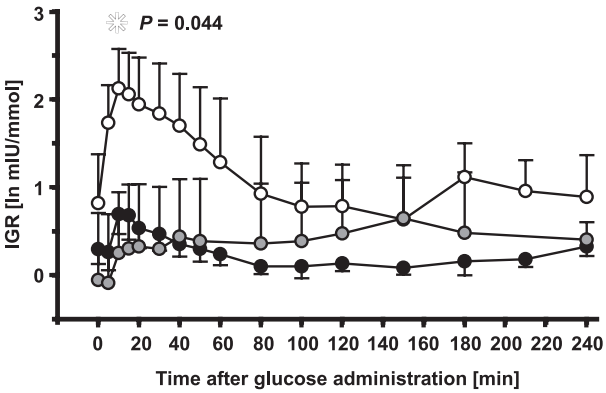

Fig 3. Mean (± SEM) log-transformed insulin-glucose ratios during late pregnancy in ewes suffering from pregnancy toxemia (PT: ●; n = 5), and in ewes with high (HR: ●, n = 7) and low risk (LR: ○, n = 5) of being affected by pregnancy toxemia after a bolus glucose injection (1 mmol/kg BW). ❋ = significantly higher IGR in the LR than in the HR ewes.

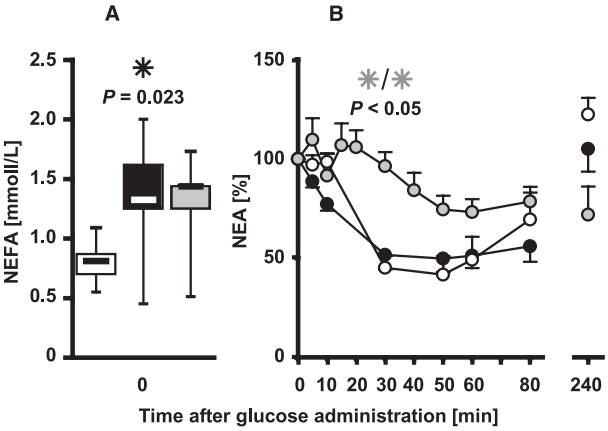

Fig 4. Baseline plasma concentrations (median, minimum, maximum, and lower and upper quantile) of nonesterified fatty acids (NEFA; Fig 4A) and percentage decrements (mean ± SEM) of the plasma NEFA levels (Fig 4B) during late pregnancy in ewes suffering from pregnancy toxemia (PT: ●; n = 5), and in ewes with high (HR: ●, n = 7) and low risk (LR: ○, n = 5) of being affected by pregnancy toxemia after a bolus glucose injection (1 mmol/kg BW). ✱ = significantly higher baseline plasma NEFA concentration in the HR than in the LR ewes. ✱/✱ = significantly higher percentage plasma NEFA concentration in the PT than in the LR and HR ewes.

hyperketonemic ewes because of the infusion of a DL-β-HB sodium salt racemate, which exhibited the same glucose utilization as normoketonemic ewes.[23] It has been established that the growing fetal-placental unit accounts for approximately 40% of the glucose disposal in ewes during late pregnancy.[24] The transplacental glucose transport is mediated by the insulin-independent glucose transporters (GLUT) 1 and 3,[25] whereas the insulin-dependent GLUT4 is predominantly located in skeletal muscle and in adipose tissue.[7] The methodical procedure of the IVGTT is not suitable for differentiating between the insulin-dependent and the insulin-independent glucose utilization.

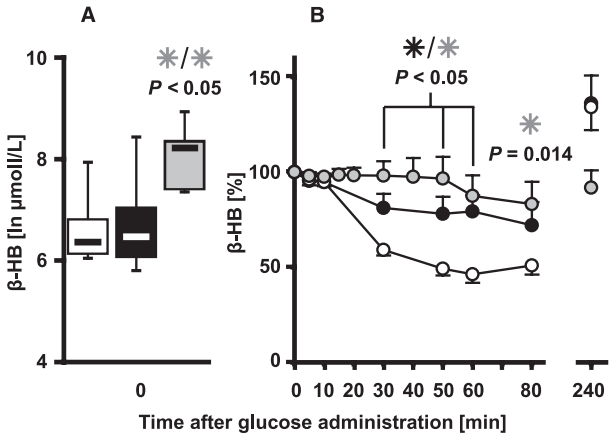

Fig 5. Baseline plasma concentrations (median, minimum, maximum, and lower and upper quantile) of β-hydroxybutyrate (β-HB; Fig 5B) and percentage decrements (mean ± SEM) of the plasma β-HB levels (Fig 5B) during late pregnancy in ewes suffering from pregnancy toxemia (PT: ●; n = 5), and in ewes with high (HR: ●, n = 7) and low risk (LR: ○, n = 5) of being affected by pregnancy toxemia after a bolus glucose injection (1 mmol/kg BW). ✱/✱ = significantly higher baseline plasma β-HB concentration in the PT than in the HR and LR ewes. ✱/✱ = significantly higher percentage β-HB concentrations in the HR and PT than in the LR ewes. ✱ = significantly higher percentage β-HB concentrations in the PT than in the LR ewes.

Thus, it appears likely that the peripheral insulin sensitivity was impaired in the PT and HR ewes, but this might have been masked by the increased insulin-independent fetal glucose uptake. Taking into account the reduced first-phase pancreatic insulin response, we assume insulin resistance in the PT and HR ewes, thus showing characteristics of early stages of NIDDM.[26]

Insulin Sensitivity with Regard to Lipolysis and Ketone Body Metabolism

Baseline lipolysis was significantly increased in the HR, but not in the PT ewes, compared with the LR group. These findings indicate higher demands for metabolic adaptations to late pregnancy in the HR compared with the LR ewes.

In all experimental groups, the plasma NEFA concentrations significantly decreased after glucose administration. Similar results were already reported in nonpregnant[27–29] and pregnant ewes[28,30] after intravenous glucose administrations and during euglycemic hyperinsulinemic clamp tests. Compared with the HR and LR ewes, the decrease in the plasma NEFA values after glucose administration was delayed in the PT ewes (Fig 4B). The decline of the plasma NEFA concentrations during the IVGTT probably relies on the inhibition of the hormone-sensitive lipase (HSL) in adipocytes[10,31] and the activation of the lipoprotein lipase (LPL) in skeletal muscle and adipose tissue,[32] by insulin which is secreted because of the glucose stimulus. Thus, the delayed NEFA clearance in the PT ewes may have been the consequence of an impaired responsiveness of these enzymes to insulin.

In contrast to the LR ewes, the plasma β-HB levels did not decrease at all after glucose administration in the HR and in the PT ewes (Fig 5B). This may be attributed to increased ketone body formation or to impaired ketone body utilization. The rate of ketogenesis depends on the activities of 3 key enzymes[33]: the adipocyte HSL; the hepatocyte acetyl CoA carboxylase; and the hepatic mitochondrial 3-hydroxy-3-methylglutaryl CoA synthase (mHS). Insulin inhibits the HSL and the mHS and stimulates the acetyl CoA carboxylase, thereby decreasing the rate of ketogenesis (for review see[11]). The HSL and the acetoacyl CoA carboxylase are dephosphorylated, while the mHS is inhibited by down regulation of the enzyme expression as a consequence of the insulin action.[34] These contrasting ways of enzyme regulation may recruit different postinsulin receptor signal cascades. It appears possible that the signal pathway contributing to the mHS inhibition, but not to the HSL and acetoacyl CoA carboxylase regulation, was disturbed in the HR and PT ewes. This resulted in an intact, albeit somewhat delayed in PT ewes, antilipolytic, but abrogated antiketogenic insulin effect. Nevertheless, only in the PT ewes did the baseline plasma β-HB values during late pregnancy exceed the reference value of 1.6 mmol/L[35] and the baseline concentrations in the LR and HR ewes. It has been demonstrated that the activity of the succinyl CoA: 3-ketoacetic CoA transferase (SCOT)—representing one of the key enzymes of ketolysis—is reduced when plasma β-HB levels exceed 5 mmol/L,[36] as recorded in the PT ewes in the present trial. Moreover, impaired ketone body disposal in ewes during different gestational stages with plasma β-HB concentrations of 5–7 mmol/L because of the infusion of DL-β-HB has been reported.[37] Thus, the increased baseline plasma β-HB values in the PT ewes may be the consequence of an impaired insulin-dependent control of the ketogenesis on one hand, and a reduced ketolysis on the other hand; whereas in the HR ewes, only the control of the ketone body formation was disturbed. A 2nd explanation for the dramatically increased baseline β-HB concentrations in the PT may be higher activities of the insulin-counteracting hormones.[11] For example, in ewes suffering from PT, higher cortisol insulin ratios have been reported than in healthy pregnant ewes.[38] Finally, liver failure may have contributed to the enhanced ketogenesis in the PT ewes. As postulated previously,[23] initial hypoglycemia may have stimulated a vicious circle, including increased gluconeogenesis, impaired NEFA oxidation, enhanced lipolysis, and ketogenesis. Nevertheless, only one of the PT ewes showed severely increased GLDH activity and severe liver lipidosis. Thus, the role of liver failure in the pathogenesis of PT could not be evaluated in this trial.

Possible Reasons for the Different Insulin Sensitivities between the Experimental Groups

The ewes in the 3 groups differed to a greater or lesser extent with regard to breed, age, and energy intake (Table 1). All 3 differences might have contributed to the different insulin sensitivities. The LR ewes were moderately overnourished during the last 4 weeks of pregnancy, which may have resulted in moderate obesity. Higher pancreatic insulin secretion and impaired glucose tolerance have been reported in distinctly[39] and moderately[40] obese sheep after overfeeding for 1–2 years. In humans, prolonged obesity and peripheral insulin resistance are accompanied by increased NEFA concentrations.[41] In the present experiment, the LR ewes showed neither impaired glucose tolerance nor elevated lipolysis and ketogenesis. Thus, it appears likely that the period of overnourishment in the LR ewes during the present trial was too short to influence the insulin action. On the other hand, the HR ewes were slightly underfed during late pregnancy. Pregnant and nonpregnant ewes, fed 50% of their energy requirements, exhibited elevated plasma NEFA concentrations[30] and decreased plasma glucose levels, while the insulin values were unaffected.[42] In a 2nd study, decreased plasma insulin values were recorded in ewes, which were fed 90% of their energy requirements during late pregnancy, compared with ewes that received an overnourishment of 10%.[43] Finally, in nonpregnant sheep, an energy supply of 18% lower than the requirements resulted in decreased plasma insulin levels, but in enhanced insulin sensitivity.[44] Taking these findings into account, it appears possible that the increased baseline plasma NEFA values, but not the insulin resistance regarding the ketone body disposal in the HR ewes, were caused by mild malnutrition. The energy supply to the PT ewes could not be evaluated. Thus, a possible influence of under- or overnutrition in the development of PT in this experimental group could not be assessed.

A 2nd important difference was the age of the ewes in the experimental groups. Fuel utilization and baseline and total energy expenditure were reported to decrease in older humans,[45] as well as the total energy expenditure in older sheep.[46] In this study, the HR ewes were significantly older than the LR ewes. Thus, the insulin resistance of the HR ewes may have been caused by the age difference between the HR and LR ewes.

Finally, the recorded insulin resistance in the HR and PT ewes may have been caused by the breeds. The high susceptibility of GBM to PT was documented earlier.[12] In the PT group too, the GBM ewes were the predominant breed. In summary, it appears likely that the impaired insulin sensitivity observed in the HR and PT ewes throughout the present experiment was breed-dependent and may explain the high risk of this breed being affected by PT.

Conclusion

The results of this study indicate a NIDDM-like impaired insulin-dependent inhibition of the ketone body formation in the HR and PT ewes, which might be a predisposing factor for PT. Further scientific work is needed to elucidate (1) whether this insulin resistance was breed-dependent or because of differences in age and energy supply and (2) the final inci-

dence, responsible for the clinical manifestation of PT in ewes susceptible to this metabolic disorder.

Footnotes

[a] Vygon Co., Aachen, Germany
[b] B. Braun Inc., Melsungen, Germany
[c] Sarstedt Co., Nürmbrecht, Germany
[d] Roche Diagnostics Co., Mannheim, Germany
[e] Siemens Healthcare Diagnostics Co., Eschborn, Germany
[f] Wako Chemicals Co., Neuss, Germany
[g] SPSS Inc., Chicago, IL

Acknowledgments

The authors thank B. Möller and T. Hantscher for their excellent care of the experimental animals. We are grateful to Prof. N. Parvizi and R. Wittig, the Institute of Farm Animal Genetics, Friedrich-Loeffler-Institute (FLI) Federal Research Institute for Animal Health, Höltystrasse 10, D-31535 Neustadt, Germany, for their technical assistance. Mrs. F. C. Sherwood-Brock, English Editorial Office, University of Veterinary Medicine Hannover Foundation is acknowledged for her careful English proofreading.

Conflict of Interest: Authors disclose no conflict of interest.

References

1. Wastney ME, Wolff JE, Bickerstaffe R. Glucose-turnover and hepatocyte glucose-production of starved and toxemic pregnant sheep. Aust J Biol Sci 1983;36:271–284.

2. Andrews A. Pregnancy toxaemia in the ewe. In Practice 1997;19:306–312.

3. Sargison ND, Scott PR, Penny CD, et al. Plasma enzymes and metabolites as potential prognostic indices of ovine pregnancy toxaemia—a preliminary study. Br Vet J 1994;150:271–277.

4. Marteniuk JV, Herdt TH. Pregnancy toxemia and ketosis of ewes and does. Vet Clin North Am Food Anim Pract 1988;4:307–315.

5. Rook JS. Pregnancy toxemia of ewes, does, and beef cows. Vet Clin North Am Food Anim Pract 2000;16:293–317.

6. Henze P, Bickhardt K, Fuhrmann H, et al. Spontaneous pregnancy toxaemia (ketosis) in sheep and the role of insulin. Zentralbl Veterinärmed A 1998;45:255–266.

7. Kahn BB. Glucose transport: Pivotal step in insulin action. Diabetes 1996;45:1644–1654.

8. Katzen HM. The effect of diabetes and insulin in vivo and in vitro on a low Km form of hexokinase from various rat tissues. Biochem Biophys Res Commun 1966;24:531–536.

9. Printz RL, Koch S, Potter LR, et al. Hexokinase II mRNA and gene structure, regulation by insulin, and evolution. J Biol Chem 1993;268:5209–5219.

10. Wang S, Soni KG, Semache M, et al. Lipolysis and the integrated physiology of lipid energy metabolism. Mol Genet Metab 2008;95:117–126.

11. Laffel L. Ketone bodies: A review of physiology, pathophysiology and application of monitoring to diabetes. Diabetes Metab Res Rev 1999;15:412–426.

12. Bickhardt K, Henze P, Ganter M. Clinical findings and differential diagnosis in ketosis and hypocalcemia in sheep. Dtsch Tierärztl Wochenschr 1998;105:413–419.

13. Oltenacu EA, Boylan WJ. Productivity of purebred and crossbred Finn sheep. I. Reproductive traits of ewes and lamb survival. J Anim Sci 1981;52:989–997.

14. Bickhardt K, Konig G. Blood values of healthy ewes of the Merino and Blackhead breed during parturition (reference values). Dtsch Tierärztl Wochenschr 1985;92:319–322.

15. Branisteanu DD, Mathieu C. Progesterone in gestational diabetes mellitus: Guilty or not guilty? Trends Endocrin Met 2003;14:54–56.

16. Chamley WA, Buckmaster JM, Cerini ME, et al. Changes in the levels of progesterone, corticosteroids, estrone, estradiol-17 β, luteinizing hormone, and prolactin in the peripheral plasma of the ewe during late pregnancy and parturition. Biol Reprod 1973;9:30–35.

17. Butterfield WJ, Keen H, Whichelow MJ. Renal glucose threshold variations with age. Br Med J 1967;4:505–507.

18. McMullen S, Osgerby JC, Milne JS, et al. The effects of acute nutrient restriction in the mid-gestational ewe on maternal and fetal nutrient status, the expression of placental growth factors and fetal growth. Placenta 2005;26:25–33.

19. Williamson DH, Mellanby J. D-(-)-3-hydroxybutyrate. In: Bergmeyer HU, ed. Methods of Enzymatic Analysis. 2nd ed. Weinheim/Bergstr: Verlag Chemie; 1970:1772–1775.

20. Fuhrmann H, Eulitz-Meder C, Geldermann H, et al. The evaluation of hormone and metabolite profiles after the infusion of glucose, propionate and butyrate in cattle. Berl Münch Tierärztl Wochenschr 1989;102:188–193.

21. Colvin HW Jr, Attebery JT, Daniels LB. Effect of diet on glucose tolerance of dairy calves one to thirteen weeks old. J Dairy Sci 1967;50:362–370.

22. Seino S, Shibasaki T, Minami K. Dynamics of insulin secretion and the clinical implications for obesity and diabetes. J Clin Invest 2011;121:2118–2125.

23. Schlumbohm C, Harmeyer J. Hyperketonemia impairs glucose metabolism in pregnant and nonpregnant ewes. J Dairy Sci 2004;87:350–358.

24. Prior RL, Christenson RK. Insulin and glucose effects on glucose metabolism in pregnant and nonpregnant ewes. J Anim Sci 1978;46:201–210.

25. Ehrhardt RA, Bell AW. Developmental increases in glucose transporter concentration in the sheep placenta. Am J Physiol 1997;273:R1132–R1141.

26. Johnson AB, Taylor R. Diabetes-mellitus. Postgrad Med J 1990;66:1010–1024.

27. Elmahdi B, Sallmann HP, Fuhrmann H, et al. Comparative aspects of glucose tolerance in camels, sheep, and ponies. Comp Biochem Physiol A Physiol 1997;118:147–151.

28. Regnault TR, Oddy HV, Nancarrow C, et al. Glucose-stimulated insulin response in pregnant sheep following acute suppression of plasma non-esterified fatty acid concentrations. Reprod Biol Endocrinol 2004;2:64-doi: 10.1186/1477-7827-2-64.

29. Kaske M, Elmahdi B, von Engelhardt W, et al. Insulin responsiveness of sheep, ponies, miniature pigs and camels: Results of hyperinsulinemic clamps using porcine insulin. J Comp Physiol B 2001;171:549–556.

30. Petterson JA, Slepetis R, Ehrhardt RA, et al. Pregnancy but not moderate undernutrition attenuates insulin suppression of fat mobilization in sheep. J Nutr 1994;124:2431–2436.

31. Jaworski K, Sarkadi-Nagy E, Duncan RE, et al. Regulation of triglyceride metabolism. IV. Hormonal regulation of lipolysis in adipose tissue. Am J Physiol Gastrointest Liver Physiol 2007;293:G1–G4.

32. Wang H, Eckel RH. Lipoprotein lipase: From gene to obesity. Am J Physiol Endocrinol Metab 2009;297:E271–E288.

33. Fukao T, Lopaschuk GD, Mitchell GA. Pathways and control of ketone body metabolism: On the fringe of lipid biochemistry. Prostaglandins Leukot Essent Fatty Acids 2004;70:243–251.

34. Arias G, Asins G, Hegardt FG, et al. The effect of fasting/refeeding and insulin treatment On the expression of the regulatory genes of ketogenesis in intestine and liver of suckling rats. Arch Biochem Biophys 1997;340:287–298.

35. Bickhardt K, Konig G, Jagerbloh A, et al. Plasma concentrations of glucose and of 3-hydroxybutyrate in ewes of different breeds and of different stages of reproduction and in ketotic ewes. Züchtungskunde 1989;61:121–130.

36. Fenselau A, Wallis K. Ketone body usage by mammals. Acetoacetate substrate inhibition of CoA transferase from various rat tissues. Life Sci 1974;15:811–818.

37. Harmeyer J, Schlumbohm C. Pregnancy impairs ketone body disposal in late gestating ewes: Implications for onset of pregnancy toxaemia. Res Vet Sci 2006;81:254–264.

38. Henze P, Bickhardt K, Fuhrmann H. The contributions of the hormones insulin, cortisol, somatotropin and total estrogen to the pathogenesis of sheep ketosis. Dtsch Tierärztl Wochenschr 1994;101:61–65.

39. Bergman EN, Reulein SS, Corlett RE. Effects of obesity on insulin sensitivity and responsiveness in sheep. Am J Physiol 1989;257:E772–E781.

40. McCann JP, Bergman EN, Reimers TJ. Effects of obesity and ovarian steroids on insulin secretion and removal in sheep. Am J Physiol 1989;256:E116–E128.

41. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 2006;444:840–846.

42. Petterson JA, Dunshea FR, Ehrhardt RA, et al. Pregnancy and undernutrition alter glucose metabolic responses to insulin in sheep. J Nutr 1993;123:1286–1295.

43. Charismiadou MA, Bizelis JA, Rogdakis E. Metabolic changes during the perinatal period in dairy sheep in relation to level of nutrition and breed. I. Late pregnancy. J Anim Physiol An N 2000;84:61–72.

44. West HJ. Maternal undernutrition during late pregnancy in sheep. Its relationship to maternal condition, gestation length, hepatic physiology and glucose metabolism. Br J Nutr 1996;75:593–605.

45. Roberts SB, Rosenberg I. Nutrition and aging: Changes in the regulation of energy metabolism with aging. Physiol Rev 2006;86:651–667.

46. Toutain PL, Toutain C, Webster AJ, et al. Sleep and activity, age and fatness, and the energy expenditure of confined sheep. Br J Nutr 1977;38:445–454.

47. Kamphues J, Coenen M, Iben C, et al. Nutrition of different species – sheep. In: Kamphues J, Coenen M, Kienzle E, eds. Supplements for Lectures and Exercises in Animal Nutrition, 11th ed. Hannover: Verlag M & H Schaper; 2008:225–233.